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## Characterization and screening of *in vitro* antimalarial and larvicidal activities of selected seaweeds from southeast coast of India against *Plasmodium falciparum* and *Anopheles stephensi*

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### ABSTRACT

**Objective:** To investigate the antimalarial and larvicidal activities of seaweeds against *Plasmodium falciparum* strain and *Anopheles stephensi* mosquito larva.

**Methods:** The three different seaweeds, *Jania rubens*, *Turbinaria ornata* (*T. ornata*) and *Ulva fasciata* were collected from Southeast India. The seaweeds were extracted using hexane and ethyl acetate solvents and evaluated for their mosquito-larvicidal and antiplasmodial property. The bioactive compounds of the seaweed extract were studied by Fourier transform infrared spectroscopy and gas chromatography-mass spectrometry.

**Results:** The hexane extract of *T. ornata* (HTO) has shown significant inhibitory effects at the IC<sub>50</sub> value 22.98 µg/mL, when compared to the positive control, chloroquine (12.32 µg/mL). The larvicidal activity of HTO on the fourth instar larvae of *Anopheles stephensi* was found to be effective with the LC<sub>50</sub> and LC<sub>90</sub> values (µg/mL) of 1.38 and 3.34. The Fourier transform infrared spectroscopy analysis of HTO revealed the presence of alkenes, aromatics, amides and amines groups, whereas in gas chromatography-mass spectrometry, 11 different bioactive components were recorded. The bioactive component, hentriacontane in the HTO might be responsible for the recorded antiplasmodial and larvicidal activities.

**Conclusions:** The finding of the present study reveals that the constituents of *T. ornata* have new sources of antimalarial and larvicidal agents in future.

## 1. Introduction

Marine algae are known to produce a wide variety of bioactive secondary metabolites and many of them show the potential for the development of novel drugs by the pharmaceutical industries. The resistance of mosquito vectors to well established drugs during the malarial infection remains to be a serious global health problem. Malaria eradication programs in the backdrop of continuously evolving drug resistance of the parasites demands the exploration of bio-resources for developing newer antimalarial agents. North-eastern India is endemic for malaria as it is characterized by high prevalence of drug resistant *Plasmodium*

*falciparum* (*P. falciparum*) parasite strain. In order to decrease the risk of chemo-resistance to most of the antimalarial drugs, the World Health Organization (WHO) has recommended artemisinin-based combination therapies (ACTs) for the management of uncomplicated *P. falciparum* malarial cases. Unfortunately, artemisinin based combination treatments have caused failures in some countries[1]. Mosquitoes serve as obligate intermediate vector for numerous diseases worldwide. Totally there are 34 genera and 3 100 species of mosquitoes out of which the three genera; *Anopheles*, *Aedes* and *Culex* are the primary vectors which mainly include the malarial parasite (*Plasmodium*), filaria (*Wucheria* and *Brugia*) and arboviruses[2]. *Anopheles stephensi* (*An. stephensi*) is the major malaria vector in India. Several reports on the occurrence of malarial cases, which states the annual incidence of 300–500 million clinically manifest cases and a death of 1.1 to 2.7 million people, globally. In all probability, some plants containing insecticidal phytochemicals that were predominantly secondary compounds were used to protect themselves against herbivorous insects[3]. Recent researches showed that seaweeds have been found to possess antimicrobial, antifeedant, anthelmintic, antidiabetic and anticancer properties[4].

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Due to their possession of bioactive substances of potential health benefits, the seaweeds have received a special attention of biomedical scientists[5,6]. For centuries the marine macro-algae, have been used as a traditional medicine to treat human parasitic infections worldwide, particularly in Japan and China. Marine algae have been reported to produce a wide variety of bioactive secondary metabolites like alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids and glycerols[7]. Some metabolites like bromophenols, carotene and steroids were isolated and purified from macroalgae. The *Ulva lactuca* commonly known as sea lettuce has long been used as food and as a traditional medicine to treat helminthic infections, fever, urinary diseases and dropsy[8]. Although many reports are available on the use of seaweeds in food industry, only limited information is available about their use in antimalarial aspect[9,10]. Hence, the present study pertains to the antiplasmodial and larvicidal activities of the selected seaweeds, *Jania rubens* (Linn.) J. V. Lamouroux, 1816 (Corallinaceae) (*J. rubens*), *Turbinaria ornata* (Turner) J. Agardh, 1848 (Sargassaceae) (*T. ornata*) and *Ulva fasciata* Delile, 1813 (Ulvaceae) (*U. fasciata*) from Southeast coast of India.

