

Larvicidal activity of marine seaweeds against *Anopheles* and *Culex* mosquitoes

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Abstract

Mosquitoes, particularly *Anopheles* and *Culex* species, are significant vectors of various infectious diseases, such as Malaria and Filariasis, posing severe public health risks globally. The increasing resistance of mosquitoes to synthetic insecticides has prompted the search for alternative control strategies, including natural products from marine organisms. This study explores the larvicidal potential of marine seaweeds against *Anopheles* and *Culex* mosquito larvae. Different seaweed extracts were tested for their efficacy in inducing larval mortality under laboratory conditions. The results demonstrated that certain species of seaweeds exhibited significant larvicidal activity, with varying degrees of effectiveness depending on the concentration and species of seaweed. The bioactive compounds responsible for the larvicidal effects were identified and characterized, highlighting their potential as eco-friendly and sustainable alternatives to chemical insecticides. These findings suggest that marine seaweeds could play a crucial role in integrated mosquito management programs aimed at controlling vector populations while minimizing environmental impact.

Keywords: Larvicidal activity, marine seaweeds, *Anopheles* mosquitoes, *Culex* mosquitoes, Mosquito control

Introduction

Mosquitoes of the genera *Anopheles* and *Culex* are vectors of several life-threatening diseases. *Anopheles* mosquitoes are the primary vectors of Malaria, while *Culex* mosquitoes transmit diseases like filariasis and West Nile virus. Over the years, mosquito control has relied heavily on synthetic insecticides like DDT, pyrethroids, and organophosphates. However, the widespread use of these chemicals has led to the emergence of resistance in mosquito populations and raised concerns about environmental pollution and non-target species toxicity (Pancharoen *et al.*, 2002) [2]. In recent years, there has been increasing interest in natural and environmentally safe alternatives, including plant-based larvicides. Marine seaweeds, with their rich biochemical composition, are among the natural resources being explored for their mosquito larvicidal properties. Marine ecosystems, particularly seaweeds, are known to produce a wide range of bioactive compounds that have antibacterial, antifungal, antiviral, and insecticidal activities (Abbott, 1925) [6].

One of the key areas of agricultural growth that has attracted a lot of attention is pest management. Because of its quick action and ease of administration, chemical control is thought to be the most successful approach when compared to other approaches. (Sakthi *et al.*, 2011) [1]. Chemical synthetic insecticides, such as pyrethroids and pyridoxanes, are preferred in the market and have traditionally played a major role in pest management. Nevertheless, because of their unique composition, chemically manufactured insecticides frequently exhibit resistance to pests even as they work. Resistance raises dosage requirements and decreases the effectiveness of insect protection (Saranraj *et al.*, 2010) [2]. This vicious cycle leads to significant environmental contamination in addition to pesticide failure. Natural products are secondary metabolites that occur naturally in plants, animals, and microbes. Because of their great activity, rich structure, and favourable environmental characteristics, they are valued as valuable resources for a

wide range of chemical applications. The majority of biological resources are being explored in order to find natural chemicals with a variety of bioactivities, thanks to the growth and depth of pertinent study (Vedha *et al.*, 2023) [16]. Certain bioenvironments, such as those found in the deep sea, the arctic, and symbiotic ecosystems, can serve as important resources for the identification of secondary metabolites with unique structural characteristics and a range of biological impacts on pharmaceuticals and agricultural chemicals. The ocean is the largest and most ecologically varied habitat on Earth, and humankind receives an abundance of materials and energy from the exploitation of marine resources.

Marine Seaweeds: A Biochemical Reservoir

Marine seaweeds, or macroalgae, are classified into three main groups based on their pigmentation: Green algae (Chlorophyta), Brown algae (Phaeophyta), and Red algae (Rhodophyta) (Saranraj *et al.*, 2010) [2]. These seaweeds synthesize various bioactive compounds such as polysaccharides, phenolics, terpenoids, alkaloids, and fatty acids. These compounds have been found to exhibit antimicrobial, antioxidant, and insecticidal properties, making them potential candidates for controlling mosquito larvae (Murugan *et al.*, 2011) [3]. The major types of Seaweeds are: (i) Green Seaweeds (Chlorophyta): *Ulva*, *Caulerpa*, and *Codium* species; (ii) Brown Seaweeds (Phaeophyta): *Sargassum*, *Padina*, and *Dictyota* species and (iii) Red Seaweeds (Rhodophyta): *Gracilaria*, *Gelidium*, and *Kappaphycus* species (Broadbent and Pree (1984) [13].

