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ANALYSIS

Variable drug bio availability of native seaweeds in India for antimalarial therapeutics

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ABSTRACT

A most important feature for the success of any planned reaction is the selection of a suitable solvent: solvents influence both chemical reactivity and reaction rates. The term "solvent polarity" lacks an exact definition, but it is generally used to encompass all of the intermolecular interactions of which the solvent is capable. Polar solvents have large dipole moments; they contain bonds between atoms with very different electronegativities, such as oxygen and hydrogen, polar reactants will dissolve in polar solvents and non-polar solvents dissolve non-polar compounds best. The present investigation was carried out to find out the antiplasmodial potential of crude seaweed extracts with three different polaritic solvents. Among the seaweed extracts tested, the crude extracts of *Sargassum wightii* and *Valaniopsis pachynema* exhibited excellent antiplasmodial activity of IC_{50} < 3.125 µg. ml^{-1} in all the three solvents (Petroleum ether, Ethyl acetate and ethyl alcohol) used at 48 h of incubation. This activity is highly comparable to the activity of positive control artemether (IC_{50} < 3.125 µg. ml^{-1}). Statistical analysis reveals that, the significant *in vitro* antiplasmodial activity (P < 0.05) was observed between the concentration and time of exposure. The chemical injury to erythrocytes was done and its shows no morphological changes in erythrocytes by all the three solvent extracts. The results revealed that, the crude seaweed extract of *S.wightii* and *V. pachynema* collected from South West coast of India possess variable drug bioavailability for the development of antiplasmodial agents.

Keywords: antimalarial drug, antiplasmodial activity, drug bioavailability, *Plasmodium falciparum,* Seaweed extracts, Solvent polarity.

1. INTRODUCTION

Malaria remains the world most devastating human parasitic infection, afflicting more than 500 million people and causing about 2.5million deaths each year. Malaria chemotherapy is challenged by the emergence of drug resistant *Plasmodium falciparum* (Hyde et al*.,* 2005). Recently, artemisinin-based combination therapies (ACTs) were recommended by the World Health Organization (WHO, 2006). However, ACTs face great pharmacological challenges with regard to variable drug bioavailability, drug interactions, and the long half life of partner drugs that have implication in the development of resistant parasite mutants (German & Aweeka, 2008; Kremsner & Krishna, 2005; Talisuna et al*.,* 2004). Recent reports suggest that, there may already be signs of the emergence of *in vitro* resistance to some artemisinins (Jambou et al, 2005; Uhlemann et al*.,* 2005). This situation indicates that, the battle against malaria could be seriously impaired, until new and efficacious drugs are developed. Chlorophyll containing organisms known to have more than 20,000 species. The macro-algae can produce bioactive compounds like antibiotics, algicides, toxins. A lot of antibiotics have been isolated from algae and show great chemical diversity (fatty acids, bromophenols, tannins, terpenoids, polysaccharides, alcohols). Most of them produce neurotoxic and hepatotoxic compounds (Metting 1996). The important form like Irish moss and carrageenan contain proteins, vitamin A, sugar, starch, vitamin B1, iron, sodium, phosphorous, magnesium, copper and calcium. They all have industrial applications as anticoagulants, antibiotics, antihypertensive agents, blood cholesterol reducers, dilatory agents, insecticides, anti-tumorogenic agents and antiplasmodial agents (Kharkwal et al, 2012; Ravikumar, 2011). Considering this great features, the present investigation has been made an attempt to test the antiplasmodial activities of seaweeds of SouthWest coast of India.

Plasmodium falciparum:
Plasmodium falciparum is a protozoan parasite, one of the species of Plasmodium that cause malaria in humans. Malaria caused by this species is the most dangerous form of malaria, with the highest rates of complications and mortality.

2. MATERIALS AND METHODS

2.1 Collection of sea weeds

The seaweeds *Amphiroa anceps* (Red Algae), *Gracilaria corticata* (Red algae), *Sargassum wightii* (Brown Algae), *Padina pavonica* (Brown algae), *Ulva lactuca* (Green algae) and *Valaniopsis pachynema* (Green algae) were collected from SouthWest coast of India, Kanyakumari, Tamil Nadu. 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.

Haematocrit:
The haematocrit (Ht or HCT), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood.

2.2 Extraction of bioactive principles

The samples were cut into pieces and kept for shade drying. Moisture free samples were subjected for percolation by soaking in 3 different polaritic solvents *viz*., petroleum ether, ethyl acetate 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}) (Jacob inbaneson et al*.,* 2012).

Parasitemia
Parasitemia is the quantitative content of parasites in the blood. It is used as a measurement of parasite load in the organism and an indication of the degree of an active parasitic infection.

2.3 Culture maintenance

The *in vitro* antiplasmodial activity of seaweed extracts 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, India) (Moore et al*.,* 1967) supplemented with O Rh^{+} serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) 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 exhibit 2% parasitaemia (Trager, 1987).