## 2. Materials and methods

### 2.1. Sample collection

Seaweed samples were collected from the Rameshwaram and Pudukcherry coasts, Southeast India and three species viz. *J. rubens*, *T. ornata* and *U. fasciata* have been identified based on standard keys[11] and the identifications have been confirmed by Dr. N. Kaliaperumal, Principal Scientist (Retd.), Central Marine Fisheries Research Institute, Mandapam Camp, Ramanathapuram District, India. The voucher specimens have been kept in the Department of Biotechnology, Periyar University, Salem. Cultures of *P. falciparum* were obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research and Indian Institute of Science, Bangalore, India. The RPMI 1640 medium, dimethyl sulphoxide, sodium bicarbonate (NaHCO<sub>3</sub>) and gentamycin sulphate were purchased from Hi-Media Laboratories Private Limited, Mumbai, India. Syringe filter (mesh 0.20 µm, Sartorius stedim Biotech, GmbH, Germany) was used. The mosquito larvae, *An. stephensi* were collected from the aquatic environments of Salem District. Biscuits were served as larval food. The larvae were kept at (25 ± 2) °C and proper photoperiod was given for their growth. Fourth instar larvae were (4–5 mm in length) used for larval bioassay purpose.

### 2.2. Preparation of seaweeds extracts

All the seaweeds were washed thrice with tap water and twice with distilled water to remove the adhering salts and the other associated animals and then dried at room temperature. From the dried materials, powder was obtained using electrical blender. Three seaweed powders (300 g each) were macerated with two solvents (1 L) viz. hexane and ethyl acetate for 2 weeks. The crude

extracts of selected seaweeds were prepared in a Soxhlet apparatus. The extracts were dried in a rotary vacuum evaporator by using the extracts and stored at low temperature (4 °C). The seaweed extracts were dissolved in dimethyl sulphoxide (mg/mL) and filtered through Millipore sterile filters (mesh 0.20 µm).

### 2.3. *P. falciparum*: Parasite cultivation

The *in vitro* antiplasmodial activity of seaweeds extracts was assessed against *P. falciparum*. The antiplasmodial activity was assessed by following the procedure of Prasannakumar and Ravikumar with slight modification[12]. Malarial parasites of *P. falciparum* were cultivated in human O Rh<sup>+</sup> red blood cells using RPMI 1640 medium containing 25 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma) supplemented with O Rh<sup>+</sup> serum (10%), 5% NaHCO<sub>3</sub> and 40 µg/mL of gentamycin sulphate. Cultures were kept at 37 °C under an atmosphere of 5% O<sub>2</sub>, 3%–5% CO<sub>2</sub> and N<sub>2</sub>. Haematocrits were adjusted at 5% and parasite cultures were used when they exhibit 2% parasitaemia.

### 2.4. *In vitro* antiplasmodial activity of seaweed extracts

The filter-sterilized crude extracts of the seaweeds at different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg/mL) were incorporated in 96 well tissue culture plate containing 200 µL of *P. falciparum* culture with fresh red blood cells diluted to 2% haematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% haematocrit and positive control was maintained with parasitized blood culture treated with chloroquine. Parasitaemia was evaluated after 48 h of incubation by Giemsa stain and the average percentage suppression of parasitaemia was calculated using the following formula:

$$\text{Average suppression of parasitaemia} = \frac{\text{Control}_{\text{average parasitaemia}} - \text{Test}_{\text{average parasitaemia}}}{\text{Control}_{\text{average parasitaemia}}} \times 100$$

### 2.5. Assessment of chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might be attributed to the extract, 200 µL of erythrocytes was incubated with 200 µg/mL of the extract at a dose equal to the high used in the antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with Giemsa stain and observed for the morphological changes under high-power light microscope. The morphological features observed on the treated erythrocytes were compared with the untreated erythrocytes.

