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Antiplasmodial potential of *Avicennia marina* (Forsk.) Vierh

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Abstract
Mangroves are enriched with various bioactive compounds is an inevitable fact. Almost all the mangroves exhibited good biological activities against human, animal and plant pathogens. In this study, the mangrove plant *Avicennia marina* is screened against *Plasmodium falciparum. Avicennia marina* (Forsk)Vierh. Is an evergreen mangrove tree found along tropical and subtropical coastlines. It is well known for being antibacterial, anticancer, antioxidant, antidiabetic and antiviral also. Leaves collected from this plant are subjected to hot continuous extraction by soxhlet type of extraction. Nine types of solvents were chosen from polar to non-polar for crude extraction. *Plasmodium falciparum* (3D7) strain is cultured according to Trager and Jensen method (1976). Among the nine solvents, a polar solvent Ethyl acetate shows 50% inhibition towards *Plasmodium falciparum* at IC_{50} Value- 50.82 µg/ml. From this study, it is observed that ethyl acetate extract of the plant *Avicennia marina* has antiplasmodial action against *Plasmodium falciparum*, which led to the development of a new antiplasmodial medication.

Keywords: Antiplasmodial action, *Avicennia marina*, *Plasmodium falciparum*, soxhlet extraction

1. Introduction
Mangroves are essential components of tropical intertidal forest communities, which are made up of woody trees and shrubs that grow along tropical coastlines of the world in the area between land and water [1]. The Indo-West Pacific region's subtropical and tropical zones are habitat to the mangrove tree *Avicennia marina* (Forssk.)Vierh, which is a member of the Acanthaceae family. It is regarded as a concise overview of mangroves that have been extensively studied for their therapeutic value [2]. Due to its remarkable resistance to environmental pressures, *A. marina* can thrive in adverse environmental conditions such gusts, anaerobic soil, extreme tides, and salt [3]. Flavonoids, tannins, steroids, saponins, alkaloids, glucosides, and triterpenoids are some of the bioactive components reported in *A. marina* [4]. For many ages, the conventional medical system has employed *Avicennia marina* [5].
The acute fever illness known as malaria is caused by *Plasmodium* parasites, which people get through bites from infected female Anopheles mosquitoes. The *Plasmodium falciparum* parasite is primarily responsible for virtually all malaria cases and fatalities, which are ascertained in the WHO African Region [6]. Natural substances, such as plant products, have contributed significantly to the development of new drugs during the past 50 years and have aided the pharmaceutical industry [7]. This study focused on the antiplasmodial properties of *Avicennia marina* leaf extracts that were extracted using nine different solvent types.

2. Materials and Methods
2.1 Collection of Plant material: The plant materials (Leaves) were gathered from T. S. Pettai, which is a part of the Pichavaram mangrove cover, on the South East coast of India, in the Tamilnadu region (Latitude 11.4110° N and Longitude 79.7954° E). A voucher specimen (Voucher No. 569) was stored at the Herbarium of the Botany Department at Annamalai University after the collected plant material was taxonomically identified. The derived plant materials are cleaned with tap water and then with distilled water to eliminate clinging salts and other related critters. For two to three weeks, the plant parts are dried in the shade.

2.2 Extract preparation: Shade-dried leaves are ground into a coarse powder (100 g), which is then exposed to hot continuous extraction using a soxhlet apparatus (in accordance with the solvent's boiling point). The solvents chosen for the extraction process include n-hexane, Diethyl ether, Ethyl acetate, chloroform, dichloromethane, acetone, n-butanol, ethanol, and methanol (300 ml).

