Chandralekha et al. International Journal of Recent Research Aspects ISSN: 2349-7688, Special Issue: Conscientious Computing Technologies, April 2018, pp. 338-342

Antimalarial activity of chosen marine halophytes from Tuticorin coast against chloroquinone sensitive Plasmodium falciparum

Chandralekha[1], Margaret Beula [2], Sundaram Ravikumar[3], Banergee Rajkumar[4], Sundaram Prasannkumar [5]

[1] Assistant Professor, Department of Chemistry, Kamaraj College, Tuticorin

[2,4] Associate professor(Rtd), Department of chemistry Scott Christian College, Nagercoil, Tamil Nadu, India,

[3] Professor, School of Marine Sciences. Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi campus, 623 409, Tamil Nadu, India,

[5]Junior Research Fellow, School of Marine Sciences. Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi campus, 623 409, Tamil Nadu, India

Abstract- **Malaria is a fatal parasitic disease transmitted by mosquitoes. The drugs resistant Plasmodium falciparum causes the most virulent form of malaria in humans and it is described as a public health disaster causing increased morbidity and mortality. Seven seaweeds species and two sea grass species were collected Tuticorin coastal area and the seaweeds and seagrass extracts of three different solvents (diethyl ether, ethyl acetate and ethyl alcohol) were tested for in vitro antiplasmodial activity against Plasmodium falciparum. It is observed that Caulerpa scalpelliformis is having the minimum inhibitory concentration (IC50) values in all the three solvent extracts. The IC50 values of crude extract from C. scalpelliformis extracted with diethylether, ethylacetate and ethanol are 27.343, 23.953 and 19.109 µg ml−1 respectively, which falls under the active category as they fall in the range IC50< 5 to < 50 µg ml−1. The in vitro antiplasmodial activity might be due to the presence of sugars, proteins, and phenols in the ethanolic extracts of seaweeds. Thus C. scalpelliformis has the highest antiplasmodial activity against the malarial human P. falciparum. When compared with the different solvent used for the extraction, the inhibitory concentrations of the seaweed and seagrass extracts differ in their polarity, and found that, the inhibitory concentration is found minimum with the ethanol solvent. It is concluded from the present study that, the ethanolic extracts of seaweeds of C.scalpelliformis possess lead compounds for the development of antiplasmodial drugs.**

Keywords: Antimalarial, Artemether, Chloroquinone, Marine halophytes, Plasmodium falciparum, Seaweeds, Seagrasses

I. INTRODUCTION

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. MATERIALS AND METHODS

Two groups of marine halophytes viz., sea grasses (Syringodium isoetifolium and Cymodocea serrulata and seaweeds (Dictyota dichotoma, Stoechospermum marginatum, Sargassum wightii, Caulerpa scalpelliformis and Valaniopsis pachynema were collected in Tuticorin coast, Tamil Nadu (Lat. 8°75'11"N; Long. 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.

3. PREPARATION OF CRUDE EXTRACTS

The samples were cut into pieces and kept for shade drying. Moisture free samples were subjected for percolation by soaking in 3 different polar solvents viz., diethylether, ethylacetate and ethanol. After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporator (>45°C) and then freeze dried (-80°C) to obtain solvent free solid residue. The extracts of seaweeds were

screened for the presence of phytochemical constituents by following the method of Sofowora (1982) and Kepam (1986). The extracts were dissolved in dimethyl sulphoxide (Hi media Laboratories Private Limited, Mumbai, India) and filtered through sterile millipore filters (mesh 0.20 µm, Sartorious Stedim Biotech GmbH, Germany). The filtrate was used for testing at different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 µg.ml-1)

Table 1. Phytochemical constituents of chosen marine halophytes extracted with different solvents

Name of the constituents	Marine halophytic plant species																				
	Syringodium isoetifolium			*Cymodocea serrulata*			*Dictyota dichotoma*			*Stoechospermum marginatum*			*Sargassum wightii*			*Caulerpa scalpelliformis*			*Valaniopsis pachynema*		
	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol	Diethylether	Ethylacetate	Ethylalcohol
Alkaloids	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Carboxylic acid	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-
Coumarins	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Flavanoids	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Quinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Saponins	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-
Xanthoproteins	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Protein	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
Resins	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-
Steroids	+			+				+			+		-	-	-	-	-	+	-	-	-
Tannins	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Sugars	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	+	+	-	-	-

(+) presence (-) absence

4. ANTIPLASMODIAL ACTIVITY

The in vitro antiplasmodial activity of the marine halophytic crude extracts 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 PrivateLimited,Mumbai, India) and40 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 seaweed and seagrass 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 and 2% parasitized 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 formula given below:

$$\text{Average \% suppression of parasitaemia} = \frac{\text{average \% parasitaemia in control} - \text{average \% parasitaemia in test}}{\text{average \% parasitaemia in control}} \times 100$$

The antiplasmodial activities of marine halophytic crude extracts were expressed by the inhibitory concentrations (IC50) of the drug that induced 50% reduction in parasitaemia compared to control (100% parasitaemia). The IC50 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 et al., (1992) and suggested that, an extract is very active if IC50< 5 µg ml−1, active IC50 < 50 µg ml−1, weakly active IC50< 100 µg ml−1 and inactive IC50 > 100 µg ml−1.

