Research Article
J. Adv. Nutri. Sci. Technol. 4(3-4): 45-51, 2024 **DOI:10.15228/ANST. 2024.v04.i03-4.p06**

Repellent Effects of *Codium flabellatum* and *Caulerpa taxifolia* Seaweed Extracts on *Aedes aegypti*

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Abstract
This study investigated the repellency activity of extracts from two green seaweeds, *Codium flabellatum* (*C. flabellatum*) and *Caulerpa taxifolia* (*C. taxifolia*), against the dengue vector *Aedes aegypti*. Extraction was conducted using ethanol as the solvent, with subsequent evaluation of repellency through the hand-introducing method. Various concentrations of seaweed extracts (20%, 30% and 40%) were prepared and tested. Results indicated moderate repellent effects across all samples, with the highest repellency observed in *C. flabellatum* at a 40% concentration, achieving 100% repellency at 6 hours. The LT_{50} values (lethal time at which 50% of mosquitoes repel due to the applied sample on human skin) showed that *C. flabellatum* at 40% concentration exhibited the highest repellency (LT_{50} value: 12 hours) against *Aedes aegypti* followed by *C taxifolia* (LT_{50} value: 10 hours) at the same concentration. Conversely, a 20% concentration of seaweed extracts demonstrated the most negligible effectiveness, with an LT_{50} value of 3 hours against *Aedes aegypti*. These findings suggest that seaweed possesses promising potential as an eco-friendly mosquito repellent and efficient biocontrol agent against mosquitoes.

Keywords: repellency activity, green seaweeds, Extraction, *Aedes aegypti*

Highlight:

- green seaweed extract is used as green organic mosquito repellent
- the highest repellency observed in 12 hours
- Seaweed extract proves the potential to take the place of existing synthetic repellent.

1.0. Introduction

The proliferation of mosquitoes in our surroundings, thriving in breeding grounds such as stagnant water and punctured tire shops, has become a growing concern (Tariq & Zafar, 2000). Many synthetic repellents containing chemicals like N-N Diethyl-Meta-toluamide (DEET), Allethrin, and Dimethyl phthalate pose risks due to adverse effects on human health (Tabassum, 2016; Asadollahi *et al*., 2019). Sprays such as DDT (Dichloro diphenyl trichloroethane), once used to combat mosquitoes, are banned in various countries due to their detrimental impact on wildlife and the environment (Hira et al., 2017: Hira et al., 2018; Kabasenche & Skinner, 2014; da Costa et al., 2019).

Mosquitoes play a significant role in transmitting mosquito-borne diseases such as dengue, yellow fever virus, Zika, filariasis, schistosomiasis, and *Japanese encephalitis* (JE), causing illness and death worldwide (Gurunathan *et al*., 2016; Asadollahi *et al*., 2029). Dengue fever, in particular, has seen a significant rise, with approximately 100 countries now affected, compared to only nine before 1970 (Tan *et al*., 2019; Yu et al., 2015). The transmission of mosquito-borne diseases affects over 700 million people annually (Shammemrani K., 2018). Malaria alone claims the lives of 3 million people yearly, with one child succumbing every 30 seconds (Alarif *et al*., 2010).

Scientific studies have investigated the insecticidal activity of volatile constituents from various algae, with Actinotrichia fragilis volatile oil showing promising results (Qari & 2018; Shameel, 2008; Pasdaran *et al*., 2016). Metabolites obtained from *Caulerpa racemose* are also alternative to synthetic insecticides (Alarif *et al*., 2010; Nagaraj *et al*., 2014).

