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Original Research Article

Antimalarial Activity of Crude Bark Extract of *Pterocarpus indicus* Willd. against *Plasmodium falciparum* Strain 3D7

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ABSTRACT

Malaria is a global concern, particularly in tropical regions. Numerous studies have been conducted on plants with antimalarial potential. This study aimed to establish the half-maximal inhibitory concentration (IC_{50}) of *Pterocarpus indicus* Willd. bark extract against *Plasmodium falciparum* strain 3D7. The extract was prepared by macerating the stem bark in three solvents (ethanol, ethyl acetate, and n-hexane). The antimalarial tests were performed using infected human erythrocytes. The Trager and Jensen method was used to culture *P. falciparum* parasites *in vitro*. A thin blood smear was prepared using 20% Giemsa dye to study parasite proliferation. Antimalarial activity tests were conducted on parasite cultures with 5% hematocrit and 1% parasitemia. The results revealed that the IC_{50} values of n-hexane, ethyl acetate, and 96% ethanol extracts were 7.14, 4.0, and 0.65 g/mL, respectively, indicating strong antimalarial activity. Alkaloids, flavonoids, tannins, phenols, and terpenoids are a few of the active chemical substances discovered in *P. indicus* Willd. Bark extract in this study. These findings suggest that the bark extract exhibits antimalarial properties even in the crude form. Thus, compounds isolated from *P. indicus* appear to be of particular importance for antimalarial research.

***Keywords*:** malaria, medicinal plants, *Pterocarpus indicus*, tropical disease

Introduction

Malaria is the most prevalent tropical illness affecting ~100 countries and 2.4 million people.[1,2,3] Every year, 100 million malaria cases are reported in Southeast Asia, with Indonesia accounting for 70% of these cases.[4] In the affected regions, chloroquine (CQ)-resistant *Plasmodium falciparum* strains have emerged owing to the widespread use of CQ against falciparum malaria. Moreover, resistance against other antimalarial medications has emerged.[5,6,7] The global emergence of drug resistance to most accessible and inexpensive antimalarial medications is concerning and necessitates the development of novel chemotherapeutics that can be easily stored and administered and are inexpensive. Traditional herbal remedies could be low-cost treatment alternatives. Plants are used to cure malaria in at least three continents: various countries in Africa, the Americas, and Asia.[8]

Pterocarpus indicus is a tropical plant that can reach a height of 15 m and is used as a traditional medicinal plant[9,10] to treat cancer sores, diuresis, sore throat, wounds, and minor injuries. It is also used for its antimalarial, antidysenteric, antidiarrheal, astringent, and laxative effects as well as a mouthwash. The leaf, wood, bark, and root of *P. indicus* can be used in the form of a decoction or crude extract to treat various common diseases, such as boils, prickly heat, bladder stones, diarrhea, dysentery, canker sores, and syphilis wounds.[11] Several studies have examined the medicinal properties of *P. indicus* as it contains antibacterial chemical components such as alkaloids, flavonoids, saponins, tannins, and triterpenoids.[12,13]

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Indonesia's status as a tropical country with megabiodiversity does not exempt it from the threat of endemic diseases, one of which is malaria. The bite of a female Anopheles mosquito can transmit the potentially fatal malarial parasite to humans. In 2018, ~228 million cases were reported globally with 405,000 fatalities. The most vulnerable age group to malaria infection is children <5 years of age, accounting for 67% (272,000) of all malaria-related deaths globally.[4] The mosquito species *P. falciparum*, *P. ovale*, *P. malariae*, *P. knowelesi*, and *P. vivax* often infect people. *P. falciparum* is the most hazardous of the four species because it produces various significant sequelae, including cerebral malaria, severe anemia, kidney failure, and other life-threatening disorders.[14]

In every malaria-endemic area, the *P. falciparum* parasite has developed and adapted, resulting in new strains with improved mosquito-infecting potential and resistance to medication. *P. falciparum* strain 3D7 is an adaptable strain that originated in Africa.[15] *P. falciparum* strain 3D7 is a CQ-sensitive isolate that is frequently utilized in antimalarial research. In some endemic locations, malaria therapy with CQ and an antifolate (sulphadoxine–pyrimethamine) is no longer effective.[16] *P. falciparum* can develop resistance to various antimalarial medicines, including CQ. Therefore, people living in endemic areas are turning to medicinal plants for treating malaria.[17]