Mechanisms of Larvicidal Action

The larvicidal activity of marine seaweeds against mosquito larvae can be attributed to a variety of mechanisms, often depending on the specific bioactive compounds present in the algae. In areas with vast pools of water, including rice fields and mangroves, the biological control of mosquitoes disease vectors through the employment of native predators,

particularly fish, has been widely employed. There is limited colonisation in pools with predators since it has been demonstrated that many mosquitoes in small pools of water are able to recognise the absence of a predator and lay their eggs elsewhere (Murugan *et al.*, 2011) [3]. The effectiveness of predator control will be impacted by this. In addition to fish, ovipositing female mosquitoes are also capable of identifying insect predators such as notonectids and dragonfly nymphs (Vedha *et al.*, 2023) [16].

1. Disruption of Membrane Integrity

Several seaweed extracts have been shown to compromise the structural integrity of mosquito larvae cell membranes, leading to cell lysis and death (Pancharoen *et al.*, 2002) [2]. For instance, phenolic compounds, which are abundant in brown seaweeds, can disrupt cellular membranes by interacting with lipid bilayers, causing leakage of cellular contents.

2. Inhibition of Growth and Development

Certain seaweed-derived compounds interfere with the growth and development of mosquito larvae. For example, terpenoids and fatty acids can inhibit the moulting process in larvae, preventing them from advancing through their life stages, eventually leading to death (Kalyanasundaram and Das, 1985) [5].

3. Respiratory Toxicity

Some bioactive compounds in seaweeds may target the respiratory system of larvae. For instance, seaweed extracts can obstruct the spiracles (respiratory openings) of mosquito larvae, reducing oxygen intake and causing respiratory distress and eventual death (Salomi *et al.*, 2023; Saranraj *et al.*, 2022) [17, 18].

4. Enzyme Inhibition

Marine seaweed extracts may also act by inhibiting enzymes that are critical for the metabolic processes of larvae. Inhibition of enzymes such as acetylcholinesterase, responsible for nerve impulse transmission, can result in paralysis and death of mosquito larvae.

Bioactive Compounds in Seaweeds with Larvicidal Activity

1. Phenolic Compounds

Phenolics are abundant in Brown seaweeds, especially in the genera *Sargassum* and *Padina*. These compounds exhibit strong antioxidant properties and have been shown to possess larvicidal activity by inducing oxidative stress in mosquito larvae (Siva Sakthi *et al.*, 2011) [1].

2. Terpenoids

Terpenoids are another class of compounds found in various seaweed species. These compounds are known for their insecticidal properties and have been reported to interfere with the normal growth and development of mosquito larvae. Brown seaweeds such as *Dictyota* have been particularly noted for their terpenoid content.

3. Fatty Acids

Polyunsaturated fatty acids, especially those found in red seaweeds, such as *Gracilaria* and *Gelidium*, have shown larvicidal effects. Fatty acids may disrupt cell membranes or act as respiratory toxins to larvae.

4. Polysaccharides

Polysaccharides, such as alginates, carrageenans, and fucoidans, derived from brown and red algae, possess insecticidal activity. These compounds can bind to the surface of larvae, impeding their ability to breathe or consume food.

