In vitro Anti-malarial Activity of Rhizome Extracts of Curcuma species

Prasanthi Donipati[1]*, Dr.S.Harasreeramulu[2], Prasanna Jyothi Donipati[3]

[1*]Research Scholar, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India - 530 013. Ph: +91 9885653508, E-mail: prashanthi.christopher@gmail.com

[2] Professor & Head of the Dept. of Biotechnology, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India-530 013. Ph: +91 9556533105, E-mail: dr.shsr@gmail.com

[3] Research Scholar, Centre for Biotechnology, Department of Chemical Engineering, A U College of Engineering, Andhra University, Visakhapatnam, A.P, India Ph: +91 8500308242, E-mail: prasannajyothidonipati@gmail.com

Corresponding Author:- Prasanthi Donipati

[*]Research Scholar, Dr.V.S.Krishna Govt. College, Visakhapatnam, A.P, India - 530 013. Ph: +91 9885653508, E-mail: prashanthi.christopher@gmail.com

Abstract

Anopheles mosquitoes carry the deadly parasite illness malaria, which kills thousands of people annually in many tropical and subtropical areas. This study set out to evaluate the effectiveness of chloroquine in treating Curcuma species malaria caused by *Plasmodium vivax*. In this investigation, the malaria pathogen *Plasmodium vivax* was employed. In an incubator with 5% CO2 and 37°C, parasitemia was cultivated in RPMI 1640 culture media supplemented with 10% human serum and 2µg/ml of gentamycin. In order to evaluate plant extracts' ability to combat malaria, we employed the susceptibility micro assay method. Significant parasitaemia inhibitions were demonstrated by ethanolic extracts of *Curcuma longa*, *Hedychium coronarium*, and *Curcuma caesia* against blood stage chloroquine-resistant *P. vivax*, with low cytotoxic effects to parasitaemia cells in vitro. These inhibitions ranged from 5.8-75.6%, 2.2-29%, and 2-29.8%. The growth of *P. vivax*, which is resistant to chloroquine, was effectively inhibited by extracts derived from six different plant species. The results supported the fractionation of these plants using a bioassay to identify strong anti-malarial chemicals or to create standardised extracts that could strengthen the anti-malarial impact *in vitro*.

Key words: Anti-malarial, Chloroquine, *Plasmodium vivax*, Curcuma species, Parasitaemia inhibitions.

Introduction

More illnesses than any other category of arthropods may be spread by mosquitoes, which have an impact on millions of people worldwide. Mosquitoes are considered the "public enemy number one" by the WHO [1]. It is brought on by a number of factors, including a shortage of new insecticides, the high price of synthetic pesticides, the need to protect the environment, negative effects on human health and other non-target populations, the fact that these pesticides are not biodegradable, a higher rate of biological magnification through ecosystems, and an increase in insecticide resistance globally [2, 3]. Historically, the primary source for novel anti-malarial medications has been plants. Thirty-three tropical nations had published 1277 plant species from 160 families for use in treating fevers and malaria up until 2003 [4].

Several nations in Africa, the Americas, and Asia are among the three continents where plants are used as a malaria treatment [5].The rhizome of *Curcuma longa* has long been used as an insect repellent and antibacterial [6]. Given the botanical origins of many contemporary medications, including quinine and artemisinin, it is imperative to explore other medicinal plants with a folkloric reputation for anti-malarial properties. This will help to ascertain the plants' safety and efficacy as well as their potential as sources of novel anti-malarial medications [7].

All believed that Chloroquine was a reliable treatment for acute vivax malaria, with a clinical and parasitological cure. But since then, Indonesia and western Oceania have reported substantial rates of chloroquine resistance [8–10]. This method has led to the development of a more complex evaluation methodology for morphological growth assessment in *P. vivax* drug-sensitivity tests, which eliminates the need for perfectly synchronised samples [11]12]. In this work, the anti-plasmodial efficacy of six traditional medicinal herbs (Table 1) against the chloroquine (CQ)-resistant *P. vivax* malaria parasite was evaluated in vitro.

