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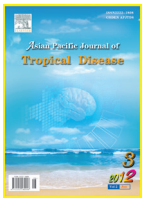

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In vitro antiplasmodial activity of ethanolic extracts of South Indian medicinal plants against *Plasmodium falciparum*

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ABSTRACT

Objective: To explore the antiplasmodial potential of *Catharanthus roseus* L (*C. roseus*), *Coccinea grandis* (*C. grandis*), *Thevetia peruviana* (*T. peruviana*), *Prosopis juliflora* (*P. juliflora*), *Acacia nilotica* (*A. nilotica*), *Azadirachta indica* (*A. indica*) (Abr. Juss) and *Morinda pubescens* (*M. pubescens*). **Methods:** The *C. roseus* L, *C. grandis*, *T. peruviana*, *P. juliflora*, *A. nilotica*, *A. indica* (Abr. Juss) and *M. pubescens* 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*. The phytochemical constituents in the potential extracts were also detected. **Results:** Of the selected plants species, the bark extract of *A. indica* (Abr. Juss) showed excellent antiplasmodial activity (IC_{50} 29.77 μg/mL) followed by leaf extract of *A. indica* (Abr. Juss) (IC_{50} 47.20 μg/mL) and leaf extract of *C. roseus* L (IC_{50} 49.63 μg/mL). The leaf, bark and flower extracts of *P. juliflora* showed IC_{50} values of more than 100 μg/mL. Statistical analysis reveals significant antiplasmodial activity ($P<0.01$) between the concentrations and time of exposure. Additionally, no chemical injury was found in the erythrocytes incubated with the ethanolic extract of all the tested plants. The *in vitro* antiplasmodial activity might be due to the presence of alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, triterpenoids, proteins and tannins in the ethanolic extracts of the tested plants. **Conclusions:** The ethanolic bark extracts of *A. indica* (Abr. Juss) possess lead compounds for the development of antiplasmodial drugs.

1. Introduction

Malaria remains one of the most serious world health problems and the major cause of mortality and morbidity in the endemic regions. There are currently 109 malarious countries and territories, 45 of which are within the World Health Organization[1]. Resistance to chloroquine and sulfadoxine-pyrimethamine has resulted in millions of deaths over the past 30 years. The malaria parasite has the ability to develop resistance to the major current anti-malarials. Chloroquine resistance was the result of a single point mutation in the transporter gene Pf*crt*[2]. Gametocytes are an important mechanism for resistance and an increased duration and density of gametocytes carriage after sulfadoxine-pyrimethamine treatment predicts development of resistance[3]. The control of malaria became nearly impossible as resistance eliminated chloroquine and sulfadoxine-pyrimethamine. Recent malaria treatment is based on artemisinin[4] and artemisinin resistance in *Plasmodium falciparum* (*P. falciparum*) has been recently detected in Cambodia[5]. Alternative antiplasmodials must be needed to overcome any deficiencies in present therapy. Previous reports on the bioactive potential of medicinal plants insisted us to choose *Catharanthus roseus* L (*C. roseus*)[6], *Coccinea grandis* (*C. grandis*)[7], *Thevetia peruviana* (*T. peruviana*)[8], *Prosopis juliflora* (*P. juliflora*)[9], *Acacia nilotica* (*A. nilotica*)[10], *Azadirachta indica* (Abr. Juss) (*A. indica*)[11] and *Morinda pubescens* (*M. pubescens*)[12] for the present study. In this connection, the present study was made an attempt to explore the antiplasmodial potential of the chosen South Indian medicinal plants against *P. falciparum*.

2. Material and methods

2.1. Plant material

Fresh samples of different plant parts (leaf, stem, flower, seeds and bark) from *C. roseus* L, *C. grandis*, *T. peruviana*, *P. juliflora*, *A. nilotica*, *A. indica* (Abr. Juss) and *M. pubescens* 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, India. The percentage of extraction and yield were described in Table 1. A sample voucher

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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, 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 to 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: Percentage of extraction (%) = Weight of the extract (g)/ Weight of the plant material (g) ×100%. The extracts of the selected medicinal plants were screened for the presence of phytochemical constituents by following the standard methods[13]. 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[13].

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* were cultivated in human O Rh^+ red blood cells using RPMI 1640 medium (HiMedia Laboratories Private Limited, Mumbai, India)[13] 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 to 5% and parasite cultures were used when 2% of them exhibited parasitaemia[13].

2.4. In vitro antiplasmodial assay

Filter sterilized extracts (100, 50, 25, 12.5, 6.25 and 3.125 μg/mL) were incorporated in a 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, while positive control was maintained with parasitized blood cell culture treated with 18.63 μg/mL chloroquine or 5.55 μg/mL artemether[13]. Parasitaemia was evaluated after 48 h by Giemsa stain and the average suppression percentage of parasitaemia was calculated by the following formula: Average suppression of parasitaemia (%) = [Average percentage of parasitaemia in control (%) - Average percentage of parasitaemia in test (%)]/Average percentage of parasitaemia in control (%) ×100%.

2.5. Calculation and analysis of antiplasmodial activity

The antiplasmodial activities of ethanolic extracts were expressed by IC_{50}, the inhibitory concentrations 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 with a linear regression equation[13]. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al*[14]. According to these 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.

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 the extract[13].

3. Results

Table 1
Yield percentage and IC_{50} of ethanolic extracts from medicinal plants.

