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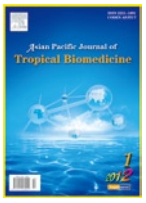

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In vitro antiplasmodial activity of chosen terrestrial medicinal plants against *Plasmodium falciparum*

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ABSTRACT

Objective: To identify the possible antiplasmodial compounds from *Carica papaya* (*C. papaya*), *Pongamia glabra* (*P. glabra*), *Phyllanthus emblica* (*P. emblica*) L, *Phyllanthus acidus* (*P. acidus*) L, *Pisonia grandis* (*P. grandis*) and *Moringa pterygosperma* (*M. pterygosperma*). **Methods:** The *C. papaya*, *P. glabra*, *P. emblica* L, *P. acidus* L, *P. grandis* and *M. pterygosperma* were collected from Ramanathapuram District, Tamil Nadu, India and the extraction was carried out in ethanol. The filter sterilized extracts (100, 50, 25, 12.5, 6.25 and 3.125 μg/mL) were tested for antiplasmodial activity against *Plasmodium falciparum* (*P. falciparum*). The potential extracts were also tested for their phytochemical constituents. **Results:** Of the selected plants species, the leaf extract of *P. emblica* L showed excellent antiplasmodial activity (IC_{50} 35.09 μg/mL) followed by leaf extract of *M. pterygosperma* (IC_{50} 42.36 μg/mL), leaf extract of *C. papaya* (IC_{50} 46.23 μg/mL) and leaf extract of *P. glabra* (IC_{50} 48.17 μg/mL). The flower extract of *M. pterygosperma* showed IC_{50} values of more than 100 μg/mL. Statistical analysis reveals 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 shows 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, carbohydrates, flavonoids, phenols, saponins, triterpenoids, steroids and tannins in the ethanolic extracts of tested plants. **Conclusions:** The ethanolic stem extracts of *P. emblica* L possess lead compounds for the development of antiplasmodial drugs.

1. Introduction

Malaria is still the most important parasitic disease in the world and caused by the genus *Plasmodium*. The development and spread of drug resistant strains of the causative agent *Plasmodium falciparum* (*P. falciparum*) has limited the effectiveness of the currently used malarial drugs. This creates the need for new antimalarial drugs. Previous findings of antimalarial agents such as quinine and artemisinin from medicinal plants also encouraged the possibility of finding new antimalarial drugs from plant source[1]. The bioactive potential of medicinal plants insisted us to choose *Carica papaya* (*C. papaya*) [2], *Pongamia glabra* (*P. glabra*)[3], *Phyllanthus emblica* (*P. emblica*) L[4], *Phyllanthus acidus* (*P. acidus*) L[5], *Pisonia grandis* (*P. grandis*)[6] and *Moringa pterygosperma* (*M. pterygosperma*)[7] for the present study. In this connection, the present study was made an attempt to explore the antiplasmodial potential of chosen terrestrial medicinal plants against *P. falciparum*.

2.Material and methods

2.1.Plant material

Fresh samples of different plant parts (leaf, stem, root, flower and fruit) from *C. papaya*, *P. glabra*, *P. emblica L*, *P. acidus L*, *P. grandis* and *M. pterygosperma* were collected from Ramanathapuram District, Tamil Nadu, India and were botanically authenticated by Prof. K. Kathiresan, Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu,

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India. The percentage of extraction and yields is depicted in Table 1. A sample voucher specimen was deposited in the herbarium facility (Sponsored by Indian Council of Medical Research, New Delhi) 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

Table 1
Yield percentage of ethanolic extracts from chosen terrestrial medicinal plants

Botanical name	Family	Local Name	Plant part used	Weight of plantpart (g)	Yield of extract (g)	Yield of extract %
C. papaya	Caricaceae	Pappaly	Leaf	25	2.84	11.36
			Stem	22	1.27	5.77
P. glabra	Fabaceae	Pungai	Leaf	23	2.84	12.35
			Fruit	20	1.15	5.75
P. emblica L	Phyllanthaceae	Perriya Nelli	Leaf	22	3.26	14.82
			Bark	21	1.33	6.33
P. acidus L	Phyllanthaceae	Chinna Nelli	Leaf	20	2.75	13.75
			Bark	19	1.18	6.21
P. grandis	Nyctaginaceae	Latcha Kattai	Leaf	24	2.98	12.42
			Bark	20	1.05	5.25
M. pterygosperma	Moringaceae	Murungai	Leaf	21	2.85	13.57
			Bark	19	1.09	5.73
			Flower	15	0.73	4.87

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 (-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[8]. 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[8].

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 Hematocrits were adjusted at 5% and parasite cultures were used when they exhibited 2% parasitaemia[8].

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[8]. 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[8]. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al*[9]. 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 posthoc test to analyze the difference and statistical significances were achieved when P<0.01.

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 in erythrocytes that were uninfected and not exposed to extract[8].