Scientific Name (Species)	Family Name	Local Name	Common Name	Parts Used
Curcuma longa	Zingiberaceae	Pasupu	Turmeric	Rhizomes
Curcuma amada	Zingiberaceae	Mamidi Allam	Mango Ginger	Rhizomes
Curcuma caesia	Zingiberaceae	Nalla Pasupu	Black Turmeric	Rhizomes
Hedychium coronarium	Zingiberaceae	Dumpa Rashtram	Ginger Lilly	Rhizomes
Curcuma zedoaria	Zingiberaceae	Kakoramu	White Turmeric	Rhizomes
Curcuma aromatic	Zingiberaceae	Kasthura Pasupu	Aromatic Turmeric	Rhizomes

Table-1: Medicinal plants in Zingiberaceae with traditional claims

Materials and methods

Collection of plants and Extract preparation

The plant samples employed in this study were gathered in the Andhra Pradesh cities of Gudala, Allavaram, and Amalapuram. After being freshly harvested, the plant components were dried in the shade and ground into a coarse powder. Rota-vapor was used to concentrate the powdered material to dryness under vacuum after it had been extracted using ethanol for six hours in a Soxhlet system.

Parasite culture

When a human host is having a blood meal, an infected Anopheline mosquito injects sporozoites into the body, causing malaria infection. Following injection, sporozoites enter the circulation and travel to the liver, where they undergo asexual multiplication in the erythrocytes (erythrocytic schizogony) and early replication (exo-erythrocytic schizogony) in the liver. Merozoites are discharged from schizonts, where they infiltrate hepatocytes and transform into exo-erythrocytic forms. In this investigation, the malaria pathogen *Plasmodium vivax* was employed. In RPMI 1640 culture medium (including 10% human serum and 2µg/ml of Gentamycin), parasitemia was cultivated at 37°C in an incubator with 5% CO2.

Determination of in vitro Anti-plasmodial Activity of Extracts

The anti-malarial efficacy of plant extracts was evaluated using the susceptibility micro assay method [13][14]. Using the Trager W. Jensen technique, *Plasmodium vivax* has been maintained in culture continuously for these tests [15]. To make stock solutions (5 mg/ml with 1% ethanol), plant extracts were dissolved in ethanol and diluted with RPMI 1640 culture medium containing 10% human serum. After sterilising these solutions, a range of concentrations including 400, 200, 100, 50, 25, 12.5, 6.25, 3.125, and 1.562 µg/ml were prepared. As a control, chloroquine was utilised, and negative controls were made with 0.1% ethanol and the culture media. 100µl of 2% parasitemia and 50µl of test sample were added to each microplate test well. For each parasite line, three duplicate experiments were performed; thick films were made, stained with Giemsa to demonstrate schizont development, and parasitaemia was measured using light microscopy. The microtitre plates were then kept in a CO2 incubator at 37 °C for 48 hours. screened 200 visible fields per slide, or 25 microscopic fields per well. Based on an anticipated 200 RBCs per microscope field,

the percentage of parasitaemia may be computed using 40,000 RBCs per culture:

$$\frac{\text{\% of Parasitemia in control - \% of Parasitemia in sample}}{\text{\% of Parasitemia in control}} \times 100$$

The linear interpolation between drug concentration measurements was used to estimate the concentration that inhibited growth by 50% (IC50) when compared to control wells [16].

Results

Anti-malarial activity of plant extracts in combination with Chloroquine

When examined under a microscope, the ring stage was the most common stage discovered. The standard chloroquine dosage (without serum added) was found to have an average parasitaemia of 48.5% (Table 2), but the Curcuma species with the highest percentage of parasite invasion inhibition had the lowest parasitaemia, 24.4%, from *Curcuma caesia*. *Curcuma amada* (0.8%) showed the lowest parasitaemia inhibition at the normal Chloroquine (control) concentration, whereas *Curcuma caesia* (5.8%) showed the maximum level of parasitaemia inhibition at the lowest Conc. of 1.562. *Curcuma caesia* (75.6%) showed the maximum parasitaemia inhibition at the standard Chloroquine (control) concentration, whereas *Hedychium coronarium* (29%), exhibited the lowest parasitaemia inhibition, was found to have the highest Conc. of 400.

Anti-malarial activity of plant extracts alone

Figure 1 and Tables (Tables 2 and 3) provide a graph that plots the percentage inhibitory activity of Curcuma species extract against the parasitized red blood cells of malaria patients infected with P. vivax. Significant parasitaemia inhibitions were seen in ethanolic extracts from six species of Curcuma, with the ranges being 5.8-75.6%, 2.2-29%, and 2-29.8%. They are *Curcuma longa*, *Curcuma caesia*, and *Hedychium coronarium*. However, the non-significant parasitaemia inhibitions of other extracts, such as *Curcuma amada*, *Curcuma zedoaria*, and Curcuma aromatic, ranged from 0.8-29.1%, 1.8-30%, and 1-58%. *Curcuma caesia* is the only species of Curcuma extract that shown persistent suppression of parasitaemia.

