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Larvicidal Effect of Seaweed *Codium Edule* extracts on *Aedes Aegypti* mosquito.

Mohammed A. Alkuriji[1], Mohammed B. Al-Fageeh[2], Fekri M. Shaher[1,3]*, Majed S. Alorf[1], and Haitham F. Almazyad[1]

[1]National Center of Agricultural Technology, Life Science & Environmental Research Institute, King Abdulaziz City for Science and Technology (KACST), Riyadh, Saudi Arabia.
[2] Director-General, General Directorate for Research & Innovation Support (GDRIS), King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia.
[3]Hodeidah University, Hodeidah, Yemen.

ABSTRACT

Aedes aegypti is the main vector of mosquito-born deadly diseases including dengue, chikungunya, Zika, and yellow fever, so it is necessary to combat it. Medicinal plants such as seaweeds can provide a safe alternative to artificial larvicidal agents since they represent rich source of bioactive constituents. This work was designed to evaluate the larvicidal activity of methanol, chloroform, ethyl acetate and aqueous extracts of *Codium edule* against *Aedes aegypti* larvae and characterization the compounds of the active fraction. Bioassay experiments were accomplished according to the standard protocol of World Health Organization. The mortality was recorded after 24 h of exposure and the LC50 and LC90 were determined by Probit analysis. The most active fraction was characterized by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Chloroform fraction exhibited the most larvicidal activity with LC50 value of 19.54 ppm. Compounds profile revealed the predominance of palmitic acid, beta-sitosterol and myristic acid with the ratio 25.75, 22.45 and 12.4 %, respectively. The Chloroform fraction of *Codium edule* proved to be a strong candidate as a safe and naturally larvicidal agent alternative to synthetic ones. Also our findings suggest further investigation for the development of effective larvicides from the natural sources.

Keywords: *Aedes Aegypti*, Beta-sitosterol, fatty acids, *Codium edule.*

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Corresponding author

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INTRODUCTION

Mosquitoes are responsible to transmit many danger diseases, including dengue, yellow fever, chikungunya, malaria, rift valley, West Nile, falarias and Zika virus. Worldwide, dengue is transmitted to poeple by *Aedes* mosquitoes bite and nowadays is considered the most rapidly spreading disease around the globe. around 2.5 billion people, 40 % of the globe's population are now at risk [1] and the disease spreads in over more than hundred countries [2] . The World Health Organization (WHO) estimates dengue cases reach to nearly 390 million, a number is around three fold more than cases number estimated for the year 2009 [3]. Four genotypes of dengue virus were reported (DENV1 – DENV2 - DENV3 - DENV4), and their symptomatic ranged from asymptomatic incidence to dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS), and may lead to loss the patient life [4]. This dengue virus with different genotypes is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes bite [5]. Later, researchers in Malaysia recorded dengue virus with new gene branch suggest the fifth genotype DENV5 [3].

Repeated use of artificial insecticides and the exposure for a long time has made resistance in mosquitoes against these chemicals [6]. Therefore, there is urgent need for develop safe, ecofriendly and biodegradable alternatives for mosquitoes control [7]. The marine ecosystem is huge and highly rich source of both natural biological and chemical products. Many of these natural products have beneficial properties such as medicinal and pharmaceutical. Recently, a significant number of marine bioactive metabolites with unique pharmacological properties have been isolated and characterized [8]. Marine biota produce natural biologically active constituents such as steroids, terpenoids, alkaloids, sterols, and other metabolites [9]. Marine algae, also known as seaweeds or macroalgae are organisms thrive in coastal areas and perform photosynthesis function. Macroalgae produce several of bioactive substances with unique chemical structures and biological functions, such as polyunsaturated fatty acids, polysaccharides, phlorotannins [10] and polyphenols [11]. These natural metabolites revealed significant bioactivities anticancer, antidiabetic, antioxidant, antibacterial and anti-inflammatory activities [11, 12] and several of these substances can be used for the establishment of unique drug agents [13]. The marine macro algae are eaten by human and used as food for animals, soil fertilizing and manufacture of phyco-colloids such as agar, alginate, carrageenan and furcellaran [14]. The healthy beneficial properties of algae were recognized over ancient time, particularly in Asia region, where the nutritional value of algae make it functional food. The marine seaweeds produce a wide range of bioactive chemical compounds with different activities [15]. The larvicidal efficacy of the commercial extract of the plant *Lantana camara* aculeate was tested against *Aedes aegypti*, *Anopheles stephensi* and *Culex quafasciatus* mosquitoes and exhibited high activity. This activity is attributed to its content of bioactive components including tannins, alkaloids, flavonoids, anthocyanins, kinenes, trepenoids, saponins and steroids [16]. Ajaegbu *et al*., 2016 have evaluated the activity of the extracts of *Spondias mombin* plant leaves where the plant extracted with different solvents and tested against *Aedes aegypti* mosquitoes. the dichloroethane extract exhibited the highest inhibition. This suggests that the type of solvent used play role on the bioassay results [17]. The hexane extract of the red seaweed *Laurencia dendroidea* exhibited strong larvicidal activity (100 % mortality) against *A. aegypti* at concentration 50 ppm [18]. In the current work, seaweed *Codium edule* was extracted and fractionated with different organic solvents and the resulting fractions were tested against the 4 instar of *A. aegypti* mosquito larvae. The most active extract was chemically characterized by Gas Chromatography-Mass Spectrometry (GC-MS) to know the major bioactive constituents.

