



## Phytochemical screening and antimalarial potency of *Mimosa pigra* L. (Fabaceae) on *Plasmodium falciparum* strains at varying concentration

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### Abstract

Malaria remains a significant global health burden, causing disease in approximately 15.5 million people and resulting in 1–3 million deaths annually, predominantly among young children. The development of effective antimalarial agents remains a critical priority in combating this disease. This study evaluated the phytochemical composition and *in-vitro* antimalarial activity of *Mimosa pigra* L. (Fabaceae) leaf extract against *Plasmodium falciparum*. Dried leaf material was extracted using ethanol, and the crude extract was subjected to phytochemical screening following standard protocols. The antimalarial efficacy was assessed at varying concentrations using an *in-vitro* culture method. Phytochemical analysis revealed the presence of seven secondary metabolites: carbohydrates, reducing sugars, saponins, alkaloids, flavonoids, tannins, and terpenes. The extract demonstrated potent antimalarial activity, achieving parasitic elimination rates of 84.8% and 80.9% at concentrations of 5,000 µg/mL and 2,000 µg/mL, respectively. These findings suggest that the bioactive secondary metabolites present in *M. pigra* may contribute to its antimalarial properties, supporting the traditional use of this plant in the treatment of malaria in Northern Nigeria.

**Keywords:** Antimalarial, phytochemical screening, *Mimosa pigra*, *Plasmodium falciparum*

### Introduction

All over the world, people are being diagnosed with different ailments on daily basis. Continuous research is being carried out to provide a lasting solution to these diseases. Medicinal plants are plants used to treat medical conditions in humans and animals, due to their possession of secondary metabolites called phytochemical. These plants can be trees, shrubs, woody, perennials, annuals, biennials, or climbers. They play a major role in many communities over the world in the treatment and prevention of disease and the promotion of general health [1]. Some plants/herbs which possess some medicinal values have been in use for the prevention and treatment of malaria in various parts of the world. Majority of orthodox medicine relied on the prescription of specific plants and herbs for healing, a practice still supported by contemporary research [2]. There is lack of information on the distribution of the biological and chemical activity in different plant parts essentially related to the difference in distribution of active compounds (or active principles) which are more frequent in some plant parts than in others [3].

### Malaria

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female anophelids' mosquitoes [4]. It is caused by five species of parasite that affect humans. All the parasites belong to the genus plasmodium; *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, *Plasmodium knowlesi*. Of all these, *Plasmodium vivax* and *Plasmodium falciparum* are most important. In most cases, *Plasmodium falciparum* is the most prevalent malaria parasite on the African continent. It is responsible for most malaria related deaths globally [1]. Symptoms of malaria

(which includes fever, headaches, chills and vomiting) appear 7 days or more (usually 10 to 15 days) after the infective mosquito bite in a non-immune individual. Malaria is preventable and curable and increased efforts are dramatically put in place in reducing its burden in many places.

*Mimosa pigra* (commonly known as giant sensitive plant, bash plant, catclaw mimosa or black mimosa) belongs to the subfamily *Mimosoideae*, in the larger family *Fabaceae* of legumes (*Leguminosae*) [5]. The generic name 'mimosa' is from the Greek meaning "to imitate or mimic". It is commonly known as prickly wood weed, mimosa, giant sensitive plant, cat claw mimosa, black mimosa in English. While in French it is called *amourette riviére*. It is called "giant sensitive plant, "Kai dafi" in Hausa, and "Enwa agogo" in Yoruba

The stem which is armed with broad-based prickles up to 7 mm long is greenish in young plants but becomes woody as the plant matures. The leaves are sensitive and fold up when touched and at nightfall [6].

Traditionally, it is used in tropical Africa as a tonic and for diarrhoea, gonorrhoea and blood poisoning. In Tanzania, the powdered leaf is taken with water to relieve swelling. In Zambia, the root ash is sprinkled over leprosy patches on the skin. The root is apparently aphrodisiac to some people and calming to others. The seed is emetic and an expectorant and is used for tooth troubles [7].

### Materials and Methods

#### Collection and Identification of Plant Materials

The fresh leaves of *Mimosa pigra* were collected at Rigasa, Kaduna state. The plant was identified and authenticated by Malam Mua'zam department of Medicinal Plant Research & Traditional Medicine, National Institute of Pharmaceutical

Research and Development (NIPRD). It was then allotted a voucher number of NIPRD.H.7043. After collection, it was air dried in a room for a period of 2 weeks. It was then crushed and reduced into small sizes using pestle and mortar. The powder was stored until needed.

