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Research Article

IN VITRO ANTIPLASMODIAL ACTIVITY OF NATIVE INDIAN SEAWEED *SARGASSUM SP.*

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ABSTRACT

Objectives: To investigate the antiplasmodial activity of three different solvent extracts of *Sargassum tenerrimum* against *Plasmodium falciparum.*

Methods: The seaweed species of *S. tenerrimum* were collected from Rameshwaram, Southeast coast of India. The collected samples were dried and extracted with three different polaritic (hexane, acetone, and ethylacetate) solvents and tested against *P. falciparum* parasite strain.

Results: Acetone extract exhibited better activity than the other two extracts. The inhibitory concentration$_{50}$ values of acetone *S. tenerrimum* were found to be 27.82 and 18.14 µg/ml at 24-48 hrs, respectively. *S. tenerrimum* crude extracts were subjected for the phytochemical analysis, and it showed the presence of steroids, alkaloids, flavonoids, tannins, glycosides, amino acids, and phenol compounds. The gas chromatography-mass spectroscopy result reveals that the presence of 10 major and minor compounds in the *S. tenerrimum* extract. In that, cyclotrisiloxane hexamethyl compounds might be responsible for the effective parasite suppression.

Conclusion: It can be concluded from the present study that the acetone extract of *S. tenerrimum* has strong antiplasmodial activity. Furthermore, the study has been extended to the isolation of the possible active compounds that is responsible for the antiplasmodial properties.

Keywords: Antiplasmodial assay, Different polaritic solvents, *Plasmodium falciparum, Sargassum tenerrimum.*

INTRODUCTION

Malaria is an endemic infectious parasitic disease that is widespread in tropical and subtropical regions of the world [1]. The World Malaria report of 2014 [2] estimated that there were approximately 283 million malaria cases among 3.3 billion people at risk in 109 countries where malaria is currently considered prevalent; 87% of these cases were reported in the African region. The disease caused nearly one million deaths, 91% of which were in Africa and 85% of these were children under 5 years [3]. Developing countries, malaria was one of the most predominant diseases; still rely on traditional medicine as a source for the treatment of this disease. *Plasmodium falciparum* the most widespread etiological agent for human malaria has become increasingly resistant to standard antimalarials, e.g., chloroquine. In Africa and elsewhere, plant extracts are still widely used in the treatment of malaria and other ailments, and up to 80% of the African population use traditional medicines for primary health care [4]. Resistance to all known antimalarial drugs, with the exception of the artemisinin derivatives, has developed to various degrees in several countries [5]. Consequently, new drugs combinations are urgently needed today for the treatment of malaria. These drugs should have novel modes of action or be chemically different from the drugs in current use. Seaweeds are used as a nutraceuticals for decades especially in oriental countries. They are a rich source of vitamins and minor elements [6,7]. There have been many reports of macroalgae - derived compounds that possesses a broad range of biological functions such as antibiotic, antiviral, antioxidant, antifouling, anti-inflammatory, cytotoxic, and antimitotic activities [5,8,9]. Among the seaweeds, *Sargassum* a genus of brown seaweed, commonly known as gulf-weed or sea holly. It belongs to the marine family *Sargassaceae* and order Fucales. It is widely distributed in tropical and temperate oceans. *Sargassum* species are found throughout tropical and subtropical areas of the world and are reported to produce metabolites of structural classes such as terpenoids, polysaccharides, polyphenols, sargaquinoic acids, sargachromenol, plastoquinones, steroids, glycerides, etc., which possesses several therapeutic activities. As it possesses many pharmacological properties, it has been considered as a medicinal food of the 21st century, and research is being carried out on it to reveal its other pharmacological properties. Hence, the present study has been undertaken to find out the antiplasmodial effects of the *Sargassum tenerrimum* extracts and its bioactive compounds against the malarial protozoan parasite *P. falciparum*.

METHODS

Collection

The seaweed was collected from the Rameshwaram coast of Southeast India. The collected seaweed sample was identified as *S. tenerrimum* using standard manual [10].

Preparation of seaweed extracts

The collected seaweed sample were brought to the laboratory and washed in running tap water to remove associated debris; then the seaweed were shadow dried for 2 weeks. During the extraction process, powdered samples (300 g) were extracted with hexane, acetone, and ethyl acetate each (500 ml) in a Soxhlet apparatus (boiling point range 50-80°C) for 48 hrs. The extracts of different solvents were separated out, and the solvents were redistilled using a rotary evaporator. Finally, crude extracts were stored at room temperature.