EXPERIMENTAL

Seaweed material

Codium edule was collected manually from their natural habitat in al-kharrar Lagoon, Red Sea, Saudi Arabia. The sample was identified by taxonomy specialist at marine biology department, Faculty of Marine Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The fresh algal material was cleaned with distilled water in order to remove salts, dust and any adhesive particles. Then kept to dry completely in shaded area at room temperature (27± 2^{o}C). The dried sample was grinded to become fine powder using electric blender.

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Codium edule

Preparing of sample fractions:

Four different extracts (Aqueous, Methanol, Ethyl acetate and Chloroform) were prepared by cold maceration of the sample powder in four different flask with shaking. Maceration repeated three times. The combined resulting filtrate evaporated by rotary evaporator to get dry extract of active ingredients.

Larvicidal test

Larvicidal test were carried out according to the method of world health organization WHO (2005) [19]. Experiments were conducted by exposing the fourth instar larvae of *A. aegypti* to a series concentration of the tested fraction. Five replicates of 20 larvae per concentration, in addition to negative control (larvae exposed to water without extract) were set up. The usual larval food was added during the experiments.

Data analysis

The larval mortality percentages in each concentration and control was recorded after 24 hours of exposure and were corrected using Abbots (1925) formula; P = PI – C /1 – C, where PI denotes the observed mortality rate and C means the natural mortality. The results expressed in terms of LC50 and LC90 units and were calculated using 'Probit' analysis [20].

RESULTS AND DISCUSSION

Applications of larval control strategy are effective approach to control the mosquito populations since targeting the eggs and larvae is easier way compared to controlling the adult insects [21]. Researchers reported in earlier studies that seaweeds have larvicidal effect against mosquito [22, 15] and show skin expeller and smoke expeller characteristics against adult mosquitoes [23,24].

Table (1) larvicidal effect of different fractions of seaweed *Codium edule* on *Aedes aegypti* larvae

Extract Solvent	Sample powder weight (g)	Yield extract (g)	LC50 ppm	LC90 ppm
Methanol	100	4.1	138.90	266.29
Ethyl acetate	100	4.7	65.21	154.41
Chloroform	100	6.2	19.54	38.73
Aqueous	100	2.9	197.23	580.14

(LC50 and LC90 = lethal concentrations that cause mortality by 50 and 90 % of the exposed larvae)

Among the *Codium edule* extracts screened here (table 1 & Fig. 1), chloroform extract was the most effective (LC50 = 19.54 ppm) against the four instar larvae of *Aedes aegypti* followed by Ethyl acetate (LC50 =

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65.21 ppm), methanol (LC50 = 138.90 ppm) and aqueous (LC50 = 197.23 ppm) extracts. Since chloroform extract of *Codium edule* exhibited strong larvicidal activity, its chemical constituents were investigated and analyzed using Gas chromatography-Mass Spectrometry (GC-MS) analysis. The GC-MS profile of the chloroform extract showed 15 peaks. Identification of the compounds was performed by comparison of their spectral fragmentation patterns with those of the available database libraries Wiley USA (Wiley Int.). Quantitative determination was carried out based on peak area integration. Based on the comparison, fifteen compounds were characterized (Table 2). The table revealed that most of compounds belonging to aliphatic hydrocarbon and sterols, where the most abundant were palmitic acid followed by beta-sitosterol and myristic acid at proportions of 25, 22 and 12 %, respectively. Considerable amounts of mono-unsaturated palmitoleic acid, Stearic acid and Lauric acid were also detected with 7.60, 6.42 and 5.66 %, respectively. These chemical components are likely to be responsible for the larvicidal activity of the chloroform fraction.

