IN VITRO ANTIPLASMODIAL ACTIVITY OF CINNAMOMUM TAMALA

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ABSTRACT

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan (a group of single-celled microorganism) belonging to the genus Plasmodium. Malaria causes symptoms that typically include fever, fatigue, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma or death. The disease is transmitted most commonly by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites travel to the liver where they mature and reproduce. Five species of Plasmodium can infect and be spread by humans. Most deaths are caused by P. falciparum because P. vivax, P. ovale, and P. malariae generally cause a milder form of malaria. The species P. knowlesi rarely causes disease in humans.

KEYWORDS: Plasmodium, P. vivax, P. ovale, and P. malariae, P. falciparum, Paroxysm, Quartan fever.

INTRODUCTION

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoan (a group of single-celled microorganism) belonging to the genus Plasmodium. Malaria causes symptoms that typically include fever, fatigue, vomiting, and headaches. In severe cases it can cause yellow skin, seizures, coma or death. The disease is transmitted by the biting of mosquitoes, and the symptoms usually begin ten to fifteen days after being bitten. If not appropriately treated, people may have recurrences of the disease months later [1], [2]. In those who have recently survived an infection, re-infection typically
causes milder symptoms. This partial resistance disappears over months to years if the person has no continuing exposure to malaria. The parasites travel to the liver where they mature and reproduce. Five species of Plasmodium can infect and be spread by humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests [2]. The disease is transmitted most commonly by an infected female Anopheles mosquito. The mosquito bite introduces the parasites from the mosquito's saliva into a person's blood. The parasites travel to the liver where they mature and reproduce. Five species of Plasmodium can infect and be spread by humans. Most deaths are caused by P. falciparum because P. vivax, P. ovale, and P. malariae generally cause a milder form of malaria. The species P. knowlesi rarely causes disease in humans. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic tests. Methods that use the polymerase chain reaction to detect the parasite's DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity [3]. The risk of disease can be reduced by preventing mosquito bites by using mosquito nets and insect repellents, or with mosquito-control measures such as spraying insecticides and draining standing water.

Signs and symptoms
The signs and symptoms of malaria typically begin 8–25 days following infection. However, symptoms may occur later in those who have taken antimalarial medications as prevention [4]. Initial manifestations of the disease—common to all malaria species—are similar to flu-like symptoms, and can resemble other conditions such as sepsis, gastroenteritis, and viral diseases [5].

The classic symptom of malaria is paroxysm—a cyclical occurrence of sudden coldness followed by shivering and then fever and sweating, occurring every two days (tertian fever) in P. vivax and P. ovale infections, and every three days (quartan fever) for P. malariae. P. falciparum infection can cause recurrent fever every 36–48 hours, or a less pronounced and almost continuous fever [6].
Cause of Malaria
Malaria parasites belong to the genus Plasmodium (Phylum apicomplexa). In humans, malaria is caused by P. falciparum, P. malariae, P. ovale, P. vivax and P. knowlesi [7], [8]. Among those infected, P. falciparum is the most common species identified (~75%) followed by P. vivax (~20%). Although P. falciparum traditionally accounts for the majority of deaths, recent evidence suggests that P. vivax malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of P.falciparum infection. Here have been documented human infections with several species of Plasmodium from higher apes; however, except for P. knowlesi—a zoonotic species that causes malaria in macaques [8].

Life cycle of the malaria parasites
In the life cycle of Plasmodium, a female Anopheles mosquito (the definitive host) transmits a motile infective form (called the sporozoite) to a vertebrate host such as a human (the secondary host), thus acting as a transmission vector. A sporozoite travels through the blood
vessels to liver cells (hepatocytes), where it reproduces asexually (tissue schizogony), producing thousands of merozoites. These infect new red blood cells and initiate a series of asexual multiplication cycles (blood schizogony) that produce 8 to 24 new infective merozoites, at which point the cells burst and the infective cycle begins a new. Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilised mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form an ookinete—a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal. Only female mosquitoes feed on blood; male mosquitoes feed on plant nectar, and do not transmit the disease. The females of the Anophelus genus of mosquito prefer to feed at night. They usually start searching for a meal at dusk, and will continue throughout the night until taking a meal. Malaria parasites can also be transmitted by blood transfusions, although this is rare [9].

![Life Cycle of the Malaria Parasite](https://en.wikipedia.org/wiki/Malaria#cite_note-29)

**Fig. 2.** The life cycle of malaria parasites.
Other merozoites develop into immature gametocytes, which are the precursors of male and female gametes. When a fertilised mosquito bites an infected person, gametocytes are taken up with the blood and mature in the mosquito gut. The male and female gametocytes fuse and form an ookinete—a fertilized, motile zygote. Ookinetes develop into new sporozoites that migrate to the insect's salivary glands, ready to infect a new vertebrate host. The sporozoites are injected into the skin, in the saliva, when the mosquito takes a subsequent blood meal [10], [11], [12].