3.Results

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

Botanical name	Herbarium voucher Number	Collection site		Plant part used	IC50 (μg/mL)
		Latitude	Longitude		
C. papaya	SMSMP017	09° 19′ 10″ N	78° 43′ 07″ E	Leaf	46.23
				Stem	65.13
P. glabra	SMSMP018	09° 21′ 45″ N	78° 49′ 41″ E	Leaf	48.17
				Fruit	54.95
P. emblica L	SMSMP019	09° 22′ 21″ N	78° 49′ 56″ E	Leaf	35.09
				Bark	71.91
P. acidus L	SMSMP020	09° 22′ 21″ N	78° 49′ 56″ E	Leaf	78.20
				Bark	73.84
P. grandis	SMSMP014	09° 19′ 01″ N	78° 44′ 13″ E	Leaf	50.11
				Bark	61.25
M. pterygosperma	SMSMP024	09° 21′ 45″ N	78° 49′ 41″ E	Leaf	42.36
				Bark	66.58
				Flower	>100
Positive control					
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 chosen terrestrial medicinal plants

Phytochemical constituents	*C. papaya* Leaf	*P. glabra* Leaf	*P. emblica* L Leaf	*M. pterygosperma* Leaf
Alkaloids	+	+	+	+
Glycosides	+	+	+	−
Carbohydrates	−	+	−	+
Coumarins	−	−	−	−
Flavanoids	+	+	+	−
Quinones	−	−	−	−
Phenols	+	−	+	−
Saponins	+	+	+	+
Triterpenoids	+	−	−	+
Proteins	−	−	−	−
Resins	−	−	−	−
Steroids	−	+	−	−
Tannins	+	+	+	+

− : Absent; + : Present

The percentage yields of extracts were ranged from 4.87 to 14.82 and were represented in Table 1. It reveals that, leaf of *P. emblica* L (14.82%) showed maximum yield followed by leaf of *P. acidus* L (13.57%). The leaf extract of *P. emblica* L (IC_{50} 35.09 μg/mL) showed excellent antiplasmodial activity, followed by leaf extract of *M. pterygosperma* (42.36 μg/mL), leaf extract of *C. papaya* (46.23 μg/mL) and leaf extract of *P. glabra* (48.17 μg/mL). Moreover, *M. pterygosperma* flower

extract was 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 reveals that, the extracts from tested medicinal plants have variety of phytochemical constituents, namely alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, triterpenoids, steroids and tannins (Table 3).

4.Discussion

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250 000-500 000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Considering the vast potentiality of plants as sources for antibacterial, antifungal, antiviral, antiplasmodial, hepatoprotective and anti-inflammatory agents, a systematic investigation was undertaken to test the local flora for antiplasmodial activity.

Among the tested extracts, leaf extract of *P. emblica* L showed IC_{50} value (35.09 μg/mL) and leaf extract of *M. pterygosperma* (IC_{50} 42.36 μg/mL) at 1.9 fold and 2.3 fold concentration of positive control chloroquine respectively. This may be due to the presence of alkaloids[10], glycosides[11], carbohydrates[12-14], flavonoids[15], phenols[12], saponins[15], triterpenoids[16], steroids[17] and tannins[12]. According to Rasoanaivo *et al*[9], 31%, 61% and 8% of extracts from medicinal plants were classified as active, weakly active and inactive respectively[9]. Pinmai *et al*[18] reported that, the water extract of *P. emblica* Linn showed antiplasmodial activity (IC_{50} 14.37 μg/mL) and ethanolic leaf extracts of *P. niruri* from different geographical distribution showed antiplasmodial activity IC_{50} ranged from 19 to more than 50 μg/mL[19]. The juice extracted from the leaves of *Moringa pterygosperma* has strong antibacterial, antimalarial properties and the paste of the leaves is used as an external application to promote healing of wounds[20]. Ngemenya *et al* reported that, a very weak antiplasmodial activity in the leaves and seeds of *C. papaya* with IC_{50} of about 60 μg/mL[21]. Petroleum ether extract of pulp of *C. papaya* showed antiplasmodial activity IC_{50} at 18.09 μg/mL[22].

The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into the erythrocytes[23] and disruption of *P. falciparum* rosettes[24] by the carbohydrates; inhibition of *P. falciparum* fatty acid biosynthesis[25] and inhibition of hemozoin biocrystallization by the alkaloids[26], inhibition of protein synthesis by triterpenoids[27], inhibition of β-haematin formation[28], decreased mitochondrial membrane potential, DNA fragmentation and cytoplasmic acidification by steroids[29]. It is concluded from the present study that, leaf extract of *P. emblica* L displayed *in vitro* antiplasmodial activity and further studies are in progress to isolate the active principles for the development of new antiplasmodial drugs.

Conflict of interest statement

We declare that we have no conflict of interest.

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