2.3 *In vitro* culture of *Plasmodium falciparum*: For *in vitro* blood stage culture, the CQ-sensitive *P. falciparum* strain 3D7 was utilised to investigate the antimalarial potency of several plant extracts. The strain 3D7 was acquired at Jawaharlal Nehru University in New Delhi. With a few modest adjustments, Trager and Jensen's candle jar technique was used in order to maintain *Plasmodium falciparum* culture. Highly synchronised ring stage P. falciparum was employed to assess antimalarial activity. Fresh O+ve human erythrocytes were used to nourish the culture, which was then incubated at 37°C with a gas combination of 5% O2, 5% CO2, and 90% N2 while containing 0.2% sodium bicarbonate, 0.5% albumax, 2% glucose, 45 g/L of hypoxanthine, and 50 g/L of gentamicin (Himedia). Fresh O+ve human erythrocytes were used to nourish the culture, which thereafter underwent incubation at 37°C with a gas combination of 5% O2, 5% CO2, and 90% N2 while containing 0.2% sodium bicarbonate, 0.5% albumax, 2% glucose, 45 g/L of hypoxanthine, and 50 g/L of gentamicin (Himedia).

2.4 Antiplasmodial assay: In 96 well tissue culture plates with 100 µL of *P. falciparum* culture and fresh red blood cells diluted to 2% hematocrit, filter sterilised leaf extracts (200, 100, 50, 25, 12.5, 6.25 µg/mL) were added. Fresh red blood cells and 2% parasitized P. falciparum diluted to 2% hematocrit served as the negative control, while a culture of parasitized blood cells treated with chloroquine served as the positive control (8). Giemsa stain was used to assess parasitaemia after 48 hours, and the average % suppression of parasitaemia was computed using the formula below:

$$\text{Average \% suppression of Parasitemia} = \frac{\text{No.of Infected RBC}}{\text{Total No.of RBC}} \times 100$$

2.5 SYBR green assay: 100 µl of SYBR green dye has been added to each well following a 48-hour incubation period. This experiment is conducted in a light-sensitive region using 0.2 µl of SYBR green dye per ml of PBS lysis solution [10]. The reading was recorded under excitation at 480 nm and emission at 520 nm.

2.6 Antiplasmodial activity calculation and analysis: The inhibitory concentration 50 (IC50), or the amount of medication that reduced parasitaemia by 50% as compared to the positive control culture with 100% parasitaemia, served as a measure of the antiplasmodial activity of mangrove leaf extracts [11].

2.7 Statistical analysis: The IC50 values were determined using the linear regression equation and Graph Pad Prism software (concentration of extract in X axis and percentage of inhibition in Y axis).

3. Results and Discussion

In the present study, Nine types of crude extracts obtained from the plant *A. marina* showed some antiplasmodial activity towards 3D7 strain of *Plasmodium falciparum*. The inhibition percentage of the crude extracts is summarized in Table 1. The concentration of the extracts taken from 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml. From the above nine types of extracts, ethyl acetate extract showed 50% inhibition towards chloroquine sensitive 3D7 strain with the IC50 value 50.82 µg/ml (Table 2) and the Positive control chloroquine gives IC50 value 41.89±1.89 µg/ml.

Eight species, including variants and synonyms, belong to the genus *Avicennia*, which was the first prominent group of mangrove plant species with potent pharmacological activities. Many bioactive substances, including alkaloids, flavonoids, phenols, saponins, tannins, glycosides, and terpenoids, have been determined to exist. Hence, there is a lot of potentials to find new biologically active phytochemicals in the many *Avicennia* mangrove species [12]. *A. marina* aerial decoction has been reported to have anti-malarial properties [13]. Previously *A. marina* leaves were reported for antimalarial activity which shows 40.20 ± 0.163% of parasitemia inhibition towards *Plasmodium falciparum* [14]. The IC50 value of *A. marina* bark extract was 49.63 µg/ml followed by percolation extraction in ethanol and water mixture (3:1) [15]. Aqueous extracts of the *A. marina* plant seedlings' shoots and roots exhibited antiplasmodial activity [16]. The inclusion of significant chemical classes like phenols and coumarins may be the cause of the *A. marina* plant extract's *in vitro* antiplasmodial activity. Hence, coumarins are potent antiplasmodial substances [17].

