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# A case study of screening for larvicidal activity of *Ulva reticulata* and *Colpomenia sinuosa* acetone extracts against *Artemia salina*

**E. Udayan[1], A. Kathiravan[1], S. Mukund[2] and R. Ranjith Kumar*[1]**

[1]Department of Botany, Madras Christian College (Autonomous), Chennai, Tamil Nadu, India.
[2]Phycospectrum Environment Research Centre, Chennai, Tamil Nadu, India
*****Corresponding Author E-mail id: ranjithkumar@mcc.edu.in

**Abstract**

Acetone extracts from *Ulva reticulata* and *Coplomenia sinuosa* were evaluated at different concentrations (10, 20, 30, 40 and 50 µl/ml) for their larvicidal activity against *Artemia salina*. After 24 hours, the viability of *Artemia salina* larvae was observed and the mortality rate was recorded. Chlorophyll ‘a’ and ‘b’ were found to be 14.12 mg/ml and 21.87 mg/ml in *Ulva reticulata*, 7. 2312 mg/ml and 20 mg/ml in *Colpomenia sinuosa* respectively in the acetone extract as a dominant derivative component. The results of this study conclude that the acetone chlorophyll extracts from *Ulva reticulata and Colpomenia sinuosa* applied at various concentrations, showed 100% mortality rate which indicates their effective larvicidal activity against *Artemia salina*.

**Key words**

*Ulva reticulata, Colpomenia sinuosa*, Seaweeds, Larvicidal activity, *Artemia salina*

## Introduction

Marine algae are the chief producers of biomass in marine habitats. In the last 30 years of the 20$^{th}$ century, aquaculture has turned into one of the pioneer fields for food industries, leading to the increased production of fish, mollusks, crustaceans and macroalgae (Titlyanov and Titlyanova, 2010). Luning *et al.,* 2003 reported that the mass production of seaweeds crossed more than 8 million tonnes of fresh weight (FWT) out of which 6 million tonnes of brown algae, 2.1 million tonnes of red algae and around 100,000 tonnes of green algae were recorded. Seaweeds play a major role in providing food and shelter to a wide range of organisms present in the coastal ecosystem (Belanche *et al.,* 2016). Seaweed extracts are a natural source of a wide variety of structures which can be used in the field of nutraceuticals, functional foods, food additives and biological agrochemicals (Joana Gill-chavez *et al.,* 2013; Jacob-lopes *et al.,* 2015).

Seaweeds are capable of producing a group of primary, secondary and other bioactive compounds with greater utilities and functions, making them as a potential source for pharmaceutical agents (Mayer and Hamann, 2002; Newman *et al.,* 2003). Certain properties such as antibacterial, anti-inflammatory, antihelminthic, antifungal, antioxidant, antiviral and antiallergenic have been found in green, brown, and red macroalgae (Newman *et al.,* 2003; Wijesinghe and Jeon, 2011). Over the years, great interest has been developed to isolate the bioactive compounds responsible for the larvicidal activity. Several studies have been carried out to demonstrate the presence of various biological activity in seaweeds such as larvicidal, antifeedant and anticoagulant (Manilal *et al.,* 2009). Campbell *et al.,* (1933) was the first person to report about the larvicidal activity of plants. Marine algal products may serve as suitable substitutes for artificial insecticides as they are environment friendly, biodegradable and readily available throughout the world. Larvicides play an important role in controlling mosquitoes at the larval stage (Poonguzhali and Nisha, 2012). In this research paper, Brine shrimp (*Artemia salina*) was used as the screening system for larvicidal activity using acetone extracted chlorophyll of *Ulva reticulata* and *Colpomenia sinuosa* collected from Kilakarai, Ramnad District, Tamil Nadu, India. The main advantage of using *Artemia salina* is that the eggs (cysts) are readily available in the market from which active larvae can be attained within 1 or 2 days.

 

## Materials and methods

### *Sample collection and preparation*

The samples of *Ulva reticulata and Coplomenia sinuosa* were collected (**Fig. 1**) in February 2018 from Kilakarai 9.2343° N, 78.7836° E, located in the Southeast coast of Tamil Nadu. The elevation of the coast is 17 m from the sea level and the weather is around 28°C, wind NE at 16 km/h, 67% humidity. The place has ample marine algal growth round the year. The shore is sandy with a rough and uneven surface. The seaweeds were collected in clean transparent polythene bags along with the seawater and brought to the lab. The samples were washed in running tap water to remove all the epiphytes and other surface debris. Then the samples were rinsed in distilled water to remove all the salts. Further, they were shade dried for a week to remove all the moisture contents. Finally, the dried biomass was powdered using a mortar and pestle.