2.4 *In vitro* antiplasmodial activity

Different concentrations of filter-sterilized crude extract from seaweeds (100, 50, 25, 12.5, 6.25 and 3.125µg ml^{-1}) was incorporated into 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% haematocrits and positive control was maintained with parasitized blood culture treated with Artemether and chloroquine (Azas et al*.,* 2002). Parasitaemia was evaluated after 24 h and 48 h by giemsa stain and the average percentage suppression of parasitaemia was calculated by the following formula: average % suppression of parasitaemia= average % parasitaemia in control−average % parasitaemia in test/average % parasitaemia in control×100.

2.5 Antiplasmodial activity calculation and analysis

The antiplasmodial activity of seaweed extracts expressed by the inhibitory concentrations (IC_{50}) of the drug that induced 50% reduction in parasitaemia compared to the control (100% parasitaemia). The IC_{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 et al., (1992) and suggested that, an extract is very active if IC_{50}< 5 µg ml^{-1}, active IC_{50} < 50 µg ml^{-1}, weakly active IC50< 100 µg ml^{-1} and inactive IC_{50} > 100 µg ml^{-1}.

2.6 Chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might attributed by the extract, 200 µl of erythrocytes was incubated with 100 µg. ml^{-1} of the extract at a dose equal to the highest used in the antiplasmodial assay. The conditions of the experiment were maintained as in the case of antiplasmodial assay. After 48 h of incubation, thin blood smears were stained with giemsa stain and observed for morphological changes under high-power light microscope. The morphological findings were compared with those erythrocytes that were uninfected and not exposed to extract (Waako et al., 2007).

3. RESULTS

Among the seaweed extracts tested, the crude extract of *S. wightii* and *V. pachynema* exhibited maximum activity (IC_{50} <3.125 µg.ml^{-1}) at 48 hrs of incubation in all the three different polaritic solvent. The petroleum ether, ethyl acetate and ethyl alcohol extract of *G.corticata* showed the IC_{50} value of 8.93 µg.ml^{-1}, 29.76 µg.ml^{-1} and 22.98 µg.ml^{-1} respectively at 48 hrs (Table 1). Statistical analysis reveals that, the significant *in vitro* antiplasmodial activity ($P < 0.05$) was observed between the concentration and time of exposure. The chemical injury to erythrocytes was done and its shows no morphological changes in erythrocytes by all the three solvent extracts. The preliminary phytochemical analysis of seaweed extracts showed a variety of phytochemical constituents *viz.,* alkaloids, carboxylic acids, coumarins, flavonoids, quinones, phenols, saponins, proteins, resins, steroids tannins and sugars (Table 2).

4. DISCUSSION

The development of parasitic resistance to frontline antimalarial drugs such as Chloroquine, antifolates and recently ART has underscored the importance of developing new drugs and drug targets to treat the disease. The continuous appearance of drug resistant *P. falciparum* strains has made the chemotherapeutic management of malaria increasingly problematic in virtually all malarious regions of the world. ART is a key ingredient in combination drug therapies recommended by the WHO for the treatment of multidrug resistant strains of *falciparum* malaria. Intense efforts are ongoing to develop ART-based combination therapies (ACTs) to extent the life of existing drugs, while major initiatives are underway to discover new antimalarials. The macro algal populations of the aquatic environments provide a vast genetic resource and biodiversity. Scientists confirmed that, algae can be utilized in a completely different manner in the drug industry. *Acochyta salicornniae* was isolated from, green alga *Ulva* species and found to produce unprecedented and structurally unusual tetramiric acid contiguous metabolites ascosalipyrrolidinones A61 & B62. The ascosalipyrrolidinones A61 has antiplasmodial activity towards *Plasmodium falciparum* strains K1 and NF54 (Osterhage et al., 2000). In the recent research it is found that, algae based proteins can inhibit the entry of the HIV virus. Seaweed extracts showed various bio potential activities such as antibacterial (Ravikumar et al, 2002, 2005, 2009; Suresh kumar et al., 2002; Shehnaz 2003;) antifungal (Ravikumar et al., 2009; Aruna et al, 2010) Antiviral (Ponce et al., 2003) Anti-inflammatory (Tan et al., 2000; Jothibai Margret et al., 2009) Termicidal (Ravikumar et al., 2011a) Larvicidal (Syed ali et al., 2013; Ravikumar et al., 2011d) and Spermicidal (Prakash 2004) Antiplasmodial (Ravikumar et al., 2011b, 2011c;). Considering these great features, the present study has been made an attempt to test the antiplasmodial potential of seaweed extracts of SouthWest Coast of India. Among the seaweed extracts tested, the extract of *S. wightii* exhibited excellent antiplasmodial activity by IC50 < 3.125 µg ml^{-1}. Based on Rasoanaivo et al (1992) recommendation the antiplasmodial compounds were categorized as follows; IC_{50}< 5 µg ml^{-1}, active IC_{50} < 50 µg ml^{-1}, weakly active IC_{50}< 100 µg ml^{-1} and inactive IC_{50} > 100 µg ml^{-1}. In this present study, the petroleum ether, ethyl acetate and ethanolic extract of *S.wightii* exhibited very active inhibition of *P. falciparum* parasites. The existence of antiplasmodial activities of this seaweed extract might be due to the presence of unique chemical classes such as alkaloids, flavonoids, sugars (polysaccharides), and phenolic compounds (Andrews et al., 2005). The antiplasmodial activities of sugars (polysaccharides) were proved to have good merozoites inhibitory activity in *P. falciparum* (Adams et al., 2005) and rosettes disruption activities (Rowe et al., 1994). Moreover, the phenolic compounds from plant extracts also proved to have *in vitro* antiplasmodial activity (Stierle et al., 1988). The polyphenols are also well documented to treat chronic diseases such as cardiovascular disease, cancer, diabetes, bacterial and parasitic infections (Sherman & Billing, 1999; Cowan, 1999). The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into erythrocytes (Adams et al., 2005) or distruption of *P. falciparum* rosttes (Carlson et al., 1992; Rowe et al., 1994) or inhibition of *P. falciparum* fatty acid biosynthesis (Tasdemir et al., 2007). Karl Gademann and Joanna Kobylinska (2009) reported that alkaloids and polyketides were found to be the major substances with antimalarial activity isolated from chordata. It was reported that the ethanolic extracts of seaweed *Caulerpa toxifolia* exhibited the antiplasmodial activity of IC_{50} 5.06 µg.ml^{-1} and *Caulerpa peltata* IC_{50} value of 16.69 µg.ml^{-1} (Ravikumar et al., 2011b). And also it was reported that the methanolic extract of seaweed *Chaetomorpha antennina* showed the antiplasmoidal activity of IC_{50} 26.37 µg.ml^{-1} (Ravikumar et al., 2011b). McPhail et al., (2007) reported that, the linear alkynoic lipopetides, dragomabin have been isolated from a red Panamanian strain of the marine micro algae (blue-green algae) *Lyngbya majuscula* and showed good antimalarial activity (IC50 = 6 µM) against chloroquine-resistant *Plasmodium falciparum*. Similar findings were reported by several authors