**Prevention**

Methods used to prevent malaria include medications, mosquito elimination and the prevention of bites. There is no vaccine for malaria. The presence of malaria in an area requires a combination of high human population density, high anopheles mosquito population density and high rates of transmission from humans to mosquitoes and from mosquitoes to humans. If any of these is lowered sufficiently, the parasite will eventually disappear from that area, as happened in North America, Europe and parts of the Middle East. However, unless the parasite is eliminated from the whole world, it could become re-established if conditions revert to a combination that favours the parasite's reproduction. Furthermore, the cost per person of eliminating anopheles mosquitoes rises with decreasing population density, making it economically unfeasible in some areas [13]. Prevention of malaria may be more cost-effective than treatment of the disease in the long run, but the initial costs required are out of reach of many of the world's poorest people. There is a wide difference in the costs of control (i.e. maintenance of low endemicity) and elimination programs between countries. For example, in China—whose government in 2010 announced a strategy to pursue malaria elimination in the Chinese provinces—the required investment is a small proportion of public expenditure on health. In contrast, a similar program in Tanzania would cost an estimated one-fifth of the public health budget [14].

**Malaria diagnosis**

Malaria diagnosis Rational antimalarial drug usage is important in order to curtail resistance development and save costs for alternative therapies that are often more expensive. Accurate and reliable diagnosis is the key to rational treatment as it will be possible to distinguish malaria from other febrile illnesses such as viral (e.g. dengue fever and
influenza), bacterial (e.g. typhoid, brucellosis, respiratory and urinary tract infections) and other acute septic syndromes. Often, in malaria endemic regions, co-morbidity may occur where malaria parasitaemia is observed in patients with febrile illness due to bacterial or viral infections. The malaria infection may still remain asymptomatic due to development of anti-disease rather than anti-parasite immunity [15].

**Anti-Malarial Drugs**

Antimalarial medications, also known as antimalarials, are designed to prevent or cure malaria. Such drugs may be used for some or all of the following:

1. Treatment of malaria in individuals with suspected or confirmed infection.
2. Prevention of infection in individuals visiting a malaria-endemic region who have no immunity. (Malaria prophylaxis).
3. Routine intermittent treatment of certain groups in endemic regions. (Intermittent preventive therapy).
4. Some antimalarial agents, particularly chloroquine and hydroxychloroquine, are also used in the treatment of rheumatoid arthritis and lupus-associated arthritis.
5. Current practice in treating cases of malaria is based on the concept of combination therapy, since this offers several advantages, including reduced risk of treatment failure, reduced risk of developing resistance, enhanced convenience, and reduced side-effects. Prompt parasitological confirmation by microscopy, or alternatively by rapid diagnostic tests, is recommended in all patients suspected of malaria before treatment is started [16], [17], [18], [19].

**Antimalarials/Antimalarial Combinations Undergoing Clinical Trials**

**Drugs/combinations in phase 3 or completed phase 3**

1. Arterolane+Piperaquine
2. Dihydroartemisinin+Piperaquine
3. Artesunate+Pyronaridine
4. Azithromycin+Chloroquine
Drugs in phase 2 trials

1. Artemisone (artemifone)
2. Novel 4-Aminoquinolones- Ferroquine, Isoquine, AQ-13
3. Mefloquine
4. Ozonides- CDRI 97/98 (by CDRI, India), OZ439 (by University of Nebraska)

Trioxaquine (Sanofi-Aventis)

1. Fosmidomycin & 4-pyridone
2. Methylene blue
3. Novel 8-Aminoquinolone-Tafenoquine

Human monoclonal antibodies (Hum Abs)

1. Tinidazole
2. Mirincamycin [16], [20]

PLANT OF INTEREST

Cinnamomum tamala

Cinnamomum tamala, Indian bay leaf, also known as tejpat, Malabar leaf, Indian bark, Indian cassia, or malabathrum, is a tree within the Lauraceae family which is native to India, Nepal, Bhutan, and China [20].

It can grow up to 20 m (66 ft) tall. It has aromatic leaves which are used for culinary and medicinal purposes. It is thought to have been one of the major sources of the medicinal plant leaves known in classic and medieval times as malabathrum (or malabathrum) [21].