### 2.6. Larvicidal activity

Larvicidal bioassay was performed based on WHO protocol[13]. *An. stephensi*, mosquito larvae were exposed to a wide range of concentration of seaweed compounds (10, 5, 2.5, 1.25 and 0.62 µg/mL) in order to find out activity. Batches of 25 healthy fourth instar larvae were transferred to the 250 mL chambers containing tap water. Three replicates were set up for each concentration and

equal number of controls was also set up with tap water. Larval mortality was observed after 24 h. Mortality percentages were calculated using Abbott's [14] formula.  $LC_{50}$  and  $LC_{90}$  values were calculated by probit analysis [15]:

$$\text{Mortality (\%)} = [(X - Y)/X] \times 100$$

where, X= survival in the untreated control and Y= survival in treated sample. Based on the preliminary screening results, extracts were subjected to dose-response bioassay for larvicidal activity against the larvae of *An. stephensi*. The number of dead larvae were counted after 24 h and the selected samples turned out to be equal in their toxic potential.

### 2.7. Statistical analysis

The antiplasmodial activity of marine seaweeds were expressed by the inhibitory concentration ( $IC_{50}$ ) values that were calculated (concentration of extract in the X-axis and percentage of inhibition by the extract in the Y-axis) using office XP software. The antiplasmodial activity was analyzed in accordance with the norms. The larvicidal activity of seaweed extract was evaluated, and their percentage of mortality data and lethal concentrations,  $LC_{50}$  and  $LC_{90}$  values were subjected to Probit analysis and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit and *Chi-square* values were calculated using the SPSS software. Extracts were assayed in triplicate and results are given as averages  $\pm$  SD. Statistical evaluation was used and the values at  $P < 0.05$  were considered statistically significant. The activity of marine seaweeds was expressed by the lethal concentrations ( $LC_{50}$  and  $LC_{90}$ ) values that were calculated using Graph pad prism version 5.

### 2.8. Preliminary phytochemical analysis

The hexane extract of *T. ornata* (HTO) was subjected to phytochemical analysis by following the standard protocol [16]. Further, the HTO was analyzed through the chromatographic techniques [Fourier transform infrared spectroscopy (FT-IR) and gas chromatography-mass spectrometry (GC-MS) analyses].

### 2.9. Characterization and identification of *T. ornata* hexane extract

The FT-IR (Perkin Elmer RX1, Germany) was used to analyze the *T. ornata* hexane extract and chloroquine, which were focused in the mid-IR region  $4000-400\text{ cm}^{-1}$  by the KBr pellet technique. The spectrum was recorded using attenuated total reflectance (ART) technique bench measurement. GC-MS analysis was performed by GC-Clarus 500 Perkin Elmer using elite 5 MS column. The temperature program were set at  $80\text{ }^{\circ}\text{C}$  to  $350\text{ }^{\circ}\text{C}$  at the rate of  $3\text{ }^{\circ}\text{C}/\text{min}$  and held at 55 min. Ion temperature was  $200\text{ }^{\circ}\text{C}$  and the scan range was: 20–500 (atomic mass unit). The functional groups with their corresponding stretches from FT-IR spectrum of HTO and chloroquine was interpreted using the software IR pal 2.0 version. Interpretation of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more

than 62 000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in Wiley and NIST libraries. The name, molecular weight and components structure of the test materials were ascertained according to Hakkim *et al.* [17].

## 3. Results

### 3.1. Antiplasmodial activity

The *in vitro* antiplasmodial activity was performed using the three different seaweeds *viz.* *J. rubens*, *T. ornata* and *U. fasciata* and extracts from hexane and ethyl acetate solvents. The average suppression rate of parasitemia was found to be highest activity with the *T. ornata* hexane extracts and lowest activity of hexane and ethyl acetate extracts of *U. fasciata*, *J. rubens* and *T. ornata* ethyl acetate extract at 24 h and 48 h. The recorded  $IC_{50}$  values ( $\mu\text{g}/\text{mL}$ ) were: 34.60 and 22.98 by *T. ornata* hexane extract, 85.95 and 79.17 by *U. fasciata*, 89.82 and 80.14 by *J. rubens*, and 18.60 and 12.32 by positive control chloroquine at 24 h and 48 h of incubation, respectively (Table 1).