Various solvents, including n-hexane, ethyl acetate, and ethanol, have been employed for macerating bark during extraction. An ethanolic extract of the bark of *Pterocarpus erinaceus* exhibited good antimalarial activity with an half-maximal inhibitory concentration (IC_{50}) of 1.27 g/mL.[18] Alkaloids, anthraquinones, flavonoids, and terpenoids are also believed to possess antimalarial activity.[19] The bark of *P. indicus* contains terpenoids, which can inhibit bacteria by lysing bacterial cell membranes.[20] *P. indicus* is considered a beneficial medicinal plant owing to the wide range of phytochemicals it contains.[21,22] However, studies regarding the antimalarial properties of *P. indicus* bark extract remain scarce. The pharmaceutical sector, particularly the herbal medications sector, can benefit from the findings of this study.

Materials and Methods

Collection of plant materials

The bark of *P. indicus* (Figure 1) was acquired from the Husada Graha Famili Park in Wiyung, Surabaya, Indonesia (7°18'12.6"S 112°41'12.7"E, altitude 10 m) and used for the extraction. The plant material was collected in March 2021 with the voucher number PBS-2021-3. The taxonomic identification was performed by Dr. Rony Irawanto, Purwodadi Botanical Garden, Pasuruan, Jawa Timur, Indonesia.

P. falciparum culture test material

P. falciparum strain 3D7 (CQ-sensitive) used in the *in vitro* antimalarial activity test was cultured at the Institute of Tropical Disease, Airlangga University. Fresh blood (packed red cell) and plasma (fresh frozen plasma) were obtained from individuals with O positive blood type from the Indonesian Red Cross in Surabaya, East Java, for propagating *P. falciparum*. Aquadest, HEPES buffer, RPMI 1640, sodium bicarbonate, gentamicin, plasma and human erythrocytes, dimethyl sulfoxide, Giemsa dye 20%, methanol, and immersion oil were used to prepare *P. falciparum* culture medium and *in vitro* test solutions.

Preparation of n-hexane, ethyl acetate, and ethanol extracts

The collected stem bark of *P. indicus* was cleaned, dried under shade at room temperature, and ground to powder using a mechanical grinder. The bark extracts were prepared using n-hexane, ethyl acetate, and ethanol. Maceration was performed in three steps: commencing with a nonpolar solvent (n-hexane), followed by a semipolar solvent (ethyl acetate), and finally a polar solvent (ethanol). Next, 500 g of the simplicial powder was soaked in 5 L of n-hexane and agitated every 24 h in a covered container. Then, the extract was filtered using a muslin cloth followed by Whatman filter paper number 1 using a Buchner funnel, concentrated using a rotary evaporator, and aerated until the solvent evaporated completely. Subsequently, the residue was remacerated and the procedure was repeated thrice to exhaustively extract the compounds from the plant material. The filtrates obtained from the successive maceration were dried under reduced pressure using a rotary evaporator at 40°C. The dried crude extract was then left in the desiccator for 24 h to dry into a powder. The dried crude extract was stored in a labeled glass bottle in the refrigerator at 4°C until further use.[26,43] Each solvent was steeped for 3 days before being tested.

Preparation of P. falciparum culture media

In vitro culture of *P. falciparum* was performed according to the Trager and Jensen method.[23]

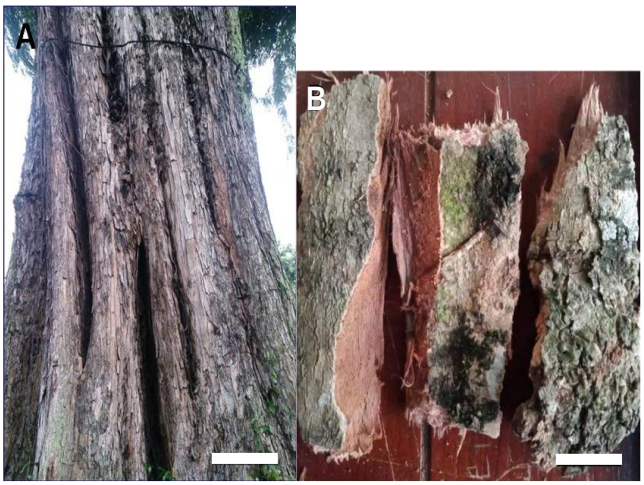

Figure 1: *P. indicus* bark as it appears while still attached to the tree (A), bar=12 cm. Pieces of tree bark that has been removed (B), bar=1.5 cm