**Fig. 1 Collected specimens a, *Ulva reticulata* b, *Colpomenia sinuosa***

### *Extraction of chlorophyll*

Two grams of powdered biomass of *Ulva reticulata and Coplomenia sinuosa* were weighed separately and 10 ml of 90% acetone was added to each biomass and sonicated for 15 minutes using an ultrasonic processor (Vibronics P2). After complete extraction, it was again centrifuged at 3000 rpm for 15 minutes and the clear supernatant was used for spectrophotometric estimation of chlorophyll pigments using double beam UV-visible spectrophotometer (Shimadzu UV – 1650 PC). The supernatant was utilized for further analysis (**Fig. 2**).


**Fig. 2 Extraction of chlorophyll by acetone a, *Ulva reticulata* b, *Colpomenia sinuosa***

### *Estimation of chlorophyll*

The concentrations of Chlorophyll 'a', 'b' and total chlorophyll were estimated using the extinction coefficients given by Arnon, (1949). The equation is as follows:

$$\text{Chlorophyll 'a'} = 12.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll 'b'} = 22.9(A_{645}) - 4.68(A_{663})$$

$$\text{Total Chlorophyll} = 20.2(A_{645}) + 8.02(A_{663})$$

***Larvicidal activity***

The eggs of Brine Shrimp, *Artemia salina* were procured from Philadelphia, USA. For hatching, these eggs were incubated for 48 hours in a small water tank filled with marine water and provided with required aeration and light for a 12 hours cycle. After 48 hours of hatching, the larvae at nauplii stage were removed and used for the experiment. 20 larvae were taken in different test tubes containing 10 ml of seawater. To this, acetone chlorophyll extracts from *Ulva reticulata and Colpomenia sinuosa* at different concentrations (10, 20, 30, 40 and 50 µl/ml) were added. Seawater was used as control. After 24 hours, the viability of the larvae was observed and the mortality rate was recorded. Larvae which were immobile and stayed at the bottom of the test tubes were considered dead. The percent of mortality of brine shrimp is calculated as follows:

**% Mortality = (No. of brine shrimp dead/ No. of brine shrimp introduced) x 100**

The experiment was carried out in triplicates.

**Results**

***Estimation of acetone extracted chlorophyll***

The main objective of this work was to study the larvicidal potential of acetone extracted crude chlorophyll from *Ulva reticulata* and *Colpomenia sinuosa*. Chlorophyll 'a' and 'b' were found to be 14.12 mg/ml and 21.87 mg/ml in *Ulva reticulata*, 7.2312 mg/ml and 20 mg/ml in *Colpomenia sinuosa*. The total chlorophyll concentration found in *Ulva reticulata* and *Colpomenia sinuosa* were 35.99 mg/ml and 27.23 mg/ml respectively. Chlorophyll contents of *Ulva reticulata* and *Colpomenia sinuosa* are tabulated in **Table 1**.

**Table 1 Chlorophyll contents in *Ulva reticulata* and *Colpomenia sinuosa***

| S.No | Seaweed species | Total Chlorophyll (mg/ml) | Chlorophyll 'a' (mg/ml) | Chlorophyll 'b' (mg/ml) |
|---|---|---|---|---|
| 1 | ***Ulva reticulata*** | 35.99 | 14.12 | 21.87 |
| 2 | ***Colpomenia sinuosa*** | 27.23 | 7.23 | 20.0 |

***Larvicidal activity***

The larvicidal potential of acetone chlorophyll extracts from *Ulva reticulata* and *Colpomenia sinuosa* showed 100% mortality rate in various concentrations (ranging from 10 µl, 20 µl, 30 µl, 40 µl and 50 µl). The results were recorded after 24 hours of incubation period. Larvicidal activity of acetone chlorophyll extracts are presented in **Fig. 3**. The percentage of mortality is tabulated in **Table 2**.