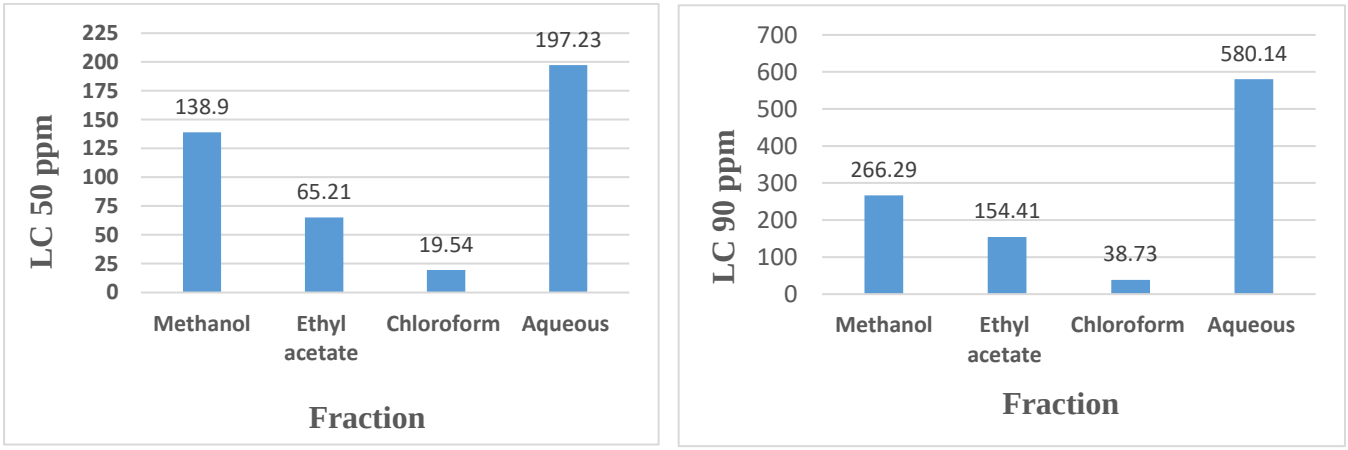
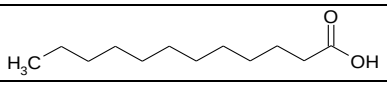
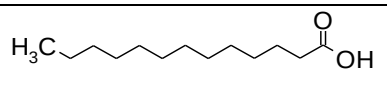
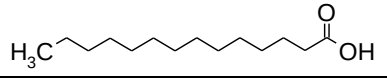
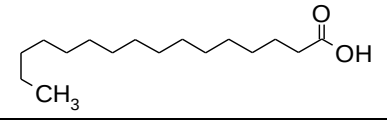


Figure (1) Relationship between *Codium edule* fractions and LC50 & LC90 of *Aedes aegypti* larvae

Beta-sitosterol was isolated from the plant *Abutilon indicum* and revealed larvicidal activity with LC50 = 11.49 ppm against *A. aegypti* larvae [25]. Additionally, our findings are in a good agreement with the previous study that showed potent mosquito larvicidal compounds with their chemical properties such as lipophilic profile [26], For example, aliphatic fatty acids with a long chain of hydrocarbon isolated from green seaweed *Cladophora glomerata* exhibited LC50 ranging from 3 to 14 ppm against *Aedes triseriatus* [27]. Also Saturated fatty acids such as capric acid, lauric acid, and myristic acid isolated from green seaweed *Cladophora glomerata* and mono-unsaturated fatty acid like palmitoleic acid exhibited larvicidal activity against *Aedes triseriatus* mosquito by LC50 ranged from 3 to 14 ppm [27]. Furthermore, the Octacosane obtained from the plant *Moschoma polystachyum* was found to show larvicidal effect by LC50=7.2 ppm against the early third instar larvae of mosquito *Culex quinquefasciatus*.