Culture maintenance

The *in vitro* antiplasmodial activity of seaweed extract was assessed against *P. falciparum* (obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India). *P. falciparum* were cultivated in human O Rh+ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, Maharashtra, India) [11] supplemented with O Rh+ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, Maharashtra, India), and 40 µg/ml of gentamycin sulfate

(HiMedia Laboratories Private Limited, Mumbai, Maharashtra, India). Hematocrits were adjusted at 5%, and parasite cultures were used when they exhibit 2% parasitemia [12].

In vitro antiplasmodial activity

Different concentrations (100, 50, 25, 12.5, 6.25, and 3.125 µg/ml) of crude extract of *S. tenerrimum* were incorporated in 96 well tissue culture plate containing 200 µl of *P. falciparum* culture with fresh red blood cells diluted to 2% hematocrit. The negative control was maintained with fresh red blood cells, and 2% parasitized *P. falciparum* diluted to 2% hematocrit, and the positive control was maintained with parasitized blood culture treated with chloroquine [13]. Parasitemia was evaluated after 48 hrs by Giemsa stain, and the average suppression of parasitemia was calculated by the following formula:

$$\text{Average suppression of Parasitaemia} \quad (\%) = \frac{\text{Ac-At}}{\text{Ac}} \times 100$$

Where Ac is average suppression of parasitaemia in control (%); At is average percentage of parasitaemia in test (%).

Antiplasmodial activity calculation and analysis

The antiplasmodial activity of seaweed extract was expressed by the inhibitory concentrations (IC_{50}) of the drug that induced 50% reduction in parasitemia compared to the control (100% parasitemia). The IC_{50} values were calculated (concentration of extract in the X-axis and percentage of inhibition by the extract in the Y-axis) using office XP (SDAS) software. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al.* [14].

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 100 µg/ml of the extract at a dose equal to the highest volume used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of the antiplasmodial assay. After 48 hrs of incubation, thin blood smears were stained with Giemsa stain and then observed for their morphological changes under the high-power light microscope. The morphological findings were compared with those erythrocytes that were uninfected and not exposed to extract [15].

Phytochemical analysis

The phytochemical screening of crude extracts from the *S. tenerrimum* acetone extracts was carried out to determine the presence of active secondary metabolites. The seaweed extract were screened for the presence of alkaloids, carbohydrates, flavonoids, tannins, glycosides, fat and fixed oil, amino acids, and phenol compounds by adopting the method of Harbone [16] and Trease and Evans [17].

Gas chromatography-mass spectrometer (GC-MS)

GC-MS analysis was followed by observing potent antiplasmodial activity in treated crude extracts. GC analysis of was performed with GC-Clarus 500 Perkin Elmer using elite 5MS columns. The qualitative and quantitative analyses of the acetone extract of *S. tenerrimum* were carried out using a CP 3800 Saturn 2200 GC-MS system. The temperature program was 80°C-350°C at the rate of 3°C/minutes and held at 50 and 55 minutes. Ion temperature was 200°C, and scan range was 20-500 (Atomic Mass Unit). The compounds were identified based on the comparison of was based on the comparison of their MS with those of Wiley and NIST libraries.

RESULTS

In vitro antiplasmodial activity

The average suppression rate of parasitemia was found to be very high in the acetone extract of *S. tenerrimum,* i.e. IC_{50} 27.82 µg/ml at 24 hrs of incubation and 18.14 µg/ml at 48 hrs of incubation Figs. 1 and 2. However, the hexane and ethyl acetate crude extract were showed the lowest activity. The IC_{50} values of the seaweed extract against *P. falciparum* strains after 24 hrs and 48 hrs incubation are listed in Figs. 1 and 2. The percentage suppression of parasitemia at 24 and 48 hrs were given in Figs. 3-5. The microscopic observation of uninfected erythrocytes added with the *S. tenerrimum* extracts did not show any morphological differences after 48 hrs of incubation.