Table 1: *In vitro* antiplasmodial activity of *Avicennia marina*

	6.25 µg/ml	12.50 µg/ml	25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml
Hexane	5.57±2.209	10.77±2.458	14.14±3.494	20.21±3.828	28.82±8.742	45.18±2.743
Diethyl ether	4.06 ±1.893	9.13±4.988	13.79±4.052	21.12±2.927	26.76±1.173	38.44±3.23
Ethyl acetate	31.99±1.724	35.36±3.863	39.02±1.749	50.96±0.375	59.89±0.473	62.28±1.457
Chloroform	5.55±1.504	6.81±2.212	10.29±2.315	15.50±3.771	19.87±3.011	33.60±2.476
Dichloromethane	11.45±0.768	14.78±3.333	16.09±3.087	19.08±1.702	21.95±2.621	25.53±4.099
Acetone	11.92±0.441	13.48±1.251	15.47±1.062	17.52±1.503	18.35±1.737	27.71±2.641
Ethanol	7.53±4.569	8.62±3.837	10.38±2.940	11.90±2.929	13.54±2.555	16.36±1.083
Methanol	6.99±1.60	10.55±2.352	16.45±1.231	18.29±2.529	19.59±2.443	23.25±2.121
Butanol	2.14±1.084	6.81±4.267	10.53±4.033	14.65±3.920	20.08±2.556	22.80±0.926

Table 2: IC50 values of Plant extracts

Solvents	IC_{50} Value(µg/ml)
n- Hexane	≥ 200
Diethyl ether	≥ 200
Ethyl acetate	50.82
Chloroform	≥ 200
*Dichlorometh*ane	≥ 200
Acetone	≥ 200
Methanol	≥ 200
Ethanol	≥ 200
Butanol	≥ 200
Chloroquinine	41.89±1.89

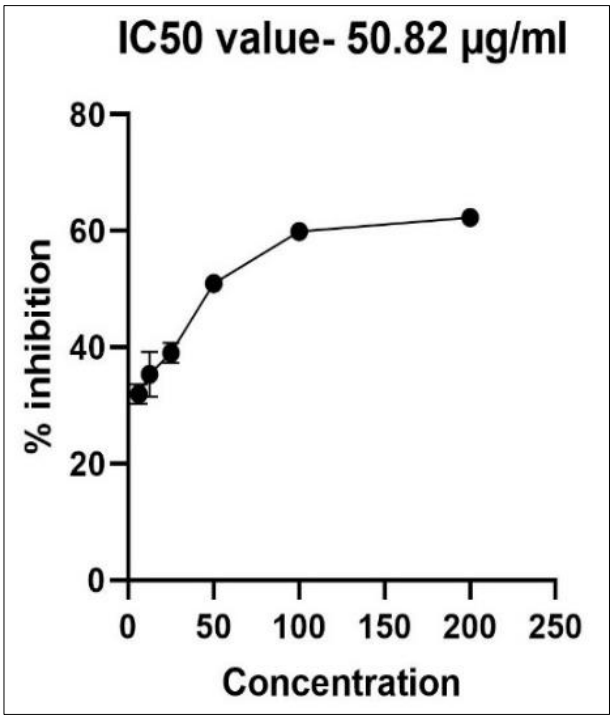

Fig 1: IC50 value of Ethyl acetate extract of *A. marina*

4. Conclusions

From the present investigation, the polar solvent Ethyl acetate shows good antiplasmodial activity with 50% inhibition towards chloroquine-sensitive *Plasmodium falciparum* when compared to all other polar and non-polar solvents. This study concluded that the plant *Avicennia marina*'s ethyl acetate leaf extract exhibits antiplasmodial activity against *Plasmodium falciparum*, which prompted the development of a novel antiplasmodial drug.

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