Table (2) GC/MS analysis of Chloroform extract of *Codium edule*

No.	Compound	Molecular formula	Molecular weight	Ratio %	Chemical structure
1	Lauric acid	$C_{12}H_{24}O_2$	200.32	5.66	
2	Tridecanoic acid	$C_{13}H_{26}O_2$	214.35	1.33	
3	Myristic acid	$C_{14}H_{28}O_2$	242.4	12.4	
4	Palmitic acid	$C_{16}H_{32}O_2$	270.4	25.75	

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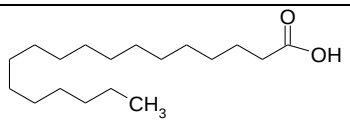
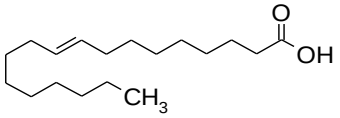
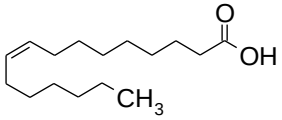
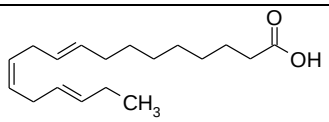
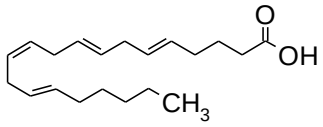
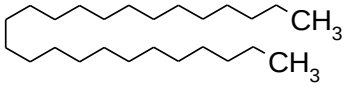
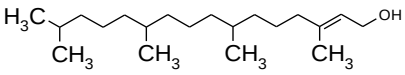
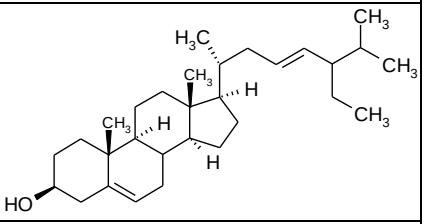
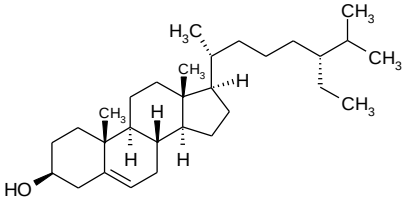
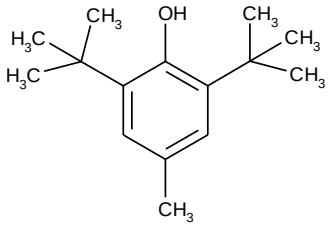
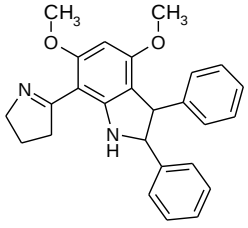
5	Stearic acid	$C_{18}H_{36}O_2$	298.4	6.42	
6	Oleic acid	$C_{18}H_{34}O_2$	296.4	1.74	
7	Palmitoleic acid	$C_{16}H_{30}O_2$	268.4	7.60	
8	Linoleic	$C_{18}H_{30}O_2$	292.3	0.43	
9	arachidonic acid	$C_{20}H_{32}O_2$	318.4	1.01	
10	Pentacosane	$C_{25}H_{52}$	352.5	0.35	
11	Phytol	$C_{20}H_{40}O$	298.4	0.55	
12	Stigmasterol	$C_{29}H_{48}O$	412.4	0.49	
13	Beta-Sitosterol	$C_{29}H_{50}O$	414.5	22.45	
14	ButylatedHydroxytoluene (BHT)	$C_{15}H_{24}O$	220.30	1.56	
15	4,6-dimethoxy-2,3-diphenyl-7-(1-pyrrolin-2-yl)indole	$C_{26}H_{24}N_2O_2$	396.5	0.89	

Until now, the investigation of seaweed extracts and isolated compounds were mainly focused for their mosquitocidal and larvicidal properties; their mechanism of action at molecular level have not been fully

illustrated compared to the insecticides originating from terrestrial plants. Rattan (2010) discussed the mode of action of insecticidal secondary metabolites derived from terrestrial plants [28].