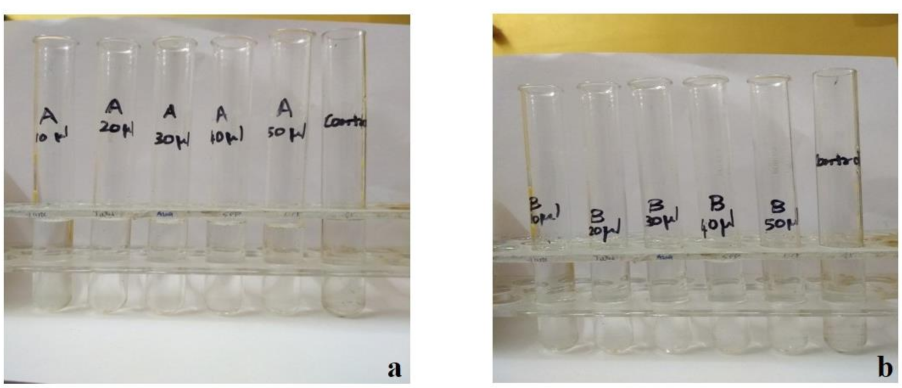


**Fig. 3 Larvicidal activity of acetone chlorophyll extracts against *Artemia salina*. a, *Ulva reticulata*; b, *Colpomenia sinuosa***

**Table 2 Larvicidal activity of acetone chlorophyll extracts from *Ulva reticulata* and *Colpomenia sinuosa* on *Artemia salina* (in % Mortality at 24 hrs)**

| Volume of Sample | Sample | |
|---|---|---|
| | ***Ulva reticulata* (%)** | ***Colpomenia sinuosa* (%)** |
| **10 µl** | 100 | 100 |
| **20 µl** | 100 | 100 |
| **30 µl** | 100 | 100 |
| **40 µl** | 100 | 100 |
| **50 µl** | 100 | 100 |

**Discussion**

Seaweeds are omnipresent in the coastal environment and several of them are reported to possess bioactive compounds with enormous biomedical applications. Fomenko *et al.,* (2016) reported that in the coming modern era, more importance would be given on marine biodiversity including macroalgae and microalgae due to the presence of novel compounds in their natural resources. Macroalgae serve as a potential source for the production of various bioactive compounds, primary metabolites, amino acids, lipids, dietary fibers, vitamins, total phenolic compounds *etc*. The results that are obtained in the present investigation supports the traditional use of the seaweeds for larvicidal activities (Poonguzhali and Nisha, 2012; Kim and Choi, 2017). The present study revealed that acetone extracted crude chlorophyll from seaweeds such as *Ulva reticulata* and *Colpomenia sinuosa* show higher efficiency in its larvicidal activity. According to Bhuvaneshwari *et al.,* (2014) leaf extracts of *Lannea coromandelica* showed 100% mortality of *Artemia salina* at a concentration of 2mg/ml. Ethanol extracts of nine different seaweeds showed larvicidal activity against *Artemia salina* (Ayesha *et al.,* 2010). Brine shrimp lethality assay was used to determine the cytotoxic activities of the seaweed species (Ara *et al.,* 1999; Kim and Choi, 2017). Ayesha *et al.,* (2010) reported that 6 ethanol extracts of seaweed species collected from the Karachi coast in Pakistan showed results for the cytotoxicity of Brine shrimps. Kim and Choi (2017) reported that methanol extracts of 6 different seaweeds (15.79%) have shown positive activity in Brine shrimp lethality. Ethanolic extract of *Gracilaria corticata* at a dose of 2 mg/ml showed 100% mortality of *Artemia salina* after 24 hours of incubation (Sreejamole and Greeshma, 2013). Moreover, *Artemia salina* is being used for preliminary cytotoxic activity studies. The present results also reveal that the two algae investigated, *Ulva reticulata* and *Colpomenia sinuosa* may possess anticancer property because of their 100% lethality against *Artemia salina* larvae.

**Conclusion**

Results of the current investigation indicate that the acetone chlorophyll extracts from *Ulva reticulata* and *Colpomenia sinuosa* were effective in its larvicidal activity which could be a tremendous alternative source for mosquito larvicides because they constitute as a potential source of bioactive chemicals that are generally free from causing harmful effects to the environment and non-target organisms. Furthermore, to the best of our knowledge, this was a first time study done in a cost-effective and environmental friendly approach which can get high public recognition. Use of these acetone chlorophyll extracts from *Ulva reticulata* and *Colpomenia sinuosa* as larvicides in mosquito control instead of synthetic insecticides could reduce the cost, adverse environmental effects, pollution and mosquito-borne diseases *inter alia*. However, further studies on the identification of active compounds from macroalgae and field trials are needed to recommend the active fraction of these algal extracts for the development of eco-friendly larvicides for controlling insect vectors.

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