#### **Extraction of Plant Materials**

150 g of the air-dried sample of the plant was percolated in 0.5L ethanol in an enclosed container, at room temperature for two weeks with occasional shaking. It was then decanted and filtered using no. 1 Whatman filter paper. The filtrate was collected into a weighed beaker, and the ethanol was allowed to evaporate at room temperature.

#### **Phytochemical Screening of Crude Extracts**

The ethanol crude extract was tested for the presence of phytochemicals using standard methods adopted by [8-10].

#### **Test for Carbohydrate (Molisch's test)**

To the extract, 3-4 drops of Molisch reagent was added followed by small quantity of concentrated Sulphuric acid and allowed to form a layer. A purple ring at the interface of the layer indicates the presence of Carbohydrates.

#### **Test for Reducing Sugar (Fehling's test)**

To about 0.5g of the extract was added 5ml of water. Equal volume (5cm<sup>3</sup> 1:1) of Fehling solution A and B were added in a test tube. The resultant mixture was boiled for two minutes. A brick red precipitate of copper (I) oxide indicates a positive test.

#### **Test for Alkaloids**

Two grams of the powdered sample was boiled in a water bath with 20ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved, another portion of the filtrate was put in 100ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run-off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5ml of dilute sulphuric acid. The various extract was then used for the following test:

#### **Wagner's test**

To the filtrate in test tube II, 1ml of wagner's reagent was added drop wise. Formation of reddish-brown precipitate indicates the presence of alkaloid.

#### **Test for Saponins**

1g of the sample in a test tube was added to 10ml of distilled water. The mixture was shaken vigorously for 30 seconds and observed. Formation of honey comb froth indicates the presence of saponins.

#### **Test for Tannins**

The extract was boiled in 50ml of distilled water for 3 minutes on a hot plate and filtered. The filtrate was used to carry out the Ferric Chloride test.

A portion of the ethanol extract was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins.

#### **Test for Flavonoids**

The extract was detanned with acetone and extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered and the filtrate was used for Lead Acetate test.

To 5ml of the detanned water extract was added 10% lead acetate solution. A coloured precipitate indicates the presence of flavonoids.

#### **Test for Terpenes**

The extract was extracted with 50 ml of ethanol (95%) and filtered. The residue was dissolved in 10ml of anhydrous chloroform and then filtered. The filtrate is used to carry out Salkowski's test.

The portion of the solution was mixed with 2ml of concentrated sulphuric acid carefully so that the acid forms a lower layer. A reddish-brown colour at the interface indicates the presence of a terpenes.

#### **Malaria Parasite Assay**

Antimalarial assay was conducted while adopting the standard method of [11,12].

#### **Preparation of Test Solution**

A stock solution of (10,000µg/ml) was prepared by dissolving extract (20mg) obtained from *Mimosa pigra* in dimethylsulphoxide (DMSO) (2ml). The following concentrations; 500µg/ml, 1000µg/ml, 2000µg/ml and 5000µg/ml were made by serial dilution.

#### **Sourcing of Malaria Parasite for Assay**

Malaria parasite of infected blood samples containing heavy parasitemia of *Plasmodium falciparum* were collected from the Department of Haematology, Bayero University Hospital, Kano. The samples were received in K3-EDTA coated disposable plastic sample bottles with tightly fitted plastic corks, and transported to the Microbiology Laboratory of Bayero University Kano.

#### **Determination of *Plasmodium falciparum* (Positive Blood Samples) Using Thin Smear Method**

Using a clean capillary tube, a small drop of each blood sample was placed at the centre of a clean glass slide at least 2mm from one end. A cover slip was placed at angle 45° in front of each drop and drawn backward to contact each drop. The drop was run along the full length of the edge of the cover slip. Smears were formed by moving the cover slip forward on each glass slide. The thin smears were immersed in methanol contained in petri dish for about 15 minutes. Geimsa's stain was dropped on each smear and allowed to stay for about 10 minutes. Excess stains were washed with clean tap water. The smears were dried in air by hanging the glass slides inverted in a rack. Each dried smear was microscopically observed under a high-power objectives (x100) using oil immersion. An average parasitemia was determined using the reading of 3 microscopic fields.

