Antimalarial activity of identified chemical classes of *Caulerpa scalpelliformis* from Tuticorin coast against chloroquinone sensitive *Plasmodium falciparum*

Chandralekha¹, Parthiban¹, Margaret Beula ², Sundaram Ravikumar³, Banergee Rajkumar², Sundaram Prasannkumar³

1. Department of Chemistry, Kamaraj College, Tuticorin Email:chandralekha1978@yahoo.com  
2. Department of chemistry Scott Christian College, Nagercoil, Tamil Nadu, India  
3. School of Marine Sciences. Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi campus, 623 409, Tamil Nadu, India

Abstract

In recent times, the marine plants, especially, the seaweeds are considered as one of the potential raw materials in the preparation of drugs to treat infectious diseases. In the present study an attempt is also made to isolate the unique chemical classes from the ethanolic crude extract of *Caulerpa scalpelliformis* by sequential extraction by column chromatography and identified by MASS, FTIR, ¹³C NMR and ¹H NMR spectroscopic techniques. Phenanthrenol derivatives and N-(2-hydroxy-1-hydroxymethyl octadec-3-enyl)-propionamide have been isolated. In order to develop an antimalarial drug from the seaweed which are known to have potentiality in the malarial infected treatment, the present study has been taken up. The *in-vitro* antiplasmodial activity of the isolated compounds viz., N-(2-Hydroxy-1-hydroxymethyloctadec-3-enyl)-propionamide and Phenanthrenol derivative were assessed against *P. falciparum* and the IC₅₀ values which falls under the active category as they fall in the range IC₅₀ < 5 to < 50 µg ml⁻¹. Thus, it has maximum inhibition of parasitemia for the treatment of malaria.

Key words: *Caulerpa scalpelliformis*, *P. falciparum*, MASS, FTIR, ¹³C NMR and ¹H NMR

1. Introduction

In the present days, especially for the production of marine natural products India has a coastline of about 8118 km and it is one of the richest marine wealth countries in the world. Increased attention is being given to this marine wealth. Seaweeds are large algae (macro algae) that grow in saltwater or marine environment. They lack true stems, roots and leaves. However, they possess a blade that is leaf like, a stripe that is stem like and a holdfast that resembles a root of the terrestrial plant. Seaweeds contain photosynthetic pigments and use sunlight to produce food and oxygen from carbon dioxide and water. Seaweeds provide valuable clues for the development of new drugs against cancer, microbial infections and inflammation (Premila *et al.*, 1996; Kim *et al.*, 1997; Okai *et al.*, 1997; Elena *et al.*, 2001). Recent works have proved that extracts from seaweeds species exhibit activity against human, animal and plant pathogens.
To identify harmless, biodegradable and environmentally safe bioactive compounds with significant pharmaceutical potential against mosquitoes larvae, Ravikumar et al., (2013) probed the larvicidal activity of DMSO extracts of the seaweeds Ulva lactuca, Caulerpa racemosa (C. racemosa), Sargassum microystum, Caulerpa scalpelliformis. Mrinalini. J. Singh (2014) reported that in Spyridia, only the methanolic extract could produce zone of inhibition against Staphylococcus aureus and Salmonella sp. whereas in Caulerpa scalpelliformis, only the petroleum ether, chloroform and methanolic extracts showed antimicrobial properties. Rabia Alghazeer et al., (2013) was found crude methanolic and water extracts of 19 marine algal species (6 Chlorophyta, 8 Phaeophyta and 5 Rhodophyta) collected from the western coast of Libya were evaluated for antibacterial activity against patho-genic bacteria (4 Gram-positive, 4 Gram-negative). Seaweeds have proved for antimicrobial activity against some human pathogens (Ravikumar et al., 2002, Sureshkumar et al., 2002, Ravikumar et al., 2005, Ravikumar et al., 2011) reported that, the seaweed species Chaetomorpha antennina showed maximum percentage than the other species.

Malaria is a fatal parasitic disease transmitted by mosquitoes. In India, 2.5 to 3 million malaria cases are being recorded annually as per the report of National Antimalaria Programme (Lal et al., 2000). Of the four malarial human plasmodia, the Plasmodium falciparum which is behind the high fatal rate has developed resistance to the common chloroquine and also to other antimalarial drugs (White, 1999). In India nearly 40% chloroquine resistant P. falciparum cases have been recorded. This has led to further research to find out new effective therapies like antibiotics. Marine plants are recently being recognized as potential sources of drug preparation for malaria.