Antimalarial test preparation

Preparation of human erythrocytes

To prepare human erythrocytes, 7.5 mL of blood and 7.5 mL of complete media were mixed in a sterile Falcon tube and centrifuged for 5 min at 1,500 rpm (the process was repeated thrice). This procedure is known as blood washing. RBC 50% was prepared by mixing cleansed blood with the same amount of complete medium until the erythrocyte concentration reached 50%.

Preparation of fresh plasma

To prepare fresh human plasma, 50 mL of fresh whole blood was centrifuged for 10–15 min at 3,000 rpm. The yellow–brown layer of plasma on top was removed (supernatant). The plasma was activated via incubation at 56°C for 30 min. Then, 15 mL activated plasma was extracted and placed in a Falcon tube that was sealed. To obtain fibrin, plasma was centrifuged at 1,500 rpm for 5 min.[23]

Observation of P. falciparum growth

A thin blood smear was prepared to monitor parasite growth. One drop of parasite cell suspension was placed on an object glass, covered with a cover slip, air-dried, fixed in 100% methanol, and air-dried again. Next, the thin blood smear was stained using Giemsa dye (20%) in Aquadest for 20 min, washed with water, and air-dried. To calculate percent parasitemia, the smear was inspected under a microscope at 10 × 100 magnification.

Antimalarial activity test

Preparation of test parasite cell suspension

For the *in vitro* antimalarial activity test, each well of a 24-well plate was filled with 1% parasitemia and 5% hematocrit. The suspension in a Petri dish (5 mL) was transferred to a sterile 15-mL capped Falcon tube and centrifuged for 5 min at 1,500 rpm. In total, 4.5 mL of the supernatant was discarded, indicating that 5% of the parasites were present in the erythrocytes and 50% of hematocrit, resulting in a total volume of 5 mL. A suspension of parasite cells with 1% parasitemia and 10% hematocrit was prepared by pouring 200 mL of 50% RBC solution into a tube, adding 10 mL of complete medium, and gently mixing the suspension with a micropipette. A thin blood smear was prepared as D0, which is the initial parasitemia level at 0 h before adding the test material and being placed into the microwell. Each well containing 500 mL of the test solution received 500 mL of the parasite cell suspension. The volume of the prepared cell suspension was sufficiently large to fill 24 wells.[23, 42]

Test sample preparation

P. indicus bark extracts obtained using n-hexane, ethyl acetate, and 96% ethanol were used. A concentration of 100, 10, 1, 0.1, and 0.01 ppm was used for each sample. Each extract was weighed at a concentration of 10 mg and then diluted in 100 mL dimethyl sulfoxide (100,000 g/mL). Then, 10 mL of the 100,000 g/mL main liquor sample was mixed with 490 L of complete medium (2000 g/mL). In total, 120 mL of the sample main liquor was pipetted into a microwell followed by 1,080 mL of complete medium (200 g/mL). A duplicate was prepared by pipetting 500 mL of the 200 g/mL solution and repeating the process. This solution was placed in the well below along with 500 mL parasitic suspension in each well. This solution is referred to as D1 (100 g/mL). Then, 120 L of 200 g/mL mother liquor sample was pipetted into a microwell and mixed with 1,080 L of complete media (20 g/mL). A duplicate was prepared by pipetting 500 mL of the 20 g/mL solution into the well below followed by the addition of 500 mL parasitic suspension into each well. This solution is referred to as D2 (10 g/mL). In total, 120 L of the main liquor sample (20 g/mL) was pipetted into a microwell and mixed with 1,080 L (2 g/mL) of complete media. A duplicate was prepared by pipetting 500 mL of the 2 g/mL solution into the well below and each well below it followed by 500 mL of the parasite suspension. This solution is called D3 (1 g/mL). Then, 120 L of the main liquor sample (2 g/mL) was pipetted into a microwell and mixed with 1,080 mL of complete media (0.2 g/mL). A duplicate was prepared by pipetting 500 mL. The 0.2 g/mL solution was then added to the well below. Then, 500 L of the parasite suspension was added to each well. This solution was

called D4 (0.1 g/mL). In total, 120 L of the mother liquor sample (0.2 g/mL) was pipetted into a microwell and mixed with 1,080 mL of complete media (0.02 g/mL). A duplicate was prepared by pipetting 500 mL of the 0.02 g/mL solution into the well below. Then, 500 mL of the parasite suspension was added to each well. This solution was referred to as D5 (0.01 g/mL).