The leaves, known as tējapattā or tejpattā in Hindi and in Nepali, tejpata in Bengali, tejpat in Assamese, and tamalpatra in Marathi and in original Sanskrit, are used extensively in the cuisines of India, Nepal, and Bhutan, particularly in the Moghul cuisine of North India and Nepal and in tsheringma herbal tea in Bhutan.
The bark is also sometimes used for cooking, although it is regarded as inferior to true cinnamon or cassia. Methanolic extract of C. tamala leaves fed at 10 mg/kg to alloxan-induced diabetic rats for 15 days resulted in significant reduction in blood glucose level, blood glycosylated haemoglobin, LPO, serum AST, and ALT, and significant increase in the antioxidant enzymes such as CAT, GSH, and SOD. C. tamala could be used as an adjunct therapy in diabetes [22].

![Indian bay leaves](https://en.wikipedia.org/wiki/Cinnamomum_tamala#cite_note-FoC-3)

**Scientific Classification**

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<tr>
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<td>Magnoliids</td>
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<tr>
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<td>Lauraceous</td>
</tr>
<tr>
<td>Genus</td>
<td>Cinnamomum</td>
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<tr>
<td>Species</td>
<td>C. Tamala</td>
</tr>
<tr>
<td>Biological Name</td>
<td>Cinnamomum Tamala</td>
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</table>
Synonyms
1. Cinnamomum albiflorum Nees
2. Cinnamomum cassia D. Don nom. illeg.
3. Cinnamomum lindleyi Lukman.
4. Cinnamomum pauciflorum var. tazia (Buch.-Ham.) Meisn.
5. Cinnamomum reinwardtii Nees
6. Cinnamomum veitchii Lukman.
8. Laurus tamala Buch.-Ham.

Actions of Cinnamomum Tamala
1. Ant diabetic activity
2. Antibacterial activity
3. Antioxidant activity
4. Anti-ulcer activity
5. Antimicrobial activity

Active Constituents
1. Beta-caryophyllene [22]
2. Linalool [23]
3. Caryophyllene oxide
4. Eugenol [24]

Medicine
Leaves of C. tamala are used in colic and diarrhoeal preparations. C. tamala leaf extracts produce a hypoglycaemic effect in experimental rats. Hydro distilled essential oils of C. tamala screened for their anti-fungal activity against Trichophyton mentagrophytes and Microsporum microsporum audounil causing ring worm diseases in animals and humans exhibited fungicidal or fungistatic toxicity and were more effective than the synthetic antifungal agents, clotrimazole, griseofulvin or nystatin. Plant parts are used in many ayurvedic preparations e.g. sudarshan, choorna and chanderprabhavati.
Other Products

The leaf extracts are used as clarifiers in dyeing procedures with myrobalans or kamala [25].

MATERIALS AND METHOD

Following chemicals were used in the project

<table>
<thead>
<tr>
<th>S No.</th>
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<th>Company Name</th>
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<tr>
<td>1</td>
<td>Dimethyl sulphoxide</td>
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<td>2</td>
<td>Agar</td>
<td>LOBA Chemical</td>
</tr>
<tr>
<td>3</td>
<td>Alcohol</td>
<td>LOBA Chemical</td>
</tr>
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<td>4</td>
<td>Nutrient broth</td>
<td>LOBA Chemical</td>
</tr>
<tr>
<td>5</td>
<td>Chloroquine-Lariago 500 mg tab</td>
<td>IPCA Labs, Ratlam</td>
</tr>
<tr>
<td>6</td>
<td>Iodine solution</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Dragendorff reagent</td>
<td>LOBA Chemical</td>
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<tr>
<td>8</td>
<td>Lead acetate</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Chloroform</td>
<td>LOBA Chemical</td>
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<tr>
<td>10</td>
<td>Fehling A &amp; B</td>
<td>LOBA Chemical</td>
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<tr>
<td>11</td>
<td>Sulphuric acid</td>
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</table>

EXPERIMENTAL WORK

Extraction

The dried plant material was powdered in a grinder at low speed and sieved through 60 mesh sieve 15. The sieved material was weighed and then soxhleted with hydro alcoholic solvent (absolute ethanol & water in 3:1 ratio) 16 till the supernatent solvent became transparent [26], [27].
Yield Obtained
Percentage Yield = "Wt of dried extract" /"Wt. of Crude drug used" × 100
Wt. of Crude drug = 50 gm.
Wt. of extract = 4.6 gm.
Percentage Yield = 5.8/50 × 100 = 9.2 %
6.3 Evaluation of anti-Plasmodial activity-The plant extract was subjected to anti-Plasmodium falciparum activity.

Method
Plate method

Organism
Plasmodium falciparum

Preparation of Medium
Five nutrient agar plates were prepared using nutrient broth powder (3.25 gm.) and agar powder (3.75 gm.) and then sterilized by autoclaving under standard conditions of 121°C temperature & 15 p.s.i for 30 minutes. After cooling and solidification these plates were then supplemented with fresh uninfected human blood in the form of a layer covering the whole plate [28].