**Table 1**

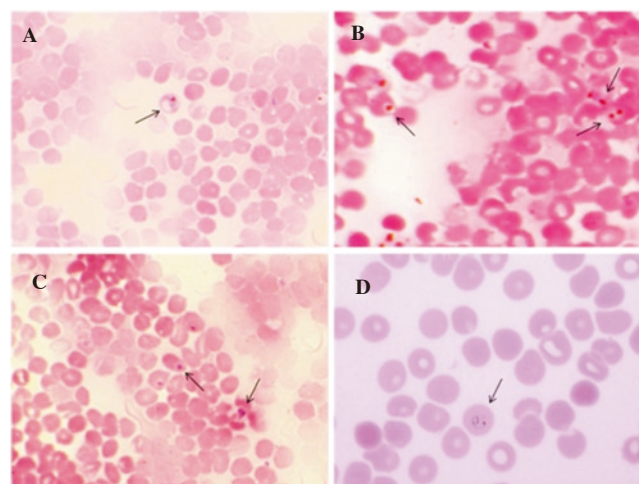
Percentage suppression of parasitemia and  $IC_{50}$  values of marine seaweeds after 24 h and 48 h.

Samples	$IC_{50}$ after 24 h <sup>*</sup>		$IC_{50}$ after 48 h <sup>*</sup>	
	Hexane	Ethyl acetate	Hexane	Ethyl acetate
<i>J. rubens</i>	89.820 $\pm$ 1.740	65.210 $\pm$ 0.700	80.14 $\pm$ 1.00	60.28 $\pm$ 0.74
<i>U. fasciata</i>	85.950 $\pm$ 0.520	> 100	79.17 $\pm$ 0.97	> 100
<i>T. ornata</i>	34.600 $\pm$ 0.630	40.420 $\pm$ 0.450	22.98 $\pm$ 0.24	36.54 $\pm$ 0.46
Control chloroquine	17.650 $\pm$ 0.016	12.320 $\pm$ 0.085		

<sup>\*</sup>Values are the average of the three replicates and found significant ( $P < 0.05$ ) between concentration and seaweeds. Data are expressed as mean  $\pm$  SD.

### 3.2. Chemical injuries of erythrocytes

The chemical injury to erythrocytes showed that there were no morphological changes in erythrocytes by the seaweed extract after 48 h of incubation when observed under light microscope which are shown in Figures 1 and 2.



**Figure 1.** *In vitro* antiplasmodial activity of marine seaweeds.

A: 24 h treated *T. ornata* hexane extract; B: 48 h *T. ornata* hexane extract; C: positive control 24 h - chloroquine; D: positive control 48 h - chloroquine. Arrow mark indicates infected *P. falciparum* parasites.

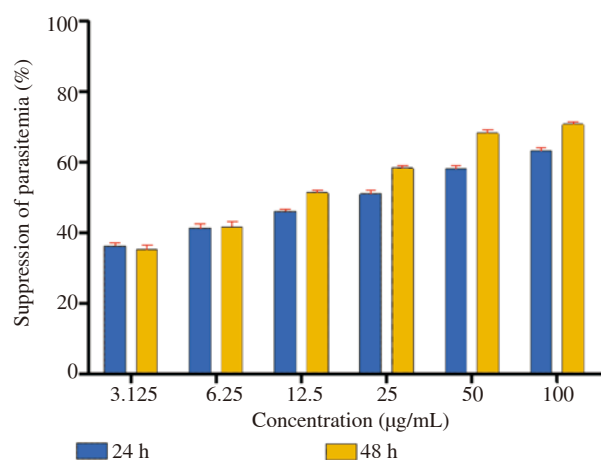


Figure 2. *T. ornata* hexane extract.

### 3.3. Larvicidal assay

The larvicidal activity of HTO was tested on fourth instar larvae of *An. stephensi*. The recorded percentage of mortality and the lethal concentrations (LC<sub>50</sub> and LC<sub>90</sub>) of the compounds are presented in Table 2. The activity of the HTO showed a maximum mortality effect on larvae of *An. stephensi*. The LC<sub>50</sub> and LC<sub>90</sub> values of HTO (*An. stephensi*) values were 1.38 and 3.34 µg/mL, respectively.

Table 2

Larvicidal activity of *T. ornata* hexane extract against *An. stephensi*.

Test larvae	Extracts	Conc. (µg/mL)	Mortality (%)	LC <sub>50</sub> 95% confidence (LCL-UCL)	LC <sub>90</sub> 95% confidence (LCL-UCL)	χ <sup>2</sup> Value
<i>An. stephensi</i>	Hexane	Control	–	1.38 (1.09–1.68)	7.84 (5.83–11.97)	3.34
		0.62	27			
		1.25	47			
		2.50	69			
		5.00	83			
		10.00	92			

LCL: Lower confidential limit; UCL: Upper confidential limit; χ<sup>2</sup> value: Chi-square value at  $P < 0.05$  significant level.

