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In vitro antiplasmodial effect of ethanolic extracts of traditional medicinal plant *Ocimum* species against *Plasmodium falciparum*

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ABSTRACT

Objective: To identify the possible antiplasmodial compounds from leaf, stem, root and flower extracts of *Ocimum canum (O. canum), Ocimum sanctum (O. sanctum)* and *Ocimum basilicum (O. basilicum)*. **Methods:** The *O. canum, O. sanctum* and *O. basilicum* were collected from Ramanathapuram District, Tamil Nadu 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 *O. canum, O. sanctum* and *O. basilicum* were tested for antiplasmodial activity against *Plasmodium falciparum (P. falciparum)*. The potential extracts were also tested for their phytochemical constituents. **Results:** The leaf extract of *O. sanctum* showed excellent antiplasmodial activity (IC_{50} 35.58 μg/mL) followed by leaf extract of *O. basilicum* (IC_{50} 43.81 μg/mL). The leaf extract of *O. canum*, root extracts of *O. sanctum* and *O. basilicum*, the stem and flower extracts of all the three tested *Ocimum* species showed IC_{50} values between 50 and 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 *O. canum*, *O. sanctum* and *O. basilicum*. The *in vitro* antiplasmodial activity might be due to the presence of alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins in the ethanolic extracts of tested plants. **Conclusions:** The ethanolic leaf extracts of *O. sanctum* possess lead compounds for the development of antiplasmodial drugs.

1. Introduction

Medicinal plants constitute an effective source of both traditional and modern medicine. Studies on plant secondary metabolites have been increasing over the last 50 years. Moreover, pharmaceutical companies are utilizing such plant based formulations in treatment of various diseases and disorders worldwide[1]. The medicinal plant species *Ocimum canum (O. canum), Ocimum sanctum (O. sanctum)* (Holy Basil) and *Ocimum basilicum (O. basilicum)* belonged to Lamiaceae family are aromatic herb found throughout India. In traditional systems of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *O. sanctum* have been recommended for the treatment of bronchitis, bronchial asthma, malaria, diarrhoea, dysentery, skin diseases, arthritis, chronic fever and insect bite[2]. Scientifically, the *Ocimum* species have proved to posses various biological activities such as antibacterial[3,4], antifungal[5,6], antioxidant[7,8], antiviral[9] and anti-diabetic[10,11]. The emergence of strains of *Plasmodium falciparum (P. falciparum)*, resistant to chloroquine and many other drugs in succession has stimulated efforts to identify new antimalarial agents. Previous discoveries and bioactive potential of *Ocimum* species insisted us to choose *O. canum, O. sanctum* and *O. basilicum* for the present study. In this connection, the present study was made an attempt to explore the antiplasmodial potential of *O. canum, O. sanctum* and *O. basilicum* against *P. falciparum*.

2. Material and methods

2.1. Plant material

Fresh samples of different plant parts (leaves, stem, root and flower) from *O. canum* (Lat. 9°21′41″ N and Long. 78°49′45″ E), *O. sanctum* (Lat. 9°21′45″ N and Long. 78°49′41″ E) and *O. basilicum* (Lat. 9°21′43″ N and Long.

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78°49′43″ E) 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 yields were described 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 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 (−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 *Ocimum* species were screened for the presence of phytochemical constituents by following the standard methods[12]. 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[12].

2.3. Parasite cultivation

The antiplasmodial activity of 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)[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 at 5% and parasite cultures were used when they exhibited 2% parasitaemia[12].

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[13]. 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[13]. This activity was analyzed in accordance with the norms of antiplasmodial activity of Rasoanaivo *et al*[14]. 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[13].

3. Results

The percentage yields of crude extracts ranged from 2.42 to 16.79 (Table 1). It reveals that, leaf of *O. sanctum* (16.79%) showed maximum yield followed by leaf of *O. basilicum* (16.22%). The leaf extract of *O. sanctum* (IC_{50} 35.58 μg/mL) showed excellent antiplasmodial activity and followed by leaf extract of *O. basilicum* (43.81 μg/mL). Moreover, the root extract of *O. canum* 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 studies reveals that, the extracts from *Ocimum* species have variety of phytochemical constituents, namely alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins (Table 3).

Table 1

Percentage yield of ethanolic extracts from *Ocimum* species.