5. RESULTS AND DISCUSSION

The antiplasmodial activity against the malarial human plasmodium Plasmodium falciparum was conducted on the extracts of the marine halophytes, sea grasses viz.,Syringodium isoetifolium and Cymodocea serrulata and seaweeds viz.,Dictyota dichotoma, Stoechospermum marginatum, Sargassum wightii, Caulerpa scalpelliformis and Valaniopsis pachynema. The antiplasmodial activities of marine halophytic crude extracts were expressed by the inhibitory concentrations (IC50) , the drug needed to induce 50% reduction in parasitaemia compared to control, 100% parasitaemia, found for 24 hours and 48 hours observation are presented in Figs. 1. The IC50 values were calculated (Concentration of extract in the X - axis and percentage of inhibition in the Y - axis) using office XP (SDAS) software. The inferences were drawn in accordance with the criteria put forwarded for antiplasmodial activity by Rasoanaivo et al., (1992). An extract is very activif IC50< 5 µg ml−1, active IC50 < 50 µg ml−1, weakly active IC50< 100 µg ml−1 and inactive IC50 > 100 µg ml−1.It is already proved that, the marine halophytic plants posses antimicrobial (Ravikumar et al., 2011; Mrinalini et al., 2014) antiplasmodial (Jacob et al., 2012; Jasmine Spavieri et al., 2013; Ghannadi et al., 2013) antibacterial (Vallinayagam et al., 2009, Ravikumar et al., 2002) activities.

The inhibitory concentrations (IC50) values for the extracts of the sea grass species Syringodium isoetifolium and Cymodocea serrulata are greater than 100, with all the three solvents, diethylether, ethylacetate and ethanol, for both 24 hours and 48 hours observations and so, according to the above criteria, these two halophytes are quite inactive against Plasmodium falciparum.But, the seaweeds, Valaniopsis pachynema has inhibitory concentration (IC50) values >100 µg ml−1with diethylether and ethylacetate extracts for 24 as well as 48 hours but has little lesser values of 94.187 and 80.625 with the ethanol extract for the same times of observation. It is therefore inferred that,Valaniopsis pachynema is inactive in its diethylether and ethylacetate

extracts and only weakly active against Plasmodium falciparumin its ethanol extract.

The activity of the remaining four sea weeds, Dictyota dichotoma, Stoechospermum marginatum, Sargassum wightii and Caulerpa scalpelliformis showed a gradual variation of inhibitory concentration (IC50). The values show a gradual decrease in the order Stoechospermum marginatum>Dictyota dichotoma> Sargassum wightii >Caulerpa scalpelliformisin the diethylether and ethanol extracts for 24 hours. The activity against Plasmodium falciparum is the reverse of this order with Caulerpa scalpelliformis exhibiting the strongest activity. In the ethylacetate extract the order is Dictyota dichotoma>Stoechospermum marginatum> Sargassum wightii >Caulerpa scalpelliformis with Dictyota dichotoma is having the least activity with IC50being86.921µg ml−1.
It is observed that Caulerpa scalpelliformis is having the least inhibitory concentration (IC50) values in all the three solvent extracts. The IC50 values for C. scalpelliformis in diethylether, ethylacetate and ethanol are 27.343, 23.953 and 19.109 µg ml−1 respectively. These three values come under the active category as they fall in the range IC50< 5 to < 50 µg ml−1. Thus C. scalpelliformis has the highest antiplasmodial activity against the malarial human plasmodium P. Falciparum. The activity shown by the standard drugs, Artemether and Chloroquine are noticed by 14.75 and 17.656 µg ml−1 for 24 hours and 7.968 and 12.81 µg ml−1 for 48 hours (Figs. 1-3). These values are closer to the inhibitory concentration values of C. scalpelliformis in the ethanol extract, thus supporting the results obtained. The brown algae Sargassum wightii is found to show the values 37.031, 22.984 and 35.578 µg ml−1 respectively with the three solvents, diethylether, ethylacetate and ethanol. The values are within the limits for active category. However the activity would be lesser than that of C. scalpelliformis. The inhibitory concentrations (IC50) determined for Stoechospermum marginatum were found to be >100, 82.562 and 71.421µg ml−1 with the three solvents diethylether, ethylacetate and ethanol respectively. Thus this halophyte is quite inactive in the diethylether extract but weakly active in the ethylacetate and ethanol extracts. This is revealed by the IC50 values of 82.562 and 71.421µg ml−1 in the ethylacetate and ethanol extracts respectively.