The primary objective of this study is to develop a natural insecticidal product utilizing indigenous seaweeds (Arumugam et al., 2018; Chan & Matanjun, 2017), potentially manufacturing mosquito repellents. Mosquitoes cause nuisance through their bites and serve as significant vectors for severe human diseases (Amerasan et al., 2012; Alarif *et al*., 2010). Effective mosquito control strategies are crucial in combating mosquito-borne diseases (Anuradha *et al*., 2016). Using synthetic insecticides poses risks to non-target organisms, including beneficial insects like honey bees, highlighting the importance of eco-friendly alternatives (Ananth *et al*., 2017; Tariq & Qadri., 2001; Mahyoub, 2018). Natural mosquito repellents offer a safer, environmentally friendly solution to mitigate the adverse environmental and human health effects (Tan et al., 2019; Khattab *et al*., 2017). Given the current situation in Pakistan, where cases of Dengue fever are rampant, natural mosquito repellents could play a pivotal role in reducing or preventing such outbreaks and other diseases transmitted by mosquitoes (Ali *et al*., 2013; Yu *et al*., 2015). It is imperative to explore alternative approaches that are environmentally sustainable, cost-effective, and biodegradable (Ali *et al*., 2013; Yu *et al*., 2015). Therefore, the current research was planned to explore the potential of green seaweeds available in the Karachi coastal

area (Pati et al., 2016) to control the impact of mosquitoes. This study provides a valuable natural seaweed extract as a natural mosquito repellent.

2.0. Material and Methods

2.1. Collection of Seaweeds

The study area encompasses a range of habitats, including small intertidal pools, rocky ledges, sublittoral zones, cobbles, shallow sand bottom flats and widely scattered boulders that exhibit a diversity of seaweeds.

Green algae specimens utilized in this investigation were collected from the Buleji coast in Karachi from February to November, excluding June, July and August (2020). These algae were collected by hand-picking and placed in plastic bags during low tides. The chosen seaweeds for this study were *Codium flabellatum* and *Caulerpa taxifolia*. *Codium flabellatum* was typically encountered in drift form, while *Caulerpa taxifolia* was firmly anchored to the substratum through its rhizoids.

2.2. Extraction:

After the collection, algae were washed thrice with tap water to remove the adhering salts, epiphytes and other detritus material (Afzal Rizvi & Shameel, 2005; Anuradha *et al*., 2016). Then, the shade is dried in a cage for a couple of weeks until it is completely dried. The algae were kept in air-tight polythene bags for further use. Some specimens, along with the holdfast, were also preserved in the form of herbarium. From the stock dried, *Codium flabellatum* was taken and soaked in ethanol in a ratio of 1:3. Similarly, *Caulerpa taxifolia* was also soaked in ethanol in a ratio of 1:3; both seaweeds were soaked for about 3 weeks/21 days. The solvents were evaporated from the crude extract by rotary evaporator at 50˚C. The crude ethanol extract was kept in air-tight glass vials and stored in a refrigerator for further use (Abbassy *et al*., 2014). The flow chart showed the extraction processes of seaweeds with ethanol (Fig.1).

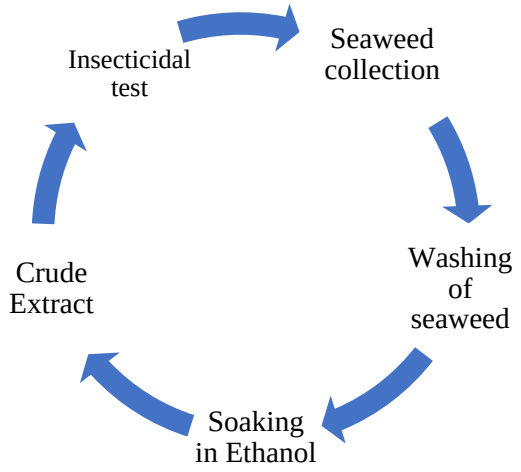

Figure 1. Flow chart of Extraction.

2.3. Testing:

2.3.1. Mosquitoes Culturing:

Mosquitoes were cultured in the laboratory, and a total of 100 male and 100 female mosquito pupae were placed in a bowl filled with tap water (Ashrafi *et al*., 1966; Ramar *et al*., 2014). This bowl was positioned within a cage measuring 30×24×24 inches. Over the course of several days, adult mosquitoes emerged from the pupae. Throughout their developmental stages, from egg to larvae, pupae, and finally to adulthood, the mosquitoes were closely monitored to ensure they remained free of viral contamination. Upon emergence, the adult mosquitoes were initially provided with a blood meal, followed by a 5% sugar solution absorbed into a piece of cotton within a petri dish.