Botanical name	Family	Local name	Plant part used	Weight of plant part (g)	Yield of extract [g (%)]	IC_{50} (μg/mL)
C. roseus L	Apocynaceae	Nithiya Kalyani	Leaf	21	2.98 (11.19)	49.63
			Stem	19	1.12 (5.89)	77.72
			Flower	20	0.85 (4.25)	51.08
C. grandis	Cucurbitaceae	Kovaikai Kodi	Leaf	22	3.17 (14.41)	69.00
T. peruviana	Apocynaceae	Arali	Leaf	20	2.82 (14.10)	73.84
			Seeds	17	0.94 (5.53)	58.83
P. juliflora	Fabaceae	Nattu Karuvai	Leaf	22	2.96 (13.45)	>100.00
			Bark	23	1.21 (5.26)	>100.00
			Flower	21	0.81 (3.86)	>100.00
A. nilotica	Fabaceae	Seemai Karuvai	Leaf	22	3.05 (13.86)	73.36
			Bark	20	1.16 (5.80)	59.80
A. indica (Abr. Juss)	Meliaceae	Vemppu	Leaf	20	2.91 (14.55)	47.20
			Bark	19	1.23 (6.47)	29.77
M. pubescens	Rubiaceae	Manjanathi	Leaf	22	3.18 (14.45)	62.70
			Bark	18	1.13 (6.28)	80.63

The percentage yields of extracts were ranged from 3.86% to 14.55% (Table 1). The results reveal that leaf of *A. indica* (Abr. Juss) (14.55%) showed maximum percentage yield followed by leaf of *M. pubescens* (14.45%). The bark extract of *A. indica* (Abr. Juss) (IC_{50} 29.77 μg/mL) showed excellent antiplasmodial activity, followed by leaf extract of *A. indica* (Abr. Juss) (47.20 μg/mL) and leaf extract of *C. roseus* L (49.63 μg/mL). Moreover, the leaf, bark and flower extracts of *P. juliflora* showed IC_{50} values of more than 100 μg/mL (Table 1). The microscopic observation of the uninfected erythrocytes incubated with the ethanolic extracts and the 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 the tested medicinal plants had a variety of phytochemical constituents, namely alkaloids, glycosides, carbohydrates, flavonoids, phenols, saponins, triterpenoids, proteins and tannins (Table 2).

Table 2
Preliminary phytochemical constituents of medicinal plants.

Phytochemical constituent	Leaf of *C. roseus*	Leaf of *A. indica*	Bark of *A. indica*
Alkaloids	+	+	+
Glycosides	−	+	+
Carbohydrates	+	+	+
Coumarins	−	−	−
Flavanoids	+	+	−
Quinones	−	−	−
Phenols	+	−	−
Saponins	+	+	−
Triterpenoids	+	+	+
Proteins	+	−	−
Resins	−	−	−
Steroids	−	−	−
Tannins	+	+	+

− : Absent; + : Present

4. Discussion

Plants, especially those used in ayurveda, can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and/or reduced toxicity. The small fraction of flowering plants that have so far been investigated have yielded about 120 therapeutic agents of known structure from about 90 species of plants. Some of useful plant drugs include vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemesinin and ephedrine among others[15]. This study showed that the extracts of South Indian medicinal plants had antiplasmodial activity against the blood stage of *P. falciparum*.

The present study was investigated with the extracts derived from different parts of *C. roseus* L, *C. grandis*, *T. peruviana*, *P. juliflora*, *A. nilotica*, *A. indica* (Abr. Juss) and *M. pubescens*. Among the tested extracts, the bark extract of *A. indica* (Abr. Juss) showed excellent antiplasmodial activity (IC_{50} 29.77 μg/mL) at 1.6-fold concentration of the positive control chloroquine. Moreover, the leaf extracts of *A. indica* (Abr. Juss) (IC_{50} 47.20 μg/mL) and *C. roseus* L (IC_{50} 49.63 μg/mL) showed good antiplasmodial activity. This may be due to the presence of alkaloids[16], glycosides[17], carbohydrates[18-21], flavonoids[22], phenols[19], saponins[22], triterpenoids, proteins[21] and tannins[19]. According to Rasoanaivo *et al*, 20%, 60% and 20% of extracts from medicinal plants were considered active, weakly active and inactive respectively[14].

Biological and pharmacological activities that are attributed to different parts and extracts of *A. indica* include antiplasmodial, antitrypanosomal, antioxidant, anticancer, antibacterial, antiviral, larvicidal and fungicidal activities. Others include antiulcer, spermicidal, anthelminthic, antidiabetic, anti-implantation, immunomodulating, molluscicidal, nematicidal, immunocontraceptive, insecticidal, antifeedant and insect repellant effects[24]. Omar *et al* have demonstrated that the purified extracts of *A. indica* and other members of the Meliaceae family showed significant *in vitro*, but poor *in vivo* antimalarial activity[25]. The water extract of *A. indica* bark showed 66.47% reduction of parasitaemia in *P. berghei* infected Swiss Albino mice[26]. The water extract of *C. roseus* leaf showed antiplasmodial IC_{50} of (36.17±7.79) μg/mL against *P. falciparum* W2[27]. These research works support the present findings. The mechanism of action might be due to the inhibition of *P. falciparum* merozoite invasion into erythrocytes[28] and disruption of *P. falciparum* rosettes[29,30] by carbohydrates; inhibition of *P. falciparum* fatty acid biosynthesis[31-35]; inhibition of hemozoin biocrystallization by alkaloids[36] and inhibition of protein synthesis by triterpenoids[37]. It is concluded from the present study that the bark extract of *A. indica* (Abr. Juss) should be an enormous resource to find out the new drugs with antiplasmodial activities. Investigations are in progress to identify the active antiplasmodial compounds of *A. indica* (Abr. Juss) extracts by bioassay-guided fractionation.

Conflict of interest statement

We declare that we have no conflict of interest.

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