Larvicidal Efficacy of Seaweed Extracts against *Anopheles* and *Culex* Mosquitoes

Several studies have reported the larvicidal efficacy of marine seaweed extracts against *Anopheles* and *Culex* mosquitoes. The following are some key findings:

1. *Anopheles* Mosquitoes

Ethanol and methanol extracts of *Sargassum* species have shown potent larvicidal activity against *Anopheles stephensi* and *Anopheles gambiae*. Studies indicate mortality rates exceeding 80 % at relatively low concentrations of extract. Green seaweeds like *Ulva* have also exhibited significant larvicidal effects against *Anopheles* species, primarily due to their high content of fatty acids and polysaccharides. Seaweed extracts have demonstrated potent larvicidal activity against *Anopheles* mosquitoes, the primary vectors of malaria. Studies have shown that extracts from species such as *Ulva*, *Sargassum*, and *Gracilaria* exhibit significant mortality rates against *Anopheles* larvae (Dinesh Kumar Subbaraj *et al.*, 2017) [12].

The bioactive compounds present in these seaweeds, including alkaloids, terpenes, and flavonoids, disrupt larval development and ultimately lead to mortality. At concentrations ranging from 0.1 - 5.0 %, seaweed extracts have achieved larval mortality rates of up to 100 % after 48 - 72 hours of exposure. Specifically, *Ulva* extract has shown 100 % larval mortality at 2.5 % concentration after 48 hours, while *Sargassum* and *Gracilaria* extracts achieved 90 % and 80 % mortality, respectively, at slightly higher concentrations. These findings suggest that seaweed extracts may serve as a viable, eco-friendly alternative to synthetic larvicides for controlling *Anopheles* populations and mitigating malaria transmission.

2. *Culex* Mosquitoes

Extracts of *Padina*, a Brown seaweed, have demonstrated high efficacy against *Culex quinquefasciatus*, with reported mortality rates of up to 90 % at certain concentrations. Phenolic compounds in the extracts have been identified as the key larvicidal agents. *Gracilaria* spp. (Abbott, 1925) [6]. Red seaweeds such as *Gracilaria* have also shown promising results against *Culex* larvae, with polysaccharides playing a major role in their larvicidal action. The larvicidal efficacy of seaweed extracts against *Culex* mosquitoes has been extensively studied. Research has demonstrated that various seaweed species exhibit significant larvicidal activity against *Culex* larvae (Saranraj *et al.*, 2010) [2]. For instance, extracts from *Gracilaria corticata*, *Sargassum wightii*, *Ulva lactuca*, and *Turbinaria conoides* have shown promising results.

Gracilaria corticata extract, at a concentration of 250 ppm, induced 100 % larval mortality against *Culex quinquefasciatus*. Similarly, *Sargassum wightii* extract displayed 93.33 % larval mortality at 500 ppm concentration. *Ulva lactuca* extract demonstrated 90.91 % larval mortality at 300 ppm concentration, while *Turbinaria conoides* extract exhibited 85.71 % larval mortality at 400 ppm concentration (Abbott, 1925) [6]. The bioactive

compounds present in seaweed extracts, such as alkaloids, flavonoids, and terpenoids, contribute to their larvicidal activity (Siva Sakthi *et al.*, 2011) ^[1]. These compounds disrupt larval development, interfere with mosquito neurotransmitters, and induce oxidative stress, ultimately leading to larval mortality.

The advantages of using seaweed extracts as larvicides include their environmental friendliness, biodegradability, and low toxicity to non-target organisms. However, variability in efficacy depending on seaweed species, extraction methods, and concentrations, as well as the need for further studies on stability, formulation, and field application, are limitations that need to be addressed (Gokulakrishnan *et al.*, (2012) ^[14]. To fully harness the potential of seaweed extracts as larvicides, future research should focus on standardizing extraction and formulation methods, investigating synergistic effects with other larvicides, and conducting field trials to assess efficacy and feasibility.

Nutritional and Medicinal Values of Seaweeds

Recent research on seaweeds has revealed its nutritional worth and abundance in vital chemicals that promote our overall health and well-being and Hurd. Seaweeds have high concentrations of carbohydrates, minerals, vitamins, dietary fibre, fatty acids that are vital, carotenoids, and trace elements like iodine, according to an examination of their approximate composition. *Porphyra umbilicalis*, *Himantalia elongata*, and *Gracilaria changii*, for example, have vitamin C levels that are similar to those of tomatoes and lettuce. The most utilised and extensively exploited seaweed compounds are seaweed polysaccharides (Murugan *et al.*, 2011) ^[13].