Table-2: Dose response on *C. caesia*, *C. amada* and *C. longa*

	Standard(Chloroquine)		***Curcuma caesia***		***Curcuma amada***		***Curcuma longa***	
Conc. In µg/ml	% of Parasitemia	% of Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition
1.562	48.5	51.5	94.2	5.8	99.2	0.8	98	2
3.125	36	64	90.1	9.9	97.1	2.9	94	6
6.25	28.1	73.9	83.3	16.7	94.4	5.6	91.2	8.8
12.5	11.8	88.2	71.7	28.3	89.3	10.7	87.1	12.9
25	3.3	96.7	68.5	31.5	85.5	14.5	86.2	13.8
50	1.2	98.6	50.1	49.9	81.1	18.9	79.2	19.8
100	0.75	99.25	46.5	53.5	77.5	22.5	75.3	24.7
200	0.01	99.89	38.2	61.8	75.2	24.8	71.2	28.8
400	0	100	24.4	75.6	70.9	29.1	70.2	29.8

Table-3: Dose response on *H. coronarium*, *C. zedoaria* and *C. aromatic*

	Standard (Chloroquine)		***Hedychium coronarium***		***Curcuma zedoaria***		***Curcuma aromatic***	
Conc. In µg/ml	% of Parasitemia	% of Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition	% Parasitemia	% Parasitemia Inhibition
1.562	48.5	51.5	97.8	2.2	98.2	1.8	99	1
3.125	36	64	95.1	4.9	94	6	98	2
6.25	28.1	73.9	96	4	95	5	94	6
12.5	11.8	88.2	89	11	91	9	84	16
25	3.3	96.7	85	15	87.4	12.6	80	20
50	1.2	98.6	81.2	18.8	83.1	16.9	73	27
100	0.75	99.25	78	22	80	20	67	33
200	0.01	99.89	74	26	75	25	59	41
400	0	100	71	29	70	30	42	58

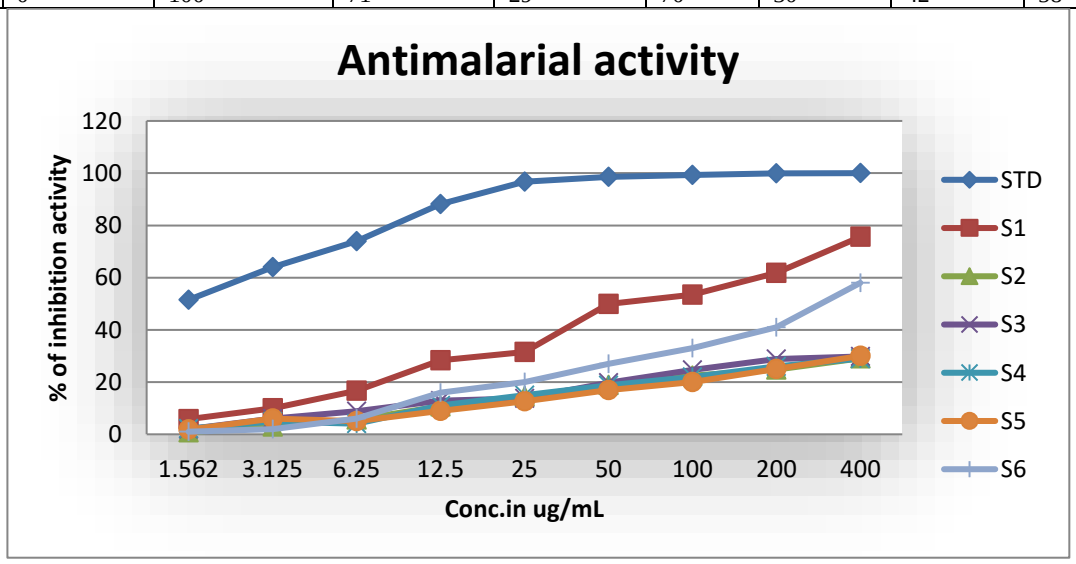

Figure-1: Percentage inhibition activity of *Curcuma species* Extracts on Parasitized RBC of P. vivax infected malaria patient. Key: STD –Chloroquine, S1- *Curcuma longa*, S2- *Curcuma amada*, S3- *Curcuma caesia*, S4- *Hedychium coronarium*, S5- *Curcuma zedoaria*, S6- *Curcuma aromatic*.