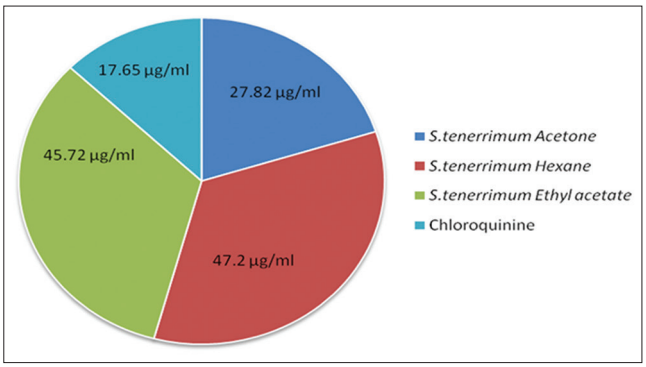


Fig. 1: Parasitemia inhibition (IC_{50}) of crude extract from *Sargassum tenerrimum* in different polaritic solvents at 24 hrs as compared with positive control. *Data expressed as mean ± standard deviation and the three replicates was found significant (p<0.05)

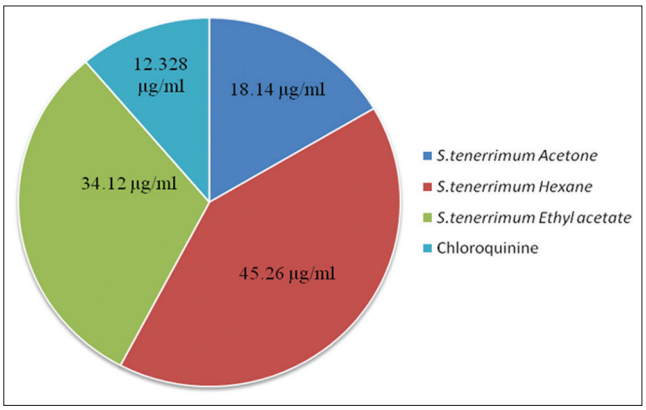


Fig. 2: Parasitemia inhibition of (IC_{50}) of crude extract from *Sargassum tenerrimum* in different polaritic solvents in 48 hrs as compared with positive control. *Data expressed as mean±standard deviation and the three replicates values was found significant (p<0.05)

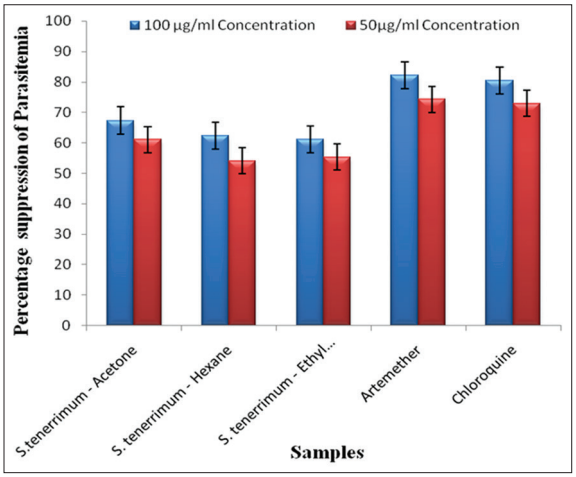


Fig. 3: Percentage suppression of parasitemia by the crude extract from *Sargassum tenerrimum* at different concentrations (µg/ml) in 24 hrs as compared with positive control

Phytochemical analysis

The phytochemical screening of seaweeds showed the presence of steroids, alkaloids, flavonoids, tannins, glycosides, amino acids, and phenolic compounds in the acetone extracts.

GC-MS analysis

A high-resolution MS equipped with a data system in combination with GC revealed the presence of bioactive components in the acetone extract of *S. tenerrimum* (which showed potent antiplasmodial activity). And then based on spectral data, it was found that the extract contained a mixture of volatile compounds (Fig. 6). The chromatogram in Fig. 6 showed 8 peaks representing their retention time corresponding to bioactive compounds. The respective molecular compounds with mass were identified by NIST library and Wiley library. The compounds found in the acetone extract of *S. tenerrimum* are listed in Table 1. The active constituents found in acetone extracts were: Cyclotrisiloxane, Hexamethyl; D-Manitol,1,1,1'-o-1,16-hexadecanediylbis; Z-Z-6,28-Heptatriactontadien-2-one; Trans,CIS-1,8-Dimethylspiro(4,5)-Decane; 3,7,11,15- Tetramethyl-2-Hexadecen-1-ol; Oleyl alcohol, Trifluoroacetate; L-(+)-Ascorbic acid 2,6-Dihexadecanoate; 1-Pentatriacontanol; N-propyl 11-octadecenoate, and fucosterol (Table 1).