Test solutions
The plant extract was used to make test solutions with concentrations 50 mg/ml, 100 mg/ml & 200 mg/ml in dimethyl sulphoxide (DMSO).

Standard solution
Standard solution of reference drug chloroquine was made at 5mg/ml concentration using a marketed formulation (Lariago®500 mg chloroquine tab. by IPCA labs, Ratlam).

Control
Pure DMSO
Inoculation of parasite

All the plates were seeded with Pfr (+) blood sample (obtained from Civil hospital, Mandsaur) at the periphery using an inoculation loop.

A well of about 3 mm. diameter was bored at the centre. Three plates contained test solutions at different concentrations, one other plate contained standard drug solution and the last one contained pure DMSO (control). The plates were then incubated at 37°C for 48 hrs and then observed through microscope for the presence of parasite in different regions of the plates. Using this information, the zone of inhibition was calculated for all the plates.

PHYTOCHEMICAL SCREENING

The extract was screened for the presence of various Phytochemical constituents:

1. Test for carbohydrates (Molish test): Few drops of alpha-naphtol solution in alcohol were added to 2-3ml of extract, shaken and few drops of conc. H2SO4 were added. Violet ring at the junction indicates the presence of carbohydrates.

2. Test for starch (Iodine test): Three ml test solution & few drops of dilute iodine solution. Blue colour indicates presence of starch.

3. Test for reducing sugars (Fehling test): Mixed 1ml Fehling A & 1ml Fehling B, boiled for 1 min. Added equal volume of test solution. Heated in boiling water bath for 5-10 min. Yellow or brick red ppt indicates the presence of reducing sugars.

4. Test for alkaloids (Dragendorff’s test): To 2-3 ml extract added few drops of dragendorff’s reagent. Orange brown ppt. indicates presence of alkaloid.

5. Test for flavonoids: To small quantity of residue added lead acetate solution. Yellow ppt. indicates flavonoids.

6. Test for saponins (Foam test): Shaken dry extract with water. Persistent foam indicates saponins.

7. Test for steroids (Salkowski test): To 2 ml of extract added 2 ml chloroform & 2 ml conc. H2SO4, shaken well. Chloroform layer appearing red & acid layer showing greenish yellow fluorescence indicates steroids.

8. Test for phenolic compounds: Added lead acetate solution to 2-3 ml of extract, white ppt. indicates phenolic compounds.
H2SO4. White ppt indicates presence of proteins. Adding NH4OH turns ppt. Orange 
[29].

RESULT

Phytochemical screening

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL TEST FOR</th>
<th>TEST</th>
<th>RESULT</th>
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<tr>
<td>Carbohydrates</td>
<td>Molish test</td>
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<tr>
<td>Starch</td>
<td>Iodine test</td>
<td>(-)</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>Fehling test</td>
<td>(-)</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Dragendorff test</td>
<td>(+)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate test</td>
<td>(+)</td>
</tr>
<tr>
<td>Saponins</td>
<td>Foam test</td>
<td>(+)</td>
</tr>
<tr>
<td>Steroids</td>
<td>Salkowski test</td>
<td>(-)</td>
</tr>
<tr>
<td>Proteins</td>
<td>Xanthoprotein test</td>
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</table>

Antiplasmodial activity

The zone of inhibition (expressed as distance from the bore in mm.) is as follows:

<table>
<thead>
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<th>S No.</th>
<th>Solution</th>
<th>Concentration</th>
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<tr>
<td>1</td>
<td>Standard</td>
<td>5 mg/ml</td>
<td>8 mm</td>
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<td></td>
<td>Test</td>
<td>5 mg/ml</td>
<td>0 mm</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>50 mg/ml</td>
<td>18 mm</td>
</tr>
<tr>
<td></td>
<td>Test</td>
<td>50 mg/ml</td>
<td>9 mm</td>
</tr>
<tr>
<td>3</td>
<td>Standard</td>
<td>100 mg/ml</td>
<td>27 mm</td>
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<td></td>
<td>Test</td>
<td>100 mg/ml</td>
<td>18 mm</td>
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<tr>
<td></td>
<td>Standard</td>
<td>200 mg/ml</td>
<td>36mm</td>
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<td>---</td>
<td>----------</td>
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<td>------</td>
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<tr>
<td>4</td>
<td>Test</td>
<td>200 mg/ml</td>
<td>26 mm</td>
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<tr>
<td>5</td>
<td>Control</td>
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</table>

**CONCLUSION**

The hydroalcoholic leaf extract of plant CINNAMOMUM TAMALA has inhibitory activity against Plasmodium falciparum which is evident from the zone of inhibition data discussed above. Therefore the plant has scope to be used in future as a source of potent antiplasmodial agent.

**REFERENCES**

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