### 3.4. Compounds identification of chromatographic analysis

The preliminary phytochemical analysis of seaweed *T. ornata* hexane extracts results have showed the presence of amino acids, steroid, tannins and phenolic compounds, respectively. The FT-IR analyses of HTO showed the peaks at 3415.49, 2972.20, 2855.73, 2362.18, 1734.68, 1618.41, 1457.54, 1379.58, 1166.06, 1061.27, 901.38, 617.91 and 477.46 cm<sup>-1</sup> (Figure 3 and Table 3). The peak at 3415.49 cm<sup>-1</sup> corresponds to the OH stretching vibration of alcohols. The peaks at 2972.20 and 2855.73 cm<sup>-1</sup> could be assigned to the CH stretching vibrations of alkanes. The peak at 2362.18 cm<sup>-1</sup> corresponds to the pH phosphine sharp stretching vibration of miscellaneous group. The peak at 1734.68 cm<sup>-1</sup> signals the C=O stretching vibration of esters. The peak at 1618.41 cm<sup>-1</sup> corresponds to the C=O stretching vibration of amides. Similarly the peaks at 1457.54 and 1379.58 cm<sup>-1</sup> were assigned to the C=H stretching vibration of alkanes. The peaks at 1166.06 and 1061.27 were assigned to the C–F stretching vibrations of alkyl halides. The peak at 901.38 cm<sup>-1</sup> signals the P–OR esters of miscellaneous group. The peaks at 617.91 and 477.46 cm<sup>-1</sup> signals the C–Br stretching vibration of alkyl halides. The standard chloroquine revealed the

presence of peaks at 3449.11, 2282.31, 1616.65, 1554.71, 1457.86, 1383.60, 1212.52, 1154.70, 1063.43, 938.53, 816.14, 601.76 and 526.57 cm<sup>-1</sup> (Figure 4 and Table 4). The peak at 3449.11 cm<sup>-1</sup> could be assigned to the NH stretching vibration of 1°, 2° amines and amides. The peak at 2282.31 cm<sup>-1</sup> consigned to the P–H stretching vibration of phosphine group. Similarly the peaks at 1616.65 and 938.53 cm<sup>-1</sup> were assigned to the C=C stretching vibrations of carboxylic acid. The peaks at 1554.71 and 1457.86 cm<sup>-1</sup> could be assigned to the alkane groups of C–O and CH<sub>2</sub> & CH<sub>3</sub> stretching vibrations, respectively. Similarly the peaks at 1383.60 and 1212.52 cm<sup>-1</sup> could be assigned to the aliphatic amines of CH<sub>3</sub> and C–N stretching vibrations. The peak at 1154.70 cm<sup>-1</sup> consigned to the S=O stretching vibration of sulfone. The peak 1063.43 cm<sup>-1</sup> responds to the C–N stretching vibration of alkenes. The peak at 938.53 cm<sup>-1</sup> corresponds to the =NOH (N–O) stretching vibration of carboxylic acids. The peak at 816.14 cm<sup>-1</sup> assigned to the C–H stretching vibration of aromatics. The peak at 601.76 cm<sup>-1</sup> could be assigned to the C–H bend vibration of alkynes. Finally the peak at 526.57 cm<sup>-1</sup> is assigned to the S–S disulfide stretching vibration of alkyhalides (Figure 4 and Table 4). Presently, the GC-MS analysis of *T. ornata* showed the 11 bioactive compounds viz. Tetradecanoic acid; 4-methoxy-6-methyl-6,7-dihydro-4H-furo(3,2-C,pyran); Cis-10-heptadecenoic acid; Dibutylphthalate; L-(+)-Ascorbic acid 2,6-dihexadecanoate; 5,8,11,14-eicosatetraenoic acid, methyl ester; Z,z,6,28-heptatriactontadien-2-one; Tetrahydropyran-12-tetradecyn-1-ol-ether; 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) (ester); Hentriacontane and Cholest-5-En-3-ol,24-propylidene,(3. beta.)- (Figures 5 and 6 and Table 5).

Table 3

Functional groups and their corresponding stretches of FT-IR spectrum of *T. ornata* hexane extract.