DISCUSSION

The main aim was to assess the therapeutic efficacy of *S. tenerrimum* as antimalarial drugs against *P. falciparum* which may be regarded as future promising phyto-therapeutics in the treatment of malaria. Seaweeds have afforded to date the highest number of compounds within a single group of marine organisms. A high percentage of recent reports concerns bioactive metabolites with interesting biological properties. In the present study, the different polaritic solvent (hexane, ethyl acetate, and acetone) extract of seaweed *S. tenerrimum* were tested for its antiplasmodial activity. Among the solvent extracts tested, the acetone crude extract of *S. tenerrimum* showed maximum inhibition (IC_{50} 18.14 µg/ml) against the *P. falciparum* at 48 hrs of incubation. Previously several studies have been conducted and estimated the antiplasmodial potential of seaweed *Sargassum* sp., i.e. the seaweed crude extract of *Sargassum boveanum* (Sargasseae family) showed the highest antimalarial activity (IC_{50} equals to 1 mg/ml) [18]. It has been reported earlier that the antiplasmodial activity of 13 species of seaweed crude extracts was tested against *P. falciparum,* and it was found to be, among the 13 seaweed extracts tested, the *Caulerpa toxifolia* extract exhibited maximum activity (IC_{50} 5.06 µg/ml); followed by the *Culerpa pellata* (IC_{50}16.69 µg/ml) [19]; It has been reported that the *Sargassum wightii* exhibited the weakest activity (IC_{50} >100) against *P. falciparum* [20]. However, it has been reported in the another study that the *Sargassum* sp. and *Sargassum myriocystem* exhibited moderate activity (61.73 and 36.06 µg/ml, respectively) [21]. And also, it is reported that the *S. wightii* extracts exhibited the maximum activity (IC_{50} <3.125 µg/ml in Petroleum ether, ethyl acetate, and ethyl alcohol extraction) against *P. falciparum* [22]. This differentiation in bioactivity is due to the collection of samples, and the presence of biochemical compounds which differs from place to place. There are several antimalarial molecules that can efficiently integrate the panel of lead compounds isolated from marine sources with new chemical backbones and sometimes with unique functional groups [23]. For instance, some seaweeds have been found to contain a powerful class of natural substances that can effectively destroy the malarial parasite. It is reported that the Ascosalipyrrolidinones A 61 have been isolated from *Ascochyta salicorniae* which is an endophytic and obligate marine fungi of green alga *Ulva* species, and it was found to exhibit activity toward *Plasmodium falciparum* strains [24]. McPhail *et al*. [25] reported that the linear lipopeptide dragomabin which is isolated from the cyanobacterium *Lyngbya majuscula* was found to have moderate (IC_{50}=6.0 µM) antimalarial activity and significant differential toxicity between the malarial parasite and mammalian cells. Linington *et al*. [26] reported that the new cyclic hexapeptides venturamides A and B isolated from *Cyanobacterium oscillatoria* sp., exhibited significant

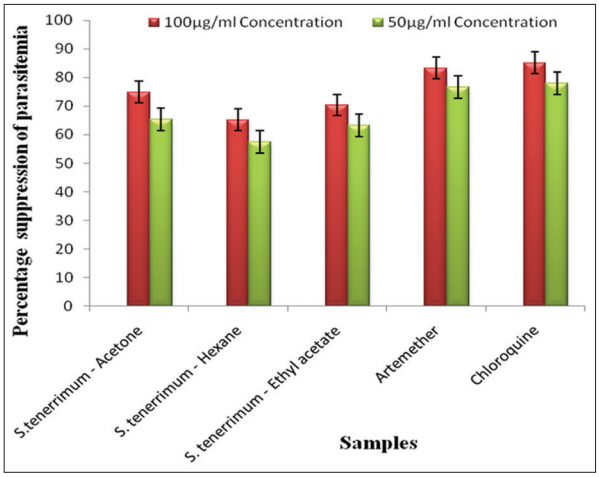


Fig. 4: Percentage suppression of parasitemia by the crude extract from *Sargassum tenerrimum* at different concentration (µg/ml) in 48 hrs as compared with positive control