(Sanon et al., 2003; Son et al., 2007; Moon et al., 2007; Chung et al., 2008; Lee et al., 2009; Ravikumar et al., 2010; Ramazani et al., 2010; Jacob Inbaneson and Ravikumar, 2012). The present finding shows better activity, when comparing with those above mentioned reports in the recent research it is found that, algae based proteins can inhibit the entry of the HIV virus.

5. CONCLUSION

The present study reveals that, the petroleum ether, ethyl acetate and ethyl alcohol crude extract of *V. pachynema and S. wightii* collected from Southwest coast of India has effective antiplasmodial protein rich compounds to treat the *falciparum* malaria.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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Table 1

IC_{50} values of chosen seaweed extracts against *Plasmodium falciparum*

S. No	Names of the Seaweeds	Suppression of Parasitaemia IC_{50} at 24 hrs			Suppression of Parasitaemia IC_{50} at 48 hrs		
		Petroleum ether	Ethyl acetate	Ethyl alcohol	Petroleum ether	Ethyl acetate	Ethyl alcohol
1.	*A. anceps*	81.10	88.37	71.90	61.25	81.59	65.12
2.	*G. corticata*	42.35	62.70	38.96	26.85	51.07	17.17
3.	*S. wightii*	28.79	<3.125	10.39	<3.125	<3.125	<3.125
4.	*P. pavonica*	42.84	54.95	37.51	8.93	29.76	22.98
5.	*U. lactuca*	90.31	64.15	41.39	77.23	55.92	31.21
6.	*V. pachynema*	11.35	<.3.125	<3.125	<3.125	<3.125	<3.125

Table 2

Positive controls

S. No	Sample name	Suppression of Parasitaemia IC_{50} at 24 hrs	Suppression of Parasitaemia IC_{50} at 48 hrs
1.	Chloroquine	<3.125	<3.125
2.	Artemether	<3.125	<3.125

Table 3
Phytochemical constituents of chosen seaweeds

S. No	Names of the seaweeds	Solvents	Alkaloids	Carboxylic acid	Coumarins	Flavonoids	Quinones	Phenols	Saponins	Xantho proteins	Proteins	Resins	Steroids	Tannins	Sugars
1.	*A. anceps*	Petroleum ether	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	-	+	-	-	-	-
		Ethyl alcohol	+	-	-	+	+	+	-	+	+	-	+	-	+
2.	*G. corticata*	Petroleum ether	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl alcohol	+	+	+	+	-	-	-	-	+	-	-	-	+
3.	*S. wightii*	Petroleum ether	-	-	-	-	-	-	-	-	-	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	-	+	-	-	-	-
		Ethyl alcohol	-	-	-	-	-	+	-	-	+	+	-	-	-
4.	*P. pavonica*	Petroleum ether	-	-	-	-	-	-	-	-	+	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	-	+	-	-	-	-
		Ethyl alcohol	+	-	-	-	-	+	-	-	-	+	-	-	+
5.	*U. lactuca*	Petroleum ether	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl alcohol	-	-	-	-	-	-	-	-	-	-	-	-	+
6.	*V. pachynema*	Petroleum ether	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl acetate	-	-	-	-	-	-	-	+	+	-	-	-	-
		Ethyl alcohol	-	+	-	-	-	+	-	-	-	+	-	-	+

(+) Presence **(-) Absence**