Data analysis

Parasitaemia percentage was calculated by counting the number of erythrocytes with malarial parasites per 1,000 erythrocytes. The following calculation yields percentage growth:

%Growth = %Parasitemia Each Test − Mean% of Parasitemia D0

Percent inhibition was calculated using the formula:

$$\%\text{Inhibition} = 100\% - (Xu/Xk) \times 100\%$$

Xu = test solution growth percentage

Xk = percentage of growth in negative control

IC_{50} Calculation

IC_{50} value is a sample concentration that inhibits parasite growth by 50%. The linear regression line equation is used to calculate IC_{50} values via Probit (probability unit) analysis, which involves drawing a curve representing the relationship between percent inhibition and the logarithm of sample concentration.

Result and Discussion

Herein, *in vitro* antiplasmodial activity was indicated by the proportion number of parasitemia (Table 1). The three different types extraction solvents used in this study demonstrated different effects on the average parasitemia proportion against *P. falciparum*. In contrast to other sample concentrations, at a concentration of 100 ppm, the proportion of parasitemia was the lowest in the n-hexane (0.76%) and ethyl acetate (2.34%) solutions. Thus, these solutions exhibit the greatest impact on lowering parasitemia. The outcomes of each treatment appeared to be better than that of the negative control (8.19). These findings reveal that with the exception of treatment with the concentration of 0.001 g/mL, all the other treatments performed in this study were successful in significantly decreasing plasmodial activity. These findings together with those for *P. erinaceus* support the plant's antimalarial activity.[24]

According to Table 2, the extraction solvents affected the growth percentage of *P. falciparum* following the administration of *P. indicus* bark extract and 48 h of incubation. Overall, the growth percentage of *P. falciparum* treated with the extracts was smaller than that of *P. falciparum* treated with the negative control. The combination of 100 ppm n-hexane extract and 96% ethanol exhibited the highest level of antimalarial activity, with 100% parasite growth suppression compared with that of 0% in the negative control group. The primary and preferred solvent in conventional settings is water. This is not surprising as some previous studies involving other plants reported similar patterns of crude aqueous plant extracts being less potent than their corresponding organic extracts.[25,26]

Table 3 displays the findings of the *P. falciparum* inhibition test. Treatment with 100 ppm ethyl acetate and 96% ethanol extract caused respective inhibitions of 100% and 79.2%. The two crude extracts are equally powerful and can be used to inhibit *P. falciparum*, which is the best outcome when compared with that of other therapies. Treatment with 96% ethanol at a concentration of 0.001 ppm exhibited the lowest inhibition (1.48%), which was close to the inhibition produced by the negative control. These outcomes are consistent with the results of studies that used *Annona muricata* and *Caesalpinia pulcherrima* extracts.[27,44]

A summary of the IC_{50} values of the different treatments are provided in Table 4. The following conditions are listed as the criteria for determining the level of activity: very active (IC_{50} < 5 g/mL), active (IC_{50} 5–15 g/mL), less active (IC_{50} 16–30 g/mL), and inactive (IC_{50} > 30 g/mL).[28] The 96% ethanol extract treatment exhibited the highest level of activity (very active) (IC_{50} value 0.65). The IC_{50} value for n-hexane was 7.14, indicating that it is active. *In vitro*, the IC_{50} values for the *P. indicus* stem bark extract against *P. falciparum* strain 3D7 varied between n-hexane, ethyl acetate, and 96% ethanol. The active chemical content of the various plants, which was extracted using various solvents, is responsible for the variation in the antimalarial activity results.[29]

Table 1: Average percent of parasitemia levels in the *in vitro* antimalarial activity test against *P. falciparum*.

