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In vitro antiplasmodial effect of ethanolic extracts of coastal medicinal plants along Palk Strait against *Plasmodium falciparum*

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ABSTRACT

Objective: To identify the possible antiplasmodial compounds from *Achyranthes aspera* (*A. aspera*), *Acalypha indica* (*A. indica*), *Jatropha glandulifera* (*J. glandulifera*) and *Phyllanthus amarus* (*P. amarus*). **Methods:** The *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus* were collected along Palk Strait and the extraction was carried out in ethanol. The filter sterilized extracts (100, 50, 25, 12.5, 6.25 and 3.125 μg/mL) of leaf, stem, root and flower extracts of *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus* were tested for antiplasmodial activity against *Plasmodium falciparum*. The potential extracts were also tested for their phytochemical constituents. **Results:** Of the selected plants species parts, the stem extract of *A. indica* showed excellent antiplasmodial activity (IC_{50}= 43.81 μg/mL) followed by stem extract of *J. glandulifera* (IC_{50}= 49.14 μg/mL). The stem extract of *A. aspera*, leaf and root extracts of *A. indica*, leaf, root and seed extracts of *J. glandulifera* and leaf and stem extracts of *P. amarus* showed IC_{50} values between 50 and 100 μg/mL. Statistical analysis revealed that, significant antiplasmodial activity (P<0.01) was observed between the concentrations and time of exposure. The chemical injury to erythrocytes was also carried out and it showed that there were no morphological changes in erythrocytes by the ethanolic extract of all the tested plant extracts. The *in vitro* antiplasmodial activity might be due to the presence of alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, and tannins in the ethanolic extracts of tested plants. **Conclusions:** The ethanolic stem extracts of *P. amarus* and *J. glandulifera* possess lead compounds for the development of antiplasmodial drugs.

1. Introduction

Malaria remains one of the most prevalent infectious diseases in the world. Traditional medicinal plants have contributed significantly to current malaria therapy. The first effective drug treatment against malaria was quinine, which was extracted from the cinchona tree. The structure of quinine was used to synthesize antimalarials, like chloroquine and primaquine. The importance of plants as effective antimalarials was further reinforced by the isolation of artemisinin from the Chinese medicinal plant, *Artemisia annua* (*A. annua*)[1]. Several plants with antiplasmodial properties have been proved as sources for novel antiplasmodial compounds[2-7]. India boasts remarkable biodiversity and rich cultural traditions of plant use.

Acalypha indica (*A. indica*) has many bioactive potential such as antibacterial, antifungal, antidiabetic, antioxidant and anti-inflammatory[8-12]. Tribal communities in India used various parts of this plant for the treatment of diseases such as asthma, cough, dog bite, rheumatism, earache, scabies, scorpion bites, snake bites and sting of centipedes, burns and eczema[13]. *Achyranthes aspera* (*A. aspera*) is one of the medicinal plants used as an emmenagogue, antiarthritic, purgative, diuretic, antimalarial, oestrogenic, antileprotic, antispasmodic, cardiotonic, antibacterial and antiviral agent[14]. *Jatropha* species such as *Jatropha glandulifera* (*J. glandulifera*) have antiviral, antifungal, antitumor, antileukemia, anti-inflammatory and insecticidal properties[15]. *Phyllanthus amarus* (*P. amarus*) has been found to have anti-diarrhea, antiviral, anticarcinogenic, antinocleeptive, anti-inflammatory, anti-diabetic and anti-lipidemic potentials[16].

Interestingly, many pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders worldwide[17]. Due to the rising prevalence of *Plasmodium falciparum* (*P. falciparum*) resistance to chloroquine and many available drugs, the treatment of malaria is becoming increasingly difficult[18]. Artemisinin resistance in *P. falciparum* has

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been recently detected in Cambodia[19]. Previous reports on bioactive potential of medicinal plants insisted us to choose *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus* for the present study. In this connection, the present study was made an attempt to explore the antiplasmodial potential of *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus* against *P. falciparum*.

2. Materials and methods

2.1. Plant material

Fresh samples of different plant parts (leaf, stem, root and seed) from *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus* were collected from Palk Strait coast, Tamil Nadu, India (Latitude 9 ° 44 ′01 ″ N and Longitude 79 ° 00 ′58 ″ E) and were botanically authenticated by Prof. K Kathiresan, Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. The percentage of extraction and yields were recorded. A sample voucher specimen was deposited in the herbarium facility (Sponsored by Indian Council of Medical Research, New Delhi, India) maintained in the Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi Campus, Thondi, Ramanathapuram District, Tamil Nadu, India. All the collected samples were washed twice with distilled water to remove the adhering dusts and other associated animals.