2.Methodology

2.1. Collection of sea weeds

The seaweed Caulerpa scalpelliformis was collected in Tuticorin coast, Tamil Nadu (Latitude 8°75’11”N; Longitude 78° 16’95”E). The collected samples were washed thrice with tap water and twice with distilled water to remove the adhering associated animals. Voucher specimen was deposited in the herbarium facility (sponsored by the Indian Council of Medical Research, New Delhi) maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Tamil Nadu, India.

2.2. Identification of bioactive metabolites in the most promising seaweed species Caulerpa scalpelliformis

2.2.1 Identification of metabolites using Thin Layer Chromatography:

Crude extract was subjected to Thin layer chromatographic (TLC) analysis on silica gel (TLC silica gel 60, 20 × 20, 0.5 mm, Merk and Co, Inc) with ethyl acetate: hexane (7:3) solvent system.
The crude extract was spotted, and the solvent front was allowed to run for approximately 16 cm. The running lane was then dried thoroughly and the elution of compound was detected at 365 nm.

2.2.2 Isolation using column chromatography

The crude extract was mixed with silica powder and kept overnight for removing moisture. Next day the crude extract was admixed with silica powder and kept ready for the isolation using column chromatography.

2.2.3 NMR, IR and Mass Spectroscopy

The dried bioactive metabolite was dissolved in ethyl acetate and filtered to remove impurities. After leaving this solution overnight at -20°C, the semisolid obtained was collected. The semisolid was again suspended in a minimum quantity of NMR solvent and analyzed by Nuclear magnetic resonance spectroscopy (NMR-400 MHz), Fourier transform spectroscopy (BRUKER, alpha-E) and Mass spectroscopy (EIMS) to determine the chemical structure.

2.3 Antiplasmodial activity

The in-vitro antiplasmodial activity of the marine halophytic crude extracts and the isolated compounds viz., N-(2-Hydroxy-1-hydroxymethyloctadec-3-enyl) propionamide and Phenanthrenol derivative were assessed against *P. falciparum* (obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Indian Institute of Science, Bangalore, India). *P. falciparum* was cultivated in human O Rh+ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India) (Moore *et al.*, 1967) supplemented with O Rh+ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited) and 40 μg ml⁻¹ of gentamycin sulphate (HiMedia Laboratories Private Limited, Mumbai, India). Haematocrits were adjusted at 5% and parasite cultures were used when they exhibited 2% parasitaemia (Trager, 1987).

Different concentrations of filter-sterilized crude extract and also the isolated compounds viz., N-(2- Hydroxy-1-hydroxymethyloctadec-3-enyl)-propionamide and Phenanthrenol derivative of chosen seaweed species (100, 50, 25, 12.5, 6.25 and 3.125 μg ml⁻¹) were incorporated into 96-well tissue culture plates 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 *P. falciparum* diluted to 2% haematocrit. Positive control was maintained with parasitized blood culture treated with Artemether and chloroquine (Azas *et al.*, 2001). Parasitaemia was evaluated after 24 h and 48 h by giemsa stain and the average percentage suppression of parasitaemia was calculated by the formula given below:

$$\text{Average } \% \text{ suppression of parasitaemia} = \frac{\text{Average } \% \text{ parasitaemia in control} - \text{average } \% \text{ parasitaemia in test}}{\text{average } \% \text{ parasitaemia in control}} \times 100$$
The antiplasmodial activities of marine halophytic crude extracts were expressed by the inhibitory concentrations (IC\textsubscript{50}) of the drug that induced 50% reduction in parasitaemia compared to control (100% parasitaemia). The IC\textsubscript{50} values were calculated (Concentration of extract in the X-axis and percentage of inhibition in the Y-axis) using office XP (SDAS) software. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo \textit{et al.}, (1992) and suggested that, an extract is very active if IC\textsubscript{50} < 5 µg ml\textsuperscript{-1}, active IC\textsubscript{50} < 50 µg ml\textsuperscript{-1}, weakly active IC\textsubscript{50} < 100 µg ml\textsuperscript{-1} and inactive IC\textsubscript{50} > 100 µg ml\textsuperscript{-1}.