Sample concentration (µg/mL)	Extract solvent type		
	n-hexane	**Ethyl acetate**	**Ethanol 96%**
D_0	0.78	0.78	0.78
Negative control	8.19	8.19	8.19
100	0.76	2,34	-
10	4.37	3.85	2.36
1	5.77	5.48	4.33
0.,1	7.18	6.7	5.95
0.01	7.95	7.87	7.05
0.001	-	-	8.08

Table 2: Average percentage growth of *P. falciparum* after administration of *P. indicus* bark extract and 48 h of incubation.

Sample concentration (µg/mL)	Extract solvent type		
	n-hexane	**Ethyl acetate**	**Ethanol 96%**
Negative control	7.41	7.41	7.41
100	0	1.56	0
10	3.59	3.07	1.58
1	4.99	4.7	3.55
0.1	6.4	5.92	5.17
0.01	7.17	7.09	6.27
0.001	-	-	7.3

In fact, a number of factors affect how differently natural ingredient extracts can inhibit the activity of microorganisms. The type of solvent used, the volume solvent used compared with the extraction material volume, temperature, pressure during extraction, and plant bioactive components are all factors that affect the extraction process. Herein, the polarity of the organic solvents used in the maceration process, such as 96% ethanol (polar), ethyl acetate (semipolar), and n-hexane (nonpolar), affected the extraction outcomes.[30,31] The maceration technique was selected because it can remove active chemicals via immersion without heating, which further protects against heat resistance and harming unstable substances. By dissolving the cell walls of the cells containing the active ingredient in an immersion system, the solvent can reach the active ingredient.[32]

Herein, the ability of *P. indicus* stem bark extract to inhibit the growth of *P. falciparum* was considered active and very active. *P. indicus* bark extract demonstrated better *P. falciparum* inhibition than that exhibited by *P. erinaceus* leaf extract[18], which showed moderate or less potent inhibition (IC_{50} value = 16–30 g/mL). Additionally, *P. erinaceus* bark extract did not exhibit antimalarial action. The active component is more soluble in n-hexane than ethyl acetate and 96% ethanol, according to the IC_{50} of the n-hexane fraction, which was higher than that of the ethyl acetate and 96% ethanol extract. It is possible that separate molecules are responsible for the antimalarial activity or that the active ingredients have similar solubilities in water and chloroform. The alkaloids are separated as they are potential antimalarial medicines and depending on pH, they can be soluble in organic solvents and water.[33,34,35]

Using natural remedies is currently the best alternative course of action. Understanding how ethnobotany is used therapeutically is crucial. Globally, >80% people use herbal and ethnomedical plants as a form of therapy and medicine.[36] Owing to its affordability and accessibility, ethnomedicine is frequently seen as the primary solution for treating illness in Africa and many other developing nations.[37] Plants are a key source of treatment for malaria in sub-Saharan Africa, where there are limited medical facilities.[38] Since ancient times, ethnomedical plants have played a crucial role in treating malaria.[39,40,41]

Traditional medicines are frequently used to treat malaria and are more accessible and less expensive than Western treatments, although they do have some drawbacks. Unpredictable efficacy, vague dosage recommendations, and unidentified short- and long-term safety effects are a few of the shortcomings. Additionally, this study only provided vague and limited details regarding the preparation of the medicine, thereby rendering replication challenging. The separation of these substances from plants is crucial and particularly worrying in light of the development of potent new antimalarials. Therefore, more research, including current clinical trials, is warranted before widely recommending such treatments.

Conclusion

These results indicate the potential developing of *P. indicus* into a new antimalarial drug candidate. However, determining the compounds and transmission-blocking strategies underlying the malaria control of *P. indicus* extracts is essential for further study. Thus, isolating compounds from these plants is important for identifying effective antimalarials. Isolating bioactive components from medicinal plants based on traditional use or ethnomedical data is highly promising for discovering effective antimalarial medication. In this regard, the highly bioactive *P. indicus*, which is widely grown in Indonesia, can potentially be a source for less expensive pharmaceutical alternatives.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 4: IC_{50} value of antimalarial activity of *Pterocarpus indicus* bark extracts in various solvents

Extract solvent type	IC_{50} (µg/mL)	Category
n-hexane	7.14	Active
Ethyl acetate	4	Very active
Ethanol 96%	0.65	Very active

Classification of antiplasmodial activity[28]

IC_{50} value (µg/mL)	Category activity
>30	Inactive
16–30	Less active
5–15	Active
<5	Very active

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