No. of peaks	Frequency (cm <sup>-1</sup> )	Bond	Functional group
1	3415.49	OH Stretch	Alcohols
2	2972.20	CH Stretch	Alkanes
3	2855.73	CH Stretch	Alkanes
4	2362.18	PH Phosphine sharp	Misc. groups
5	1734.68	C=O Stretch	Esters
6	1618.41	C=O Stretch	Amides
7	1457.54	C=H Stretch	Alkanes
8	1379.58	CH Stretch	Alkanes
9	1166.06	C–F Stretch	Alkyl halides
10	1061.27	C–F Stretch	Alkyl halides
11	901.38	P–OR esters	Misc. groups
12	617.91	C–Br Stretch	Alkyl halides
13	477.46	C–Br Stretch	Alkyl halides

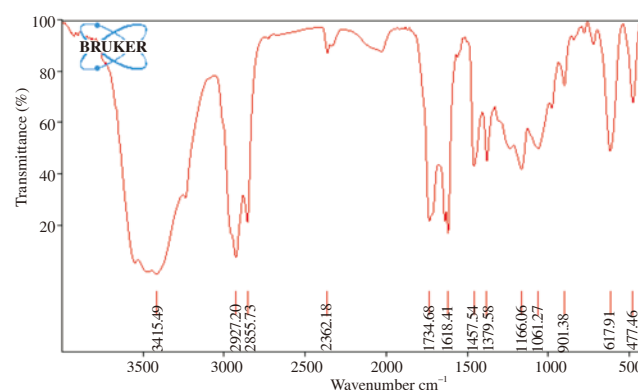


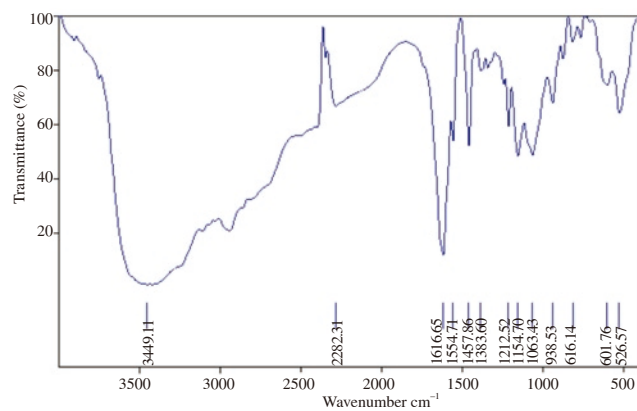
Figure 3. FT-IR analysis of *T. ornata* hexane extract.



**Table 4**

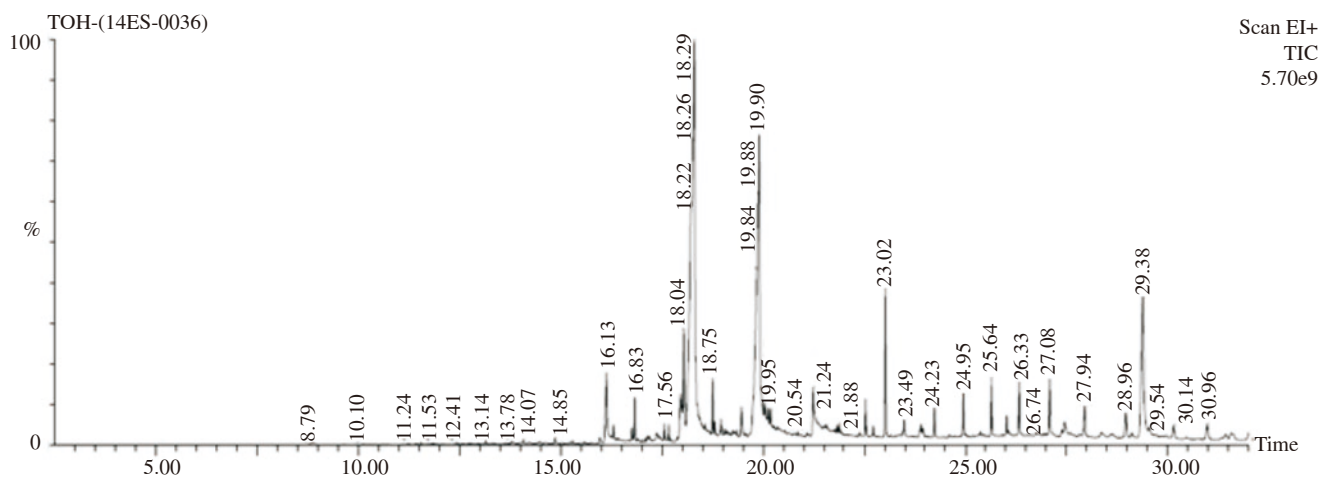
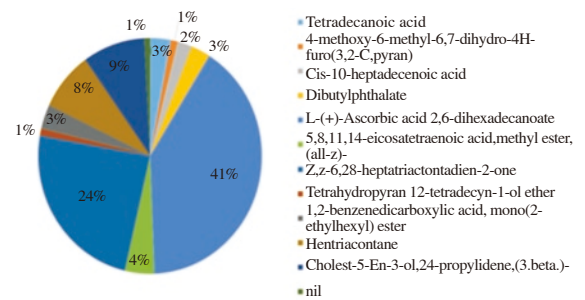
Functional groups and their corresponding stretches of FT-IR spectrum of chloroquine.