2.2. Extract preparation

Shade dried samples were subjected for percolation by soaking in ethanol. After 21 days of dark incubation, the filtrate was concentrated separately by rotary vacuum evaporation (> 45 ℃) and then freeze dried at −80 ℃ to obtain solid residue. The percentage of extraction was calculated by using the following formula: % of extraction= Weight of the extract (g)/ Weight of the plant material (g) × 100. The extracts of selected medicinal plants were screened for the presence of phytochemical constituents by following the standard methods[20,21]. The ethanolic extracts were dissolved in dimethyl sulphoxide (HiMedia Laboratories Private Limited, Mumbai, India) and filtered through Millipore sterile filters (mesh 0.20 μm, Sartorious Stedim Biotech GmbH, Germany). The filtrates were used for testing at different concentrations of 100, 50, 25, 12.5, 6.25 and 3.125 μg/mL[6].

2.3. Parasite cultivation

The antiplasmodial activity of medicinal plant extracts was assessed against *P. falciparum* obtained from the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India. *P. falciparum* are cultivated in human O Rh^+ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India) supplemented with O Rh^+ serum (10%), 5% sodium bicarbonate (HiMedia Laboratories Private Limited, Mumbai, India) and 40 μg/mL of gentamycin sulphate (HiMedia Laboratories Private Limited, Mumbai, India). Hematocrits were adjusted at 5% and parasite cultures were used when they exhibited 2% parasitaemia[6].

2.4. In vitro antiplasmodial assay

Filter sterilized extracts (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% hematocrit. Negative control was maintained with fresh red blood cells and 2% parasitized *P. falciparum* diluted to 2% hematocrit, positive control was maintained with parasitized blood cells culture treated with chloroquine and artemether[6]. Parasitaemia was evaluated after 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 activities of ethanolic extracts were 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 X axis and percentage of inhibition in Y axis) using Office XP (SDAS) software with linear regression equation. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al*[22]. According to this norms, an extract is very active if IC_{50} < 5 μg/mL, active 5 μg/mL < IC_{50} < 50 μg/mL, weakly active 50 μg/mL < IC_{50} <100 μg/mL and inactive IC_{50} > 100 μg/mL. Analysis of variances was performed by ANOVA procedures followed by a specific post-hoc test to analyze the difference and statistical significances were achieved when P< 0.01[6].

2.6. Chemical injury to erythrocytes

To assess any chemical injury to erythrocytes that might be attributed to the extract, 200 μL of erythrocytes were incubated with 100 μg/mL 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 microscopy. The morphological findings were compared with those erythrocytes that were uninfected and not exposed to extract[6].

3. Results

The percentage yields of extracts ranged from 2.72% to 15.95% and were represented in Table 1. It revealed that, leaf of *J. glandulifera* (15.95%) showed maximum yield followed by leaf of *A. indica* (14.56%). The IC_{50} value of the tested medicinal plants against *P. falciparum* are listed in Table 2. The stem extract of *A. indica* (IC_{50}= 43.81 μg/mL) showed excellent antiplasmodial activity and followed by stem extract of *J. glandulifera* (49.14 μg/mL). Moreover, the leaf, root and seed extract of *A. aspera* and root extract of *P. amarus* showed IC_{50} value of more than 100 μg/mL (Table 2).

The microscopic observation of uninfected erythrocytes incubated with the ethanolic extracts and uninfected erythrocytes from the blank column of the 96-well plate showed no morphological differences after 48 h of incubation. The preliminary phytochemical study revealed that the extracts from chosen coastal medicinal plants have variety of phytochemical constituents, namely alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins and tannins (Table 3).

Table 1
Yield percentage of ethanolic extracts from medicinal plants.

Botanical name	Plant part used	Weight of plant part (g)	Yield of extract g (%)
A. aspera	Leaf	20	2.35 (11.75)
	Stem	41	2.52 (6.15)
	Root	58	1.58 (2.72)
	Seed	42	1.34 (3.19)
A. indica	Leaf	25	3.64 (14.56)
	Stem	23	1.71 (7.43)
	Root	21	0.74 (3.52)
J. glandulifera	Leaf	21	3.35 (15.95)
	Stem	79	4.12 (5.22)
	Root	60	1.94 (3.23)
	Seed	42	2.03 (4.83)
P. amarus	Leaf	28	3.38 (12.07)
	Stem	31	1.67 (5.38)
	Root	20	0.68 (3.40)

Table 2
IC_{50} value of medicinal plants extracts against *P. falciparum*.