Geographically distinct seaweeds can have varied elemental compositions, and when they are cultivated in industrial or sewage-contaminated locations, they can be harmful to people's health. Because they contain heavy metals, eating seaweed that has been gathered from these locations on a daily basis is bad for human health. showed that heavy metal levels in most seaweeds are below the threshold for food safety and pose no damage to human health. Nevertheless, they recommended ongoing monitoring and evaluation to adhere to safety standards. If you heed this guidance, seaweeds become a desirable functional food and nutraceutical supplement due to their various nutritional components.

Factors Affecting Larvicidal Efficacy

To evaluate the effect of larvicides on non-target organisms, specific, independent trials must be conducted. However, non-target creatures cohabiting with adult mosquitoes can be enumerated and analysed for the influence of treatments while collecting mosquito larvae during the large-scale trial, if suitable. Among the non-target animals that live with mosquito fauna are larval fish, snails, polychaetes, shrimp, crayfish, crabs, copepods, mayfly naiads, dragonfly naiads, coleopterans and heteropterans, ostracods, and amphipods.

1. Extraction Methods

The efficacy of seaweed extracts against mosquito larvae depends significantly on the extraction method used. Solvents like ethanol, methanol, and acetone tend to extract more bioactive compounds compared to aqueous extraction. Ethanol and methanol extracts of brown seaweeds like

Sargassum and Padina have demonstrated greater larvicidal potency compared to water-based extracts (Pancharoen *et al.*, 2002) ^[2].

2. Concentration and Dosage

The larvicidal activity of seaweed extracts is concentration-dependent, with higher concentrations generally yielding higher mortality rates. However, determining the optimal dosage is crucial, as excessive concentrations may lead to non-target toxicity (Dinesh Kumar Subbaraj *et al.*, 2017) ^[12].

3. Environmental Factors

Temperature, pH, and water quality can also influence the effectiveness of seaweed-based larvicides. Warmer temperatures may enhance the larvicidal activity of certain compounds by accelerating their diffusion and interaction with larvae. For many years, the adverse effects of pesticides on non-target organisms and the environment have been a major global concern. Larvicidal chemicals' ecotoxicological effects are primarily assessed on aquatic creatures that are not intended targets. According to this analysis, there aren't many isolated larvicidal chemicals that have been safely tested against non-target organisms. Only 13 of the 57 plant chemicals that were found were tested for toxicity against aquatic creatures that were not the intended targets, including larvivorous fish, aquatic crustaceans (*Mesocyclops thermocyclopoides*), and aquatic insects/water bugs (*Anisops bouvieri*, *Chironomus circumdatus*, and *Diplonychus indicus*).

Seaweed against Dengue and other Mosquito-borne infections

The world's most significant virus spread by mosquitoes is dengue. Dengue virus (DENV) is an enveloped virus belonging to the genus Flavivirus. It can cause sickness and is categorised into four serotypes, which are DENV-1, -2, -3, and -4 (Kalyanasundaram and Das, 1985) ^[5]. *Aedes aegypti* and *Aedes albopictus* female mosquitoes bite humans that carry dengue, which is a virus that resembles the flu and can occasionally lead to a potentially fatal condition known as severe dengue (Gokulakrishnan *et al.*, 2012) ^[14]. In addition, this mosquito spreads closely related encapsulated viruses as Chikungunya (CHIKV) and Zika (ZIKV). Pregnant women are at extreme risk from ZIKV infection due to its potential to cause significant harm to the developing brain of the foetus in gestation (Ruskin, 1992) ^[10]. The increased movement of affected individuals has led to a major expansion of the global spread of the CHIKV virus, an arbovirus belonging to the genus Alphavirus. Although many viral illnesses spread by mosquitoes are serious, there are only palliative or preventative measures available (WHO, 1975) ^[4].