3. Results and Discussion

3.1. Identification of active chemical classes

In the present study the unique chemical classes present in the crude extract of \textit{C. scalpelliformis} were separated by sequential extraction using column chromatography. Further analysis by the \textsuperscript{13}C NMR, \textsuperscript{1}H NMR, MASS and FTIR spectroscopic techniques revealed that, the ethanol crude two unique chemical classes \textit{viz.,} (N-(2-hydroxy-1-hydroxymethyloctadec-3-yl)-propionamide (Fig.1) and phenanthrenol derivatives (Fig.2).

![Structure of the identified chemical class in column fraction no. 1 of C. scalpelliformis](image1)

![Structure of the identified chemical class in column Fraction no. 2 of C. scalpelliformis (Phenanthrenol derivative)](image2)

3.2. Antiplasmodial activity in identified chemical classes of \textit{Caulerpa scalpelliformis}

Malaria is a fatal parasitic disease transmitted by mosquitoes. In India, 2.5 to 3 million malaria cases are being recorded annually as per the report of National Antimalaria Programme (Lal et al., 2000). Of the four malarial human plasmodia, the \textit{Plasmodium falciparum} which is behind the high fatal rate has developed resistance to the common chloroquine and also to other antimalarial
drugs (White, 1999). In India nearly 40% chloroquine resistant *P. falciparum* cases have been recorded. This has led to further research to find out new effective therapies like antibiotics. Marine plants are recently being recognized as potential sources of drug preparation for malaria. These two unique chemical classes from the ethanolic crude extract of *C. scalpelliformis* viz., phenanthrenol derivatives and (N-(2-hydroxy-1-hydroxymethyl-octadec-3-enyl)-propionamide were subjected to the antiplasmodial activity analysis. The IC<sub>50</sub> values obtained for the phenanthrenol derivatives was 20.562 µg ml<sup>-1</sup> and for the (N-(2-hydroxy-1-hydroxymethyl-octadec-3-enyl)-propionamide was 12.812 µg ml<sup>-1</sup> for 24 hrs of study (Fig.3). For the 48 hours of study the values for these two chemical classes were 14.75 µg.ml<sup>-1</sup> and 7.484 µg.ml<sup>-1</sup> (Fig.4) respectively. It is also observed that the suppression of parasitaemia shown by the identified chemical classes for 48 hours of study is much close to that of the standard drugs as revealed by the IC<sub>50</sub> values of 7.484 and 14.75 µg.ml<sup>-1</sup> for N-(2-Hydroxy-1-hydroxymethyl-octadec-3-enyl)-propionamide and the Phenanthrenol derivatives and 7.968 and 12.81 µg.ml<sup>-1</sup> for the standard Artemether and Chloroquine drugs.

Fig.3. Percentage suppression of parasitaemia(IC<sub>50</sub>) values of isolated chemical classes from *C. scalpelliformis*

![Fig.3](image1.png)

Fig.4. Percentage suppression of parasitaemia(IC<sub>50</sub>) of isolated chemical classes from *C. scalpelliformis* at 48 hrs.

![Fig.4](image2.png)
Conclusion

The two unique chemical classes from the ethanolic crude extract of *C. scalpelliformis* viz., phenanthrenol derivatives and (N-(2-hydroxy-1-hydroxymethyl octadec-3-enyl)propionamide were subjected to the antiplasmodial activity. The phenanthrenol derivatives showed IC\(_{50}\) value of 20.562 µg.ml\(^{-1}\) and the (N-(2-hydroxy-1-hydroxymethyloctadec-3-enyl)-propionamide showed IC\(_{50}\) of 12.812 µg.ml\(^{-1}\) in 24 hrs (Fig.3). In 48 hrs, the IC\(_{50}\) values for these two chemical classes were 14.75 µg.ml\(^{-1}\) and 7.484 µg.ml\(^{-1}\) respectively (Fig.4). Thus the present work carried out to investigate the inhibitory effect of certain selected marine halophytes in the first part, has established that the seaweed *Caulerpa scalpelliformis* in its ethanolic extract has significant activity against the deadly *Plasmodium falciparum* which is behind the very high mortality rate of malaria.

References