Extracts and drugs	Plant part used	IC_{50} (μg/mL)
A. aspera	Leaf	>100.00
	Stem	76.75
	Root	>100
	Seed	>100.00
A. indica	Leaf	56.89
	Stem	43.81
	Root	69.00
J. glandulifera	Leaf	70.94
	Stem	49.14
	Root	86.92
	Seed	61.73
P. amarus	Leaf	94.19
	Stem	83.53
	Root	>100.00
Chloroquine	–	18.63
Artemether	–	5.55

Values are found significant between concentrations and time of exposure (P<0.01).

Table 3
Preliminary phytochemical constituents of ethanolic stem extracts of medicinal plants.

Phytochemical constituents	*A. aspera*	*A. indica*	*J. glandulifera*	*P. amarus*
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Coumarins	–	–	–	–
Flavanoids	–	+	+	–
Quinones	–	–	–	–
Phenols	–	–	+	–
Saponins	+	–	–	+
Triterpenoids	+	+	–	–
Proteins	+	–	–	–
Resins	–	–	–	–
Steroids	–	–	–	–
Tannins	–	+	–	+

–: Absent; +: Present.

4. Discussion

Malaria is still a major public health problem, especially in tropical and sub-tropical regions. It is estimated that, in 2006, 3.3 billion people were at risk of contracting malaria and that it causes nearly one million deaths each year. Mostly of African children aged below 5 years are susceptible to this disease. In Sub-Saharan regions, 45 countries were endemic for malaria in 2008[23]. Artemisinin, isolated from the well-known Chinese medicinal plant *A. annua*, is one of the best compounds used to treat multi-drug resistant strains of *P. falciparum*. However, artemisinin-resistant malaria parasites were recently detected in Cambodia[24]. The development and spread of drug resistant strains of the causative agent *P. falciparum* has limited the effectiveness of the currently used malarial drugs. This creates the need for new antimalarial drugs. Plants have over the years proved to be a good source of chemotherapeutic agents.

The present study was investigated with different parts extracts of *A. aspera*, *A. indica*, *J. glandulifera* and *P. amarus*. Among the tested extracts, the stem extracts of *A. indica* and *J. glandulifera* showed IC_{50} value (43.81 μg/mL, 49.14 μg/mL respectively) at 2.3 fold and 2.6 fold concentration of positive control chloroquine respectively. This might be due to the presence of glycosides, alkaloids, flavonoids, phenol and tannins[3,25-27]. According to Rasoanaivo *et al* 14%, 57% and 29% of extracts from medicinal plants were classified as active, weakly active and inactive respectively[22]. *A. indica* and *J. glandulifera* belonging to Euphorbiaceae family are first reported to possess antiplasmodial properties. Moreover, some other plants species belonging to this same family possess antiplasmodial properties. Udobang *et al* reported that, the leaf extract of *Acalypha wilkensiana* dose-dependently reduced parasitaemia induced by chloroquine sensitive *Plasmodium berghei*[28]. *Acalypha fruticosa* methanolic extract showed antiplasmodial at IC_{50} value of 10.7 μg/mL against clinical isolates of *P. falciparum* and this might be due to the presence of tannins, terpenoids, flavonoids and polysaccharides[29]. *Jatropha curcas* extract showed antiplasmodial with IC_{50} value of more than 50 μg/mL[30].

The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into the erythrocytes and disruption of *P. falciparum* rosettes by the carbohydrates[31-33]; inhibition of *P. falciparum* fatty acid biosynthesis[34], inhibition of hemozoin biocrystallization by the alkaloids and inhibition of protein synthesis by triterpenoids[35,36]. It is concluded from the present study that ethanolic extracts from *A. indica* and *J. glandulifera* displayed *in vitro* antiplasmodial activity and warrant further investigation of these plants as potential sources of antiplasmodial agents. Additional *in vitro* and *in vivo* work aimed at understanding the mechanisms of action of the active plant species and isolating and characterising the bioactive constituents is underway in our laboratories and will be reported on in due course.

Conflict of interest statement

We declare that we have no conflict of interest.

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