The results obtained for the activity studies for 48 hours for all of these halophytes have also shown the same trend. However the inhibitory concentration (IC50) values are lesser than those of the values observed for 24 hours. If the inhibitory concentrations of the halophyte extracts of the three solvents, which differ in their polarity, are compared, it is found that the values are the least with the ethanol extract, both in the 24 hours as well as in the 48 hours length of analysis. This indicates that the activity is more with the ethanol solvent than with the other two solvents. This higher activity of the ethanol extract could be attributed to the high polarity, relative polarity- 0.654; polarity index – 5.2, of ethanol due to its hydroxyl (OH) group, with the high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules and thus, ethanol can dissolve both polar and non-polar substances. The polarity values of the other two solvents are less. For instance, relative polarity-0.54; polarity index -2.8; for diethylether relative polarity-0.228; polarity index – 4.4 for ethylacetate . Therefore the ethanol solvent could definitely extract more bioactive components from the halophytes making its extract more active. It clearly showed that, the ethanol crude extract has the maximum suppression of parasitaemia at very low concentration when compared to other solvent and seaweed species. This might be due to the presence of several phytochemical constituents viz., alkaloids, carboxylic acid, coumarins, flavanoids, phenols, saponins, xanthoproteins, protein, resins, steroids and sugars respectively (Table 1) present in the ethanol crude extract from Caulerpa scalpelliformis when compared with the other crude extracts of the chosen halophytes.

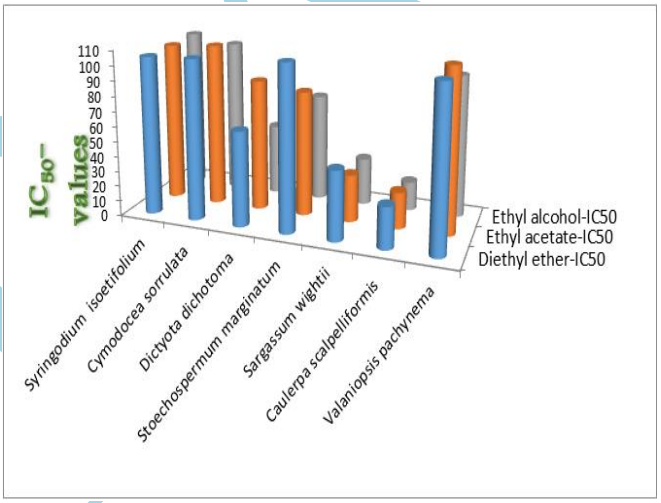

Fig. 1. Picture showing the suppression of parasitaemia (IC50) of marine seaweeds and seagrass crude extracts at 24 hrs

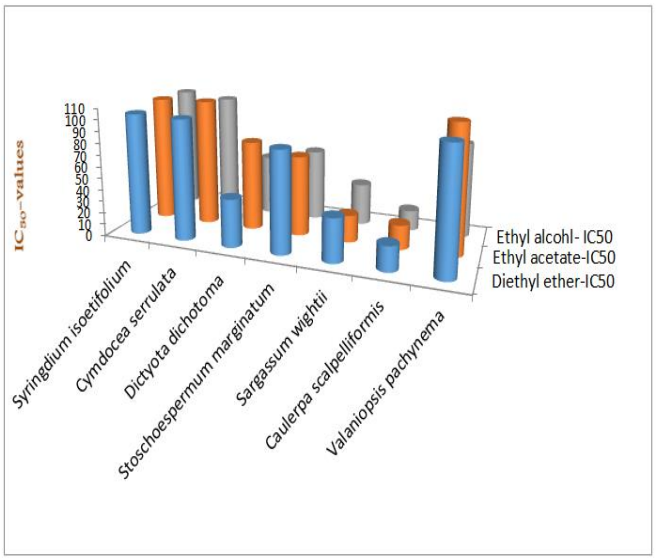

Fig. 2. Percentage suppression of paraistemia (IC50) of marine seaweeds and seagrass crude extracts at 48 hrs