No. of peaks	Frequency (cm <sup>-1</sup> )	Bond	Functional group
1	3449.11	NH stretch	1°, 2° amines, amides
2	2282.31	P-H	Phosphine group
3	1616.65	C=C stretch	Carboxylic acid
4	1554.71	C-O stretch	Alkanes
5	1457.86	CH <sub>2</sub> & CH <sub>3</sub>	Alkanes
6	1383.60	CH <sub>3</sub>	Aliphatic amines
7	1212.52	C-N stretch	Aliphatic amines
8	1154.70	S=O	Sulfone
9	1063.43	C-N stretch	Alkenes
10	938.53	=NOH (N-O)	Carboxylic acids
11	816.14	C-H	Aromatic
12	601.76	C-H Bend	Alkynes
13	526.57	S-S disulfide asym	Alkylhalides

**Figure 4.** FT-IR analysis of standard chloroquine.**Table 5**

GC-MS analysis of *T. ornata* hexane extract.

S. No.	RT	Name of the bioactive components	Mol. Formula	Mol. Weight
1.	16.134	Tetradecanoic acid	C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228
2.	16.829	4-methoxy-6-methyl-6,7-dihydro-4H-furo(3,2-C,pyran)	C <sub>9</sub> H <sub>12</sub> O <sub>3</sub>	168
3.	17.960	Cis-10-heptadecenoic acid	C <sub>17</sub> H <sub>32</sub> O <sub>2</sub>	268
4.	18.040	Dibutyl phthalate	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
5.	18.300	L-(+)-Ascorbic acid 2,6-dihexadecanoate	C <sub>38</sub> H <sub>68</sub> O <sub>8</sub>	652
6.	18.755, 21.236	5,8,11,14-eicosatetraenoic acid, methyl ester, (all-z)-	C <sub>21</sub> H <sub>34</sub> O <sub>2</sub>	318
7.	19.460, 19.900	Z,z-6,28-heptatriacontadien-2-one	C <sub>37</sub> H <sub>70</sub> O	530
8.	22.526	Tetrahydropyran-12-tetradecyn-1-ol ether	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294
9.	23.022	1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278
10.	24.947, 25.643, 27.078, 27.939, 28.964	Hentriacontane	C <sub>31</sub> H <sub>64</sub>	436
11.	29.384	Cholest-5-En-3-ol, 24-propylidene-, (3.β.)-	C <sub>30</sub> H <sub>50</sub> O	426

**Figure 5.** GC-MS analysis of *T. ornata* crude hexane extract.**Figure 6.** Percentage of bioactive components present in *T. ornata* hexane extract.

## 4. Discussion

Several studies have demonstrated that seaweeds are excellent source of components like polysaccharides, tannins, flavonoids, phenolic acids, bromophenols and carotenoids that are exhibiting different biological activities. Our present studies have revealed that, hexane and ethyl acetate extracts of *T. ornata* have shown maximum activity than the extracts of *U. fasciata* and *J. rubens*. The inhibitory concentrations values (IC<sub>50</sub>) of seaweed extracts against *P. falciparum* after 24 h and 48 h of incubation are shown in Table 1 and Figures 1 and 2. The presently recorded high IC<sub>50</sub> value (12.32 µg/mL) of chloroquine could be related to the possible increased resistance