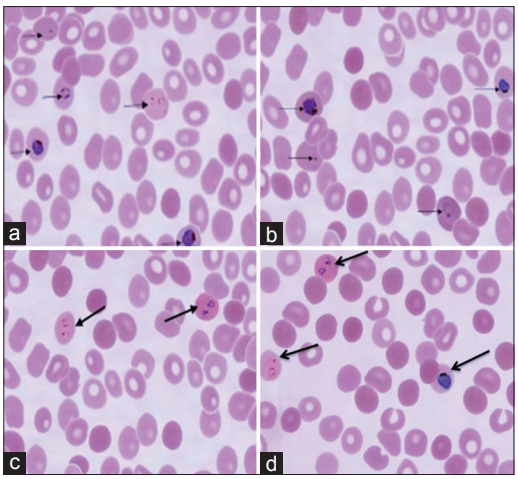


Fig. 5: Suppression of parasitemia by the crude acetone extract of *Sargassum tenerrimum.* (a) Negative control; (b) Acetone extract of *S. tenerrimum* at 24 hrs; (c) Acetone extract of *S. tenerrimum* at 48 hrs; (d) Positive control chloroquine at 48 hrs

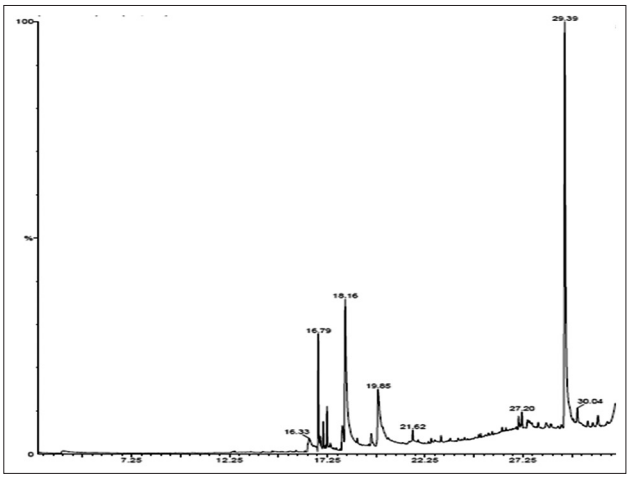
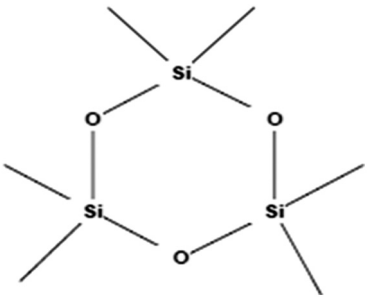
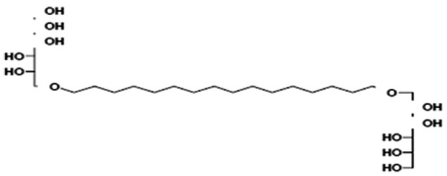
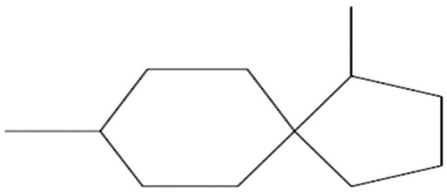
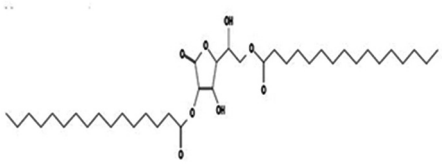
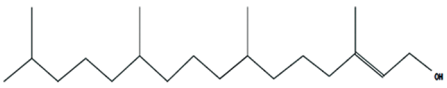
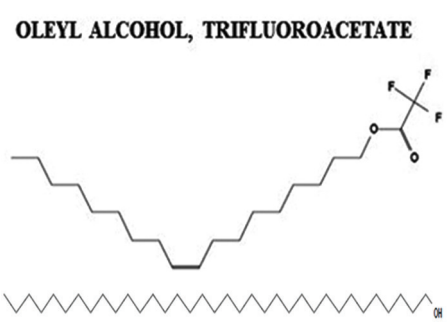


Fig. 6: Gas chromatography-mass spectroscopy chromatogram of acetone extract of *Sargassum tenerrimum*

antiplasmodial activity against W2 chloroquine-resistant *P. falciparum* strain (IC_{50}=5.6-82 µM). Researchers believed that the bromophycolide