Discussion

All over the world, malaria is a disease mostly seen in tropical regions and caused by Plasmodium species, blood parasites. These parasites are spread to humans by the female anopheles mosquito. More people die from malaria than from any other infectious illness in the world. The need for a malaria vaccine is critical since medication resistance, insecticide-resistant insects, inadequate management methods, illness recurrence, and rising tourism are all contributing to the disease's increasing worldwide burden. Clinical experiments on potential malaria vaccines have shown dismal results, including low titers and poor effectiveness. This area accounts for nine out of 10 occurrences of the illness, which results in over a million fatalities yearly [17]. Pregnant women and children have a high death rate. P. vivax malaria is seldom fatal, but it causes significant morbidity, particularly in children and pregnant women; low birth weight has been linked to the infection [18].

The recommended treatment for *P. vivax* malaria is chloroquine, yet resistance to this medication has been shown in a number of countries recently. The first reports of *P. vivax* resistance to chloroquine date back to 1989 [19], as well as from India [20], [21]. Our goal was to gather more information on *P. vivax's* therapeutic response to chloroquine in order to evaluate the medication's continued sufficiency. Giemsa 10% (pH 7.2) was used to stain thick and thin streaks for 20 minutes. *Plasmodium vivax* was identified using a thin smear. Separate samples were collected on Whatmann N_3 filter paper for genetic analysis and chloroquine assays. The samples were then air-dried and kept at room temperature in sealed bags in the dark.

Using 2.0 ml perchloric acid (0.3 M), 1.0 ml acetonitrile, and 5.0 ml phosphate buffer (pH 2; 0.05 M) containing internal standard, the blood spots were extracted for about an hour. Positive *P. vivax* cases should receive treatment with chloroquine at the full therapeutic dose of 25 mg/kg spread over three days. Relapses of vivax malaria are caused by the liver's hypnozoites. In India, the recurrence rate for vivax malaria is around 30%. Primaquine may be administered under supervision for 14 days at a dose of 0.25 mg/kg per day in order to prevent it.

Pregnant women, newborns, and those with G6PD deficiency should not use primaquine. If the patient has any side effects, such as dark urine, yellow conjunctiva, bluish staining of the lips, nausea, vomiting, or stomach discomfort, he should be urged to cease taking primaquine right once and contact his doctor right away. As previously demonstrated, growth inhibition was estimated and parasitemia in all groups was tracked. The weight and parasitaemia standard deviation data were calculated using Microsoft Excel® 2002. Graph Pad Prism 4 was used to calculate the percentage of parasitaemia in relation to the number of days after infection. The variations in techniques employed by various laboratories may also account for the discrepancy in Chloroquine levels compared to those reported in other investigations.

Using the susceptibility micro assay method, the current study has determined which plant extracts have anti-plasmodial efficacy. In their ethanolic extracts, six plants (79%) were shown to have potential anti-malarial effects. The extraction of particular classes of phytochemical elements from non-polar molecules into polar compounds is the theoretical goal of this extraction approach [22]. From an alternative perspective, the majority of these plants also showed at least 1% of CQ anti-plasmodial activity, suggesting that they might serve as a source of prospective anti-malarial drugs.

With *P. vivax* that was resistant to chloroquine, three medicinal plant extracts shown clear anti-plasmodial action in this investigation. *Curcuma longa*, *Curcuma caesia*, and *Hedychium coronarium* are these. The anti-parasiticidal qualities of Curcuma plant species, including *C.* zedoaria, *C. longa, C. aromatica*, and others, have been well investigated [23]. Table 2 displays the outcomes of the *in vitro* studies conducted using plant extracts against *P. vivax* that is resistant to chloroquine.

Conclusion

This study has made an effort to highlight medicinal plants that are allegedly utilised in or linked to the treatment of malaria. These therapeutic plants may have unidentified anti-malarial qualities that might be used as a model to create an affordable anti-malarial medication. Chloroquine is still the first-line treatment for *P. vivax* infections in this region, but in order to manage the current malaria burden in endemic areas, alternate approaches like insecticide spraying, insecticide-treated bed nets, long-lasting insecticidal nets, and combination drug therapies should be utilised to the fullest extent possible. Regular monitoring is required to detect further development of parasite resistance.

Malaria is a major parasite illness spread by vectors that is a major public health concern worldwide. It is particularly common in developing and impoverished nations. The development of an affordable human malaria vaccine that does not require complex technical knowledge or time-consuming purifying methods is urgently needed. With no harmful side effects, six extracts from the rhizomes *Curcuma longa, Hedychium coronarium,* and *Curcuma caesia* demonstrated the highest anti-plasmodial activity against the blood stage of *P. vivax* resistant to chloroquine. The ethnopharmacological approach seems to be a viable method for identifying plant metabolites that might serve as models for the creation of novel derivatives with enhanced characteristics.

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