of *P. falciparum* and similar findings were earlier reported by Ravikumar *et al.*[3]. Earlier study of the antiplasmodial effects of the seaweed species, *Caulerpa taxifolia* and *Caulerpa peltata* have found potent effects at the IC<sub>50</sub> values of 5.06 and 16.69 µg/mL, respectively[18]. The present preliminary phytochemical analyses of hexane extracts have revealed the presence of amino acids, steroid, tannins and phenolic compounds. Some previous researchers have reported the presence of steroids, alkaloids, phenols, flavonoids, saponins, tannins, glycosides and triterpenoids from several seaweeds, which was supported by our present findings[19]. Also, there were earlier reports on the early-stage of gametocytocidal activity of the MMV Malaria Box which was a collection of 400 compounds with known activity against the asexual stages of *P. falciparum*[20,21]. In this previous study, result mentioned the experimental verification of early stage gametocytocidal activity in the development of new antimalarial activity. These studies had confirmed that effects of tryptanthrin and the derivatives NT1, T8, epoxomicin and chloroquine were investigated on the development of stage II to stage V gametocytes[22]. Although marine algae have been recognized as attractive sources of known bioactive compounds, very little research has been focused on antiprotozoal activity. Antimalarial lead compounds (metabolites) were present in marine algae; *e.g.* sargaquinoic acid, sargahydroquinoic acid, sargaquinal and fucoxanthin, which were isolated from the *Sargassum heterophyllum*. However, it has been recently reported that nitidine may possess a chloroquine like mechanism of action because of its capacity to bind to heme and to inhibit β-hematin formation[23]. During the present larvicidal experimental study, using the active hexane extracts of *T. ornata* under various concentrations, the extracts of *T. ornata* have showed high mortality percentage on fourth instar larvae of *An. stephensi*. Watanabe *et al.*[24] found that polyhalogenated monoterpenes, aplysiaterpenoid A and telfairine isolated from the seaweed, *Plocamium* sp. showed insecticidal properties against *Anopheles gambiae* mosquito larvae. The earlier researchers have also demonstrated that Indian marine plant extracts possesses potential larvicidal activity[25]. Smith[6] has also reported the presence of diverse secondary metabolites in several seaweeds that showed significant larvicidal activity.

The FT-IR spectrum shows the molecular configuration of different functional groups present in the *T. ornata* hexane extract (Figure 3 and Table 3). The peak corresponding to 3415.49 cm<sup>-1</sup> indicates the presence of aliphatic amines, alkynes and alkenes groups with polymeric association. The FT-IR spectrum of positive control, chloroquine is shown in Figure 4 and Table 4. FT-IR spectra of chloroquine formulation have shown bend peak at 3462 cm<sup>-1</sup> N-H stretching, 2933 cm<sup>-1</sup> C-H

stretching, 1657 cm<sup>-1</sup> C=C stretching, 1562 cm<sup>-1</sup> N-H bending, 1403 cm<sup>-1</sup> bending and at 1012 cm<sup>-1</sup> C-N stretching respectively as shown by earlier report[26]. Similar findings pertaining to other seaweeds were reported earlier by several other researchers[27]. The GC-MS analysis of *T. ornata* showed the 11 bioactive compounds are presented in Figures 5 and 6 and Table 5. The earlier researcher reports on fucoxanthin and sargaquinal showed good antiplasmodial activity toward a chloroquine-sensitive strain of *P. falciparum*[28]. The endemic marine red alga *Plocamium cornutum* (Turner) Harvey shows antiplasmodial activity in organic extracts. Interestingly, compounds bearing the 7-dichloromethyl substituent showed significantly higher antiplasmodial activity towards a chloroquine sensitive strain of *P. falciparum*[29]. Recently, the presence of bioactive flavonoids, tannins, and steroids in marine algae, *J. rubens*, *Corallina mediterranea* and *Pterocladia capillacea* has been found[30,31]. Likewise, the presently observed antiplasmodial effects could be related to the phytochemical constituents of the HTO. Based on the virulence-reduction performance of the seaweed-based hentriacontane, a new antimalarial compound by Deharo *et al.* has been developed[31]. In our study, the hentriacontane accounted for 8.057% of the spectral peak of HTO, which might have been specifically responsible for the observed antiplasmodial effect. Therefore these findings could stimulate the development of new antiplasmodial agent. Further investigations are in progress to isolate the active compound from the seaweed, *T. ornata* that possess the antiplasmodial activity.

To sum up the present study, the crude hexane extract of *T. ornata* has showed significant inhibitory effect on the malarial parasite, *P. falciparum* and mosquito larva *An. stephensi*. Further studies are warranted to isolate the bioactive compound responsible for the antimalarial activity. The findings demonstrate that, natural products remain one of the most important sources of medicines against the malarial parasite.

### Conflict of interest statement

We declare that we have no conflict of interest.

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