He reporetd that insecticides derived from terrestrial plants affect the mitochondrial, cholinergic, octopaminergic and gamma-aminobutyric acid (GABA) systems. For instance, terrestrial botanical compounds exhibit a significant inhibition of acetylcholinesterase (AChE) enzymes [29] and affect mitochondrial activity by block sodium and potassium ion exchange [30], inhibiting calcium channels [31] and blocking the action of nerve cell membrane [32]. Seaweed constituents also showed important inhibition effects on cholinergic system. Early study described that the plastoquinones and farnesylacetone derivatives isolated from brown seaweed *Sargassum sagamianum* block acetylcholinesterase enzymes obtained from bovine red blood cells [33]. In addition, the red seaweed *Plocamium telfairiae* was investigated and the isolated aplysia terpenoid A and telfairine metabolites exhibited a potent larvicidal activity against mosquito *Anopheles gambiae*. Their mode of action is in line with cyclodiene [34]. Cyclodiene is known organochloride insecticide that has the ability to block GABA-gated chloride channel, leading to neuronal inhibition, hyper-excitation of the central nervous system, convulsion, and finally death [35].

Deformities

Morphological features of the treated larvae were tested under the microscope. Any difference between the treated larvae and the control ones was reported as deformity. Chloroform extract of *Codium edule* caused clear malformations appeared as prolongation (larvae body become longer than normal), pigmentation (larvae body was covered by dark colour) and chest swelling (Fig. 2).

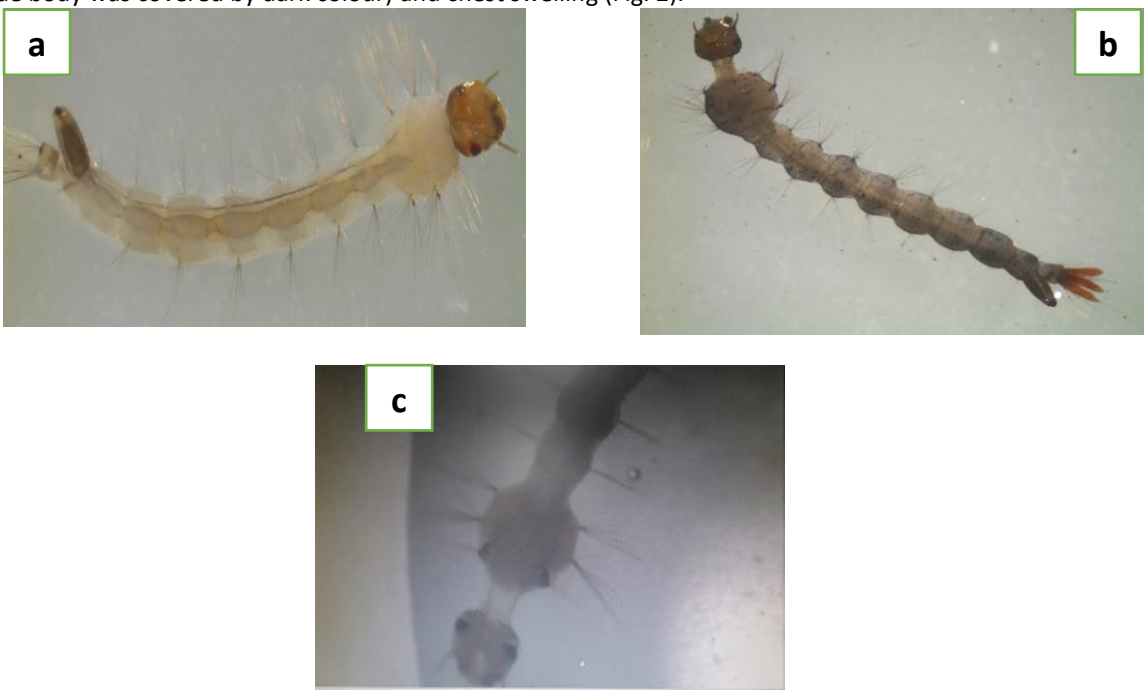


Figure 2: a. Normal larvae of Aedes aegypti b. Prolongation and Pigmentation of larvae body c. chest swelling and Elongation in the neck (treated with *Codium edule*)

CONCLUSION

Overall findings of this study demonstrate that chloroformic extract of marine algae *Codium edule* is a promising candidate for the eco-friendly, safe and biodegradable larvicides as an alternate to chemical larvicides that are being currently used in mosquito control strategies. Screening by GC-MS revealed the dominance of aliphatic and steroidal constituents, suggest their role in larvicidal activity. Further investigations are needed to isolate the individual components and understand more about the bioactivities of seaweeds and marine organisms in general to discover natural and effective mosquitocidal and larvicidal agents.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise

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