Botanical name	Plant part	Weight of plant part(g)	Yield of extract	
			(g)	%
O. canum	Leaf	102	15.62	15.31
	Stem	59	3.57	6.05
	Roots	58	1.68	2.90
	Flower	42	1.73	4.12
O. sanctum	Leaf	78	13.10	16.79
	Stem	49	2.85	5.82
	Roots	55	1.51	2.75
	Flower	40	1.64	4.10
O. basilicum	Leaf	82	13.30	16.22
	Stem	52	3.25	6.25
	Roots	60	1.45	2.42
	Flower	45	1.86	4.13

Table 2
IC_{50} value of *Ocimum* species extracts against *P. falciparum*.

Botanical name	Plant part	IC_{50} (μg/mL)
O. canum	Leaf	53.50
	Stem	63.19
	Roots	>100.00
	Flower	82.08
O. sanctum	Leaf	35.58
	Stem	53.50
	Roots	87.40
	Flower	71.91
O. basilicum	Leaf	43.81
	Stem	63.67
	Roots	78.69
	Flower	76.75
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 leaf extracts of *Ocimum* species.

Phytochemical constituents	Botanical name		
	O. canum	*O. sanctum*	*O. basilicum*
Alkaloids	+	+	+
Glycosides	+	+	+
Coumarins	–	–	–
Flavanoids	+	+	+
Quinones	–	–	–
Phenols	+	+	+
Saponins	+	+	+
Triterpenoids	+	+	+
Proteins	+	+	+
Resins	+	+	+
Steroids	+	+	+
Tannins	+	+	+

– : Absent; + : Present.

4. Discussion

Malaria is a major health problem in many developing countries. The drugs resistant *P. falciparum* causes the most virulent form of malaria in humans and it is described as a public health disaster causing increased morbidity and mortality. The development and spread of drug resistant strains of the causative agent *P. falciparum* have limited the effectiveness of the currently used malarial drugs. In Kenya, chloroquine has been discontinued as the first line treatment for malaria due to overwhelming presence of resistant *P. falciparum* strains[15]. This creates the urgent need for new antimalarial drugs. Plants have always been considered to be a possible alternative and rich source of new drugs, and most of the antimalarial drugs in use today such as quinine and artemisinin were either obtained directly from plants or developed using chemical structures of plant-derived compounds as templates[16]. *Ocimum* species provide a rich source of structurally diverse secondary metabolites. Several studies have demonstrated that, *Ocimum* species are an excellent source of components such as alkaloids, glycosides, flavonoids, phenols, saponins, triterpenoids, proteins, resins, steroids and tannins have exhibited different biological activities[17-19].

Studies on antiplasmodial activities with *Ocimum* species extracts are too limited; in this connection, the present study was investigated with different plant parts of *O. canum*, *O. sanctum* and *O. basilicum*. Among the tested extracts, *O. sanctum* showed IC_{50} value (35.58 μg/mL) at 1.9 fold concentration of positive control chloroquine. This might be due to the presence of glycosides[20], alkaloids[21], triterpenoids[22], flavonoids[23], phenols[12], saponins[23], proteins[24], resins[13], steroids[25] and tannins[12]. According to Rasoanaivo *et al*[14], 17%, 75% and 8% of extracts from selected *Ocimum* species were classified as active, weakly active and inactive respectively. The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *O. sanctum* possess moderate activity against the chloroquine-sensitive (3D7) strain of *P. falciparum*[26]. *O. bacilicum* and *O. suave* extracts showed good *in vitro* anti-malarial activity against the chloroquine-resistant strain of *P. falciparum*[27]. The methanolic leaf extract of *O. tenuiflorum* (IC_{50} 31 μg/mL) and *O. gratissimum* (IC_{50} 32 μg/mL) showed antiplasmodial activity against *P. falciparum*[28]. The methanol, water and dichloromethane extract of *O. americanum* and *O. gratissimum* showed antiplasmodial activity against chloroquine resistant strain W2 of *P. falciparum*[29]. The dichloromethane, dichloromethane/methanol (1:1), methanol and purified water extract of *O. americanum* showed antiplasmodial activity against *P. falciparum*[30].

The mechanism of action might be due to the inhibition of *P. falciparum* merozoites invasion into the erythrocytes[31] and disruption of *P. falciparum* rosettes[32] by the carbohydrates; inhibition of *P. falciparum* fatty acid biosynthesis[33], inhibition of hemozoin biocrystallization by the alkaloids[34], inhibition of protein synthesis by triterpenoids[35], inhibition of β-haematin formation[36], decreased mitochondrial membrane potential, DNA fragmentation and cytoplasmic acidification by steroids[37]. It is concluded from the present study that, ethanolic extracts from *Ocimum* species were shown for the first time to display *in vitro* antiplasmodial activity and warranted further investigation 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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