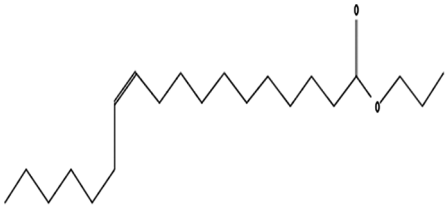
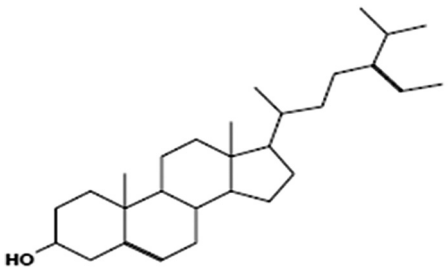
compounds are substances produced by the seaweeds as a chemical defense against marine fungi attacks, but they also appear to be effective against the malaria parasite [27]. In this present study, 10 compounds were identified using GC-MS analysis in the *S. tenerrimum* acetone extract. The earlier report CH_3OH-CH_2CL_2 extract of *Stechnoperum marginatum*, with diterpenes comprising over 50% of the compounds and, i.e. two separate diterpenes, 5(R), 16(S)-diacetoxyspata-13,17-diene and 5(R), 16(S)-dihydroxyspata-13,17-diene were isolated from

Table 1: Chemical structure of *S. tenerrimum* acetone extract

Name of the compound	Structure	Mole form	Mole weight	RT	Peak area %
Cyclotrisiloxane, Hexamethyl		$C_6H_{18}O_3Si_3$	222	27.033	0.899
Z, z-6,28-heptatriactontadien-2-one		$C_{37}H_{70}O$	530	16.769	4.989
D-Mannitol, 1,1'-O-1,16-Hexadecanediylbis		$C_{28}H_{58}O_{12}$	586	16.349	2.790
Trans, cis-1,8-dimethylspiro[4.5]decane		$C_{12}H_{22}$	166	17.044	1.253
L-(+)-ascorbic acid 2,6-dihexadecanoate		$C_{38}H_{68}O_8$	652	18.165	24.969
3,7,11,15-Tetramethyl-2-hexadecen-1-ol		$C_{20}H_{40}O$	296	17.234	1.904
Oleyl alcohol, trifluoroacetate		$C_{20}H_{35}O_2F_3$	364	18.025	3.191
1-Pentatriacontanol		$C_{35}H_{72}O$	508	19.495	0.978

(*Contd*...)

Table 1: (*Continued*)

Name of the compound	Structure	Mole form	Mole weight	RT	Peak area %
N-propyl 11-octadecenoate		$C_{21}H_{40}O_2$	324	19.845	14.735
Fucosterol		$C_{29}H_{48}O$	412	29.394	40.593

S. tenerrimum: Sargassum tenerrimum, RT: Retention time

S. marginatum [28]. The bioactive compounds identified using GC-MS analysis in the present study is much similar to the above-said report. In the previous studies, the essential oils of various brown algae have been investigated isolating 8 compounds from *Dictyota dichotoma*, 12 compounds from dichotoma, 4 from *Petalonia fascia*, 4 from *Scytosiphon lomentaria*, and 14 compounds from *Colpomenia sinuosa*, accounting for the essential oils, respectively [29]. Several of these compounds were screened *in vitro* against both chloroquine (CQ) sensitive and resistant *P. falciparum* isolates and were found to exhibit moderate antiplasmodial activity, with compounds 7-deacetoxy-7-oxogedunin and 2-hydroxymethyl-2,3,22,23-tetrahydroxy-2,6,10,15,19,23-hexamethyl-6,10,14,18 tetracosatetraene showing IC_{50} values of 6 and 7 μM, respectively [30-33]. In our study, *S. tenerrimum* was matched with major compounds of cyclotrisiloxane hexamethyl and the antiplasmodial activity exhibited by this compound (IC_{50} 18.14 μg/ml) can be comparable to the above-said reports.

CONCLUSION

The different polaritic solvent (hexane, acetone, and ethyl acetate) extracts of seaweed *S. tenerrimum* were tested for its antiplasmodial activity, and the acetone extract showed maximum activity (IC_{50} 18.14 μg/ml) at 48 hrs of incubation. The GC-MS results revealed that the unique chemical classes present in the *S. tenerrimum* might be the responsible for the antiplasmodial effect against *P. falciparum*. In our study, *S. tenerrimum* was matched with major compounds of cyclotrisiloxane hexamethyl. Further studies are highly warranted for the antiplasmodial activity of identified unique chemical classes present in the *S. tenerrimum* and also the safety and efficacy through animal model studies.

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