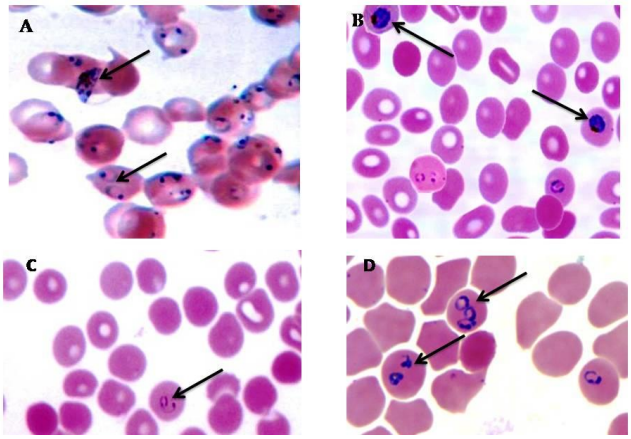

Fig. 3. Picture showing the parasitaemia in control and treated

A – Negative control; B – Treated with diethylether extract of C.scalpelliformisat 48 h;
C – Treated with ethylalcohol extract of C.scalpelliformis at 48 h; D – Treated with ethylacetate extract of C. scalpelliformisat 48 h.

6. CONCLUSION

It is found that treatment with ethanol crude extract obtained from Caulerpa scalpelliformis has clearly showed maximum suppression of parasitaemia at low concentration as compared to other solvents obtained from other seaweeds/sea grass species. It is therefore, further proceeded to separate the bioactive chemical classes from the ethanol extract of the seaweed Caulerpa scalpelliformis

7. REFERENCES

[1] Azas, N., Laurencin, N., Delmas, F., Di Giorgio, C., Gasquet, M., Laget, M. and Timon David, P. 2001. Synergistic in vitro antimalarial activity of plant extracts used as traditional herbal remedies in Mali. Parasitol Res., 88: 165–171.

[2] Ghannadi, A., Plubrukarn, A. and Yegdaneh, A. 2013.Screening for antimalarial and acetylcholinesterase inhibitory activities of some Iranian seaweeds .Res Pharm Sci., 8(2): 113–118.

[3] Jacob Inbaneson, S., and Ravikumar,S., 2012. In vitro antiplasmodial activity of PDDS-coated metal oxide nanoparticles against Plasmodium falciparum, Applied Nanoscience, 197 – 201.

[4] Jasmine Spavieri, Andrea Allmendinger, Marcel Kaiser, Maurice Ayamba Itoe, Gerald Blunden, Maria, M., Mota. and Deniz Tasdemir. 2013.Assessment of Dual Life Stage Antiplasmodial Activity of British Seaweeds .Mar. Drugs.11: 4019-4034.

[5] Kepam, W., 1986. Qualitative organic analysis (Spectrochemical techniques). Ed. II. Mc Graw Hill, London, 40 – 58.

[6] Rasoanaivo, P., Ratsimamanga, U., Ramanitrhasimbola, D., Rafatro, H., and Rakoto Ratsimamanga, A. 1992. Criblage dextraits de plantes de Madagascar pour recherche dactivite antipaludique et deffet potentialisateur de la chloroquine. J. Ethnopharmacol., 64: 117-126.

[7] Lal, S., Sonal ,G.S. and Phukan,P.K. 2000. Status of Malaria in India. Journal of Indian Academy of Clinical Medicine. 5: 19-23.

[8] Moore, G.E., Gerner, R.E. and Frankin, H.A. 1967. Cultures of normal human leukocytes. J Am Med Assoc., 199: 519–524.

[9] Mrinalini, J. and Singh. 2014. Studies on the antimicrobial potency of the marine algae - Caulerpa scalpelliformis and Spyridia species. International Journal of Biological Research2 (2). 131-133

[10] Ravikumar ,S., Ramanathan,G., Inbaneson, S.J. and Ramu,A. 2011. Antiplasmodial activity of two marine polyherbal preparations from Chaetomorpha antennina and Aegiceras corniculatum against Plasmodium falciparum. Parasitol Res.,108: 107-113

[11] Ravikumar, S., Anburajan, L., Ramanathan, G. and Kaliaperumal,N. 2002. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens. Seaweed. Res. Utln. Assoc.,1: 95-99

[12] Sofowora, A., 1982. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd.

[13] Trager, W., 1987. The cultivation of Plasmodium falciparum: applications in basic and applied research in malaria. Ann Trop Med Parasitol., 82: 511–529.

[14] Vallinayagam, K., Arumugam, R., Ragupathi Raja Kannan, R., Thirumaran, G. and Anantharaman, P. 2009.Antibacterial Activity of Some Selected Seaweeds from Pudumadam Coastal Regions Global. Journal of Pharmacology. 3 (1): 50-52

[15] White, N. 1999. Antimalarial drug resistance and mortality in falciparum malaria.Tropical Medicine and International Health 4:469-470.