2.3.2. Repellency Test:

The Repellency study used the Hand Introducing (HI) method (Tariq & Qadri, 2001). Approximately (200) mosquitoes were kept in a cage (30×24×24”); these mosquitoes remained blood-starved for 3-4 days before the sample test. On the day of the assay, the test individual had no interaction with lotion, perfumes or fragrant soaps. The crude extract of seaweed was dissolved in ethanol to make 3 different concentrations viz. 20%, 30% and 40%. On the dorsal side of the right hand, an aliquot (0.5ml) of the test solution was applied to the particular area, which is 3cm×3cm.

In contrast, the remaining part of the treated hand was covered by wearing double gloves. The treated hand was exposed to the cage, and the number of bites and visits were counted for 5 minutes after every hour. Each day, one sample was applied. Swollen spots have been observed because of the biting of mosquitoes. The initial reading was considered a 0-hour reading. No skin irritation from the seaweed extract has been found (Sundar *et al*., 2013). After the testing of the sample, a 5% sugar solution was placed in a cage for those mosquitoes which could not suck blood due to the strong fumes of the samples. So, these mosquitoes were fed on that solution.

The repellency was calculated using the formula:

$$\text{Average biting \%} = \frac{\text{total no.of biting in 5 minutes}}{\text{total no.of visits in 5 minutes}} \frac{\text{total no.of biting in 5 minutes}}{\text{total no.of visits in 5 minutes}} \times 100$$

Repellency % = Control biting (100 %) – Average biting % in 5 minutes

Different concentrations were prepared using the formula:

$$\text{Concentration in \%} = \frac{\text{wt.in grams}}{\text{solvent in ml}} \frac{\text{wt.in grams}}{\text{solvent in ml}} \times 100$$

20 % = 1/5 × 100

30 % = 1/3.3 × 100

40 % = 1/2.5 × 100

3.0 Results and Discussion

The current investigation assessed the mosquito-repellent activity of ethanol extracts (Hane & Raj 2018) from two seaweeds, *C. flabellatum* and *C. taxifolia,* against *Aedes aegypti* (Ahmad et al. 2016). In this study, crude extracts of *C. flabellatum* and *C taxifolia* dissolved in ethanol were prepared to make stock solutions and applied to a 3cm×3cm area of the right hand. These extracts are protected against mosquito bites without causing allergic reactions or irritation to the test subjects (Kumaresan et al., 2015). Additionally, the effectiveness of the repellent activity depends on the strength of the seaweed extract. The most substantial repellent effect against *Aedes aegypti* mosquitoes was observed with *C. flabellatum* and *C. taxifolia* at a concentration of 40% concentration, whereas *C. flabellatum* and *C. taxifolia* at a concentration of 20 % exhibited the weakest repellency against the same mosquitoes. The findings revealed that both *C. flabellatum* and *C. taxifolia* demonstrated a minimal repellency of about 50% for 3 hours at a concentration of 20% (Table. 1). Conversely, at a concentration of 30%, both seaweed extract (Raj et al., 2018) displayed a moderate, ranging from 80% to 90% for 3 to 4 hours (Table. 2). Notably, the strongest repellency, reaching approximately 100%, was observed for 5-6 hours with both *C. flabellatum* and *C taxifolia* at a concentration of 40% (Table. 3).

Table.1: Repellency at 20% concentration.

Duration of Repellency in hours	Seaweed Extract			
	Codium flabellatum		*Caulerpa taxifolia*	
	Biting rate in %	Repellency rate in %	Biting rate in %	Repellency rate in %
0	01	99	00	100
1	16	84	14	86
2	30	70	35	65
3	50	50	50	50
4	66	34	63	37
5	84	16	76	24
6	99	01	99	01

Table.2: Repellency at 30% concentration.