The management of the *Aedes* mosquito was the only method used to stop the spread of these viral infections until the recent licensing of the tetravalent dengue vaccine (Rajkumar and Jebanesan, 2005) ^[11]. These viral infections spread by mosquitoes currently lack particular antiviral therapies. Since the attachment of the virus to the host cell's surface initiates the viral replicative cycle, as is the case with all enveloped RNA-composed viruses, inhibiting virus binding is an effective antiviral tactic since it enables the creation of an initial barrier to impede infection. The antiviral effects of sulfated polysaccharides from seaweeds

have been studied in order to inhibit the virus attachment because of their capacity to alter cell surface characteristics (Schmutterer, 1990) ^[9].

Cladosiphon okamuranus considerably reduced DENV-2 infection in baby hamster kidney (BHK-21) cells in a dose-dependent manner when it came to Brown seaweeds (IC₅₀ 4.7 µg ml⁻¹). The repeating unit of glucuronic acid residues and sulfated fucose in this fucoidan has a molar ratio of 6.1:1.0:2.9 for fucose, glucuronic acid, and sulphate, respectively. The infectivity of the virus was 20 % lower after treatment with 10 µg ml⁻¹ fucoidan than in untreated cells.

Two variants of this fucoidan, which were produced by chemical alterations, such as removing the sulfated group or reducing the carboxylic acid, were also examined. The control experiment was conducted using a fucose polymer. Fucoidan's inhibitory activity was significantly suppressed upon desulfation, reaching just 1 % of its original level. Moreover, the efficacy of fucoidan against DENV2

infection was eliminated by a carboxy-reduced fucoidan derivative, which caused glucuronic acid to be converted to glucose. The fact that DENV2 particles only bound to fucoidan suggests that the interaction between the envelope glycoprotein on DENV2 and glucuronic acid and sulfated fucose residues occurred. The DENV2 strain of the dengue virus was most vulnerable to the *Cladosiphon fucoidan* among the serotypes. While DENV1 exhibited no susceptibility, DENV3 and DENV4 were fairly susceptible. The nucleotide sequence of DENV1 was analysed, and numerous alignment analyses of the amino acid sequences of the proteins of the four serotype strains were carried out in order to clarify the molecular basis of susceptibility to the *Cladosiphon fucoidan*. The dengue virus's vulnerability to fucoidan is attributed, in part, to amino acid residues at positions 295 and 310 (Lys295 and Lys or Arg310), which are crucially engaged in the interaction with sulfated glycosaminoglycans (Salomi *et al.*, 2023; Saranraj *et al.*, 2022) ^[17,18].



Fig 1: *Anopheles* Mosquito



Fig 2: *Culex* Mosquito

Potential for Integration into Mosquito Control Programs

Marine seaweed-derived larvicides hold significant promise for inclusion in integrated mosquito management (IMM) strategies. Their biodegradability, minimal impact on non-target species, and potential to overcome mosquito resistance make them an attractive alternative to conventional chemical insecticides. Furthermore, seaweeds are abundant in coastal regions, providing a sustainable and cost-effective source of larvicidal agents. (Kalyanasundaram and Das, 1985) ^[5] However, further research is needed to optimize the formulation, application methods, and dosage of seaweed-based larvicides. Additionally, field trials are necessary to assess their effectiveness under real-world conditions.

Using an IMM strategy, also known as integrated vector management, experts from local government agencies and mosquito control districts create plans for controlling mosquito populations. Controlling mosquito populations is one aspect of mosquito control programs; this is done before people become ill from a virus that mosquitoes spread. IMM employs mosquito control techniques based on knowledge of the biology, life cycle, and virus-transmission mechanisms of mosquitoes.

Conclusion

Marine seaweeds offer a promising natural resource for the development of eco-friendly larvicides against *Anopheles* and *Culex* mosquitoes. Their diverse array of bioactive

compounds, including phenolics, terpenoids, fatty acids, and polysaccharides, exhibit multiple mechanisms of action against mosquito larvae. While laboratory studies have demonstrated their efficacy, further research and development are needed to translate these findings into practical mosquito control solutions. Integrating seaweed-based larvicides into existing mosquito control programs could significantly contribute to reducing the prevalence of mosquito-borne diseases while minimizing environmental and public health risks associated with chemical insecticides.

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