Duration of Repellency in hours	Seaweed Extract			
	Codium flabellatum		*Caulerpa taxifolia*	
	Biting rate in %	Repellency rate in %	Biting rate in %	Repellency rate in %
0	00	100	00	100
1	00	100	03	97
2	00	100	10	90
3	00	100	14	86
4	01	99	19	81
5	06	94	26	74
6	15	85	35	65

7	35	65	50	50
8	50	50	63	37
9	64	36	84	16
10	86	14	99	01
11	99	01	-	-

As presented in Table (4), considering the LT_{50} values (the time required for 50% of the mosquitoes to repel), it is evident that the extract of *C. flabellatum* at a concentration of 40% (LT_{50} value: 12 hours) exhibited notable effectiveness against *Aedes aegypti*. This was followed by the extract of *C taxifolia* at the same concentration, with an LT_{50} value of 10 hours. Conversely, the extracts of *C. flabellatum* and *C taxifolia* at a concentration of 20% demonstrated the most negligible efficacy, with an LT_{50} value of 3 hours (Khattaba & Temraz 2017)

Table. 3: Repellency at 40% concentration.

Duration of Repellency in hours	**Seaweed Extract**			
	Codium flabellatum		***Caulerpa taxifolia***	
	Biting rate in %	**Repellency rate in %**	**Biting rate in %**	**Repellency rate in %**
0	00	100	00	100
1	00	100	00	100
2	00	100	00	100
3	00	100	00	100
4	00	100	00	100
5	00	100	01	99
6	00	100	02	98
7	02	98	06	94
8	06	94	17	83
9	13	87	30	70
10	22	78	50	50
11	38	62	66	34
12	50	50	78	22
13	65	35	99	01
14	77	23	-	-
15	99	01	-	-

Table. 4: LT_{50} value (lethal time at which 50% of mosquitoes repel).

Extract Concentration	**LT_{50} (in hours)**
Codium flabellatum 20%	3 hours
Codium flabellatum 30%	8 hours
Codium flabellatum 40%	12 hours
Caulerpa taxifolia 20%	3 hours
Caulerpa taxifolia 30%	7 hours
Caulerpa taxifolia 40%	10 hours

During the evening hours, individuals typically employ various methods such as coils, pads, lotion, smoke, mist, mosquito nets, Mospel, King, etc., to deter mosquitoes, commonly *Culex* and *Anopheles*, but hardly *Aedes*. However, these methods are often ineffective against *Aedes* mosquitoes, which are diurnal and active during daylight hours in schools, universities, colleges, factories, and offices. Synthetic repellents, although effective for up to 3 hours (Movahhedin et al., 2014; Tariq & Qadri, 2001; Khalid et al., 2018), pose safety concerns, particularly for infants who tend to put their hands in their mouth, posing a significant risk.

Interests in plant-based repellents diminished with the advent of synthetic alternatives (Vimaladevi *et al*., 2012), despite earlier reports indicating the strong insecticidal properties of Caulerpin and Caulerpinic acid found in genus *Caulerpa* (Alarif *et al*., 2010). Similar repellent activity against *Aedes aegypti* was observed with *Caulerpa racemosa* when prepared in acetone extract (Esteves *et al*., 2019; Madhusudan et al., 2011). However, research on repellent activity against mosquitoes using *Codium flabellatum* has been scarce. Previous reports have indicated that *C taxifolia* possesses nematicidal activity, while *C. flabellatum* exhibits anti-leishmanial and antibacterial properties (Khan *et al*., 2012). The biological activities of marine seaweed extracts (Ahmed et al.,2011) may be attributed to compounds such as saponin, flavonoids, phenolics, terpenoids and alkaloids present in seaweeds (El Gamal, 2010; El-Din 2015; Etcherla & Rao 2014;

Sundar *et al*., 2013; Kolanjinathan et al. 2014). These findings underscore the potential of seaweeds as an eco-friendly approach to mosquito control (Govindarajan *et al*., 2011).

Conclusion

Our findings demonstrate that treatments utilizing seaweed extracts, specifically *C. flabellatum* and *C. taxifolia*, show significant potential for mosquito-repellent activity against *Aedes aegypti* while ensuring safety. Further investigations are warranted to explore the broader biological activities of seaweeds.

Acknowledgement

We acknowledge the support of the Department of Botany, University of Karachi and the Department of Zoology, University in supporting this study.

Conflict of interests

There is no conflict of interest among authors.

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Received: July 5th 2024 **Accepted: August 21th 2024**