#### **Separation of the Erythrocytes (5% Parasitaemia) from the serum of the Blood Samples**

50% dextrose solution (0.5cm<sup>3</sup>) was added to each of the blood sample (5cm<sup>3</sup>) defibrinated and then centrifuged at 2500rpm for 15minutes in a spectra merlin centrifugation machine. Supernatant layer was separated from the

sediment. The later was diluted with 2-3 drops of normal saline and centrifuged at 2500rpm for 10minutes. The resulting supernatants were decanted. Samples with higher parasitemia (above 5%) were diluted with fresh malaria parasite negative erythrocytes.

**Preparation of *Plasmodium falciparum* Culture Medium**

Venous blood (2cm3) from the main vein of white healthy rabbit pinnae was withdrawn using a disposable 5cm3 syringe (BD 205 WG). This was defibrinated by allowing it to settle for at least one hour. The defibrinated blood was centrifuged at 1500rpm using spectre merlin centrifuged for 10minutes and the supernatant layer was collected in a sterilized tube. The sediment was further centrifuged at 1500rpm for 5minutes, and the supernatant layer was added to the first test tube. The sediment was discarded and the serum collected was supplemented with the salt of RPMI 1640 salt medium and sterilized 50µg/ml gentamacin sulphate.

**In-Vitro Assay of the Activity of the Extract on *Plasmodium falciparum* Culture**

A test solution (0.1ml) and the culture medium (0.2ml) were added into a test tube containing 5% parasitemia erythrocytes are and mixed thoroughly. The sensitivity of the of the parasitemia erythrocytes and mixed thoroughly. The sensitivity of the parasites to each tested fraction at 500, 1000, 2000, and 5000µg/ml was determined microscopically at 370C after 24 and 48 hours of incubation. The incubation was carried out under a bell jar system with a lighted candle that ensured the condition being atmospherically inert (about 5% O<sub>2</sub>, 2% and 93% nitrogen gas).

**Determination of the Activity**

At the end of the incubation periods usually between 24 to 48 hours, a drop of a thoroughly mixed aliquot of the culture medium was smeared on microscope slides and stained by Giemsa’s staining techniques. The mean number of erythrocytes appearing as blue discoid cells containing life rings of the parasites (that appeared red pink) was estimated and the average percentage elimination by samples was determined. The activity of the tested samples was calculated as the percentage elimination of the parasite after incubation periods of 24 and 48 hours, using the formula below;

$$\% = \frac{N}{Nx} \times 100 \dots\dots\dots\text{equation 1}$$

Where % = percentage activity of the extracts  
 N = total number of cleared Red Blood Cells (RBC)  
 Nx = Total number of parasitized RBC

**Results and Discussion**

**Result of Phytochemical Screening**

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to drug discovery and development [9].

The phytochemical analysis of the ethanol extracts of *Mimosa pigra* shows the distribution of various secondary metabolites as shown in the table below. These include; tannins, flavonoids, reducing sugar, saponins, carbohydrates, terpenes, alkaloid.

However, previous studies on the phytochemical analysis of the leaves of *Mimosa pigra* showed the presence of Flavonoids, Alkaloids, Tannins, and Carbohydrate [13]. Additionally [14], reported the presence of Saponins, Tannins and Flavonoids, triterpenes in the leaves. These agree with the result of this research.

**Table 1:** Phytochemicals Test Results

Phytochemical Test	Result on the Ethanol Extract
tannins	+ve
flavonoid	+ve
reducing sugar	+ve
saponins	+ve
carbohydrate	+ve
terpenes	+ve
alkaloid	+ve

**Keys:** +ve = present -ve = absent

**Result of Antimalarial Activity of ethanol Extract**

The results of anti-malaria activity of the extract were shown in Table 2. The microscopic examination of Geimsa’s stained slides for the fractions at 5000µg/ml showed a lesser number of parasites after 24 and 48 hours and has the most interesting anti-plasmodial activity. These observations suggest that the antimalaria activity of the extract is cytotoxic for *Plasmodium falciparum*, at 5000mg/ml and 2000mg/ml with a percentage (%) elimination of 84.8% and 80.9% respectively, when compared with the Standard (control) with percentage elimination of 88.6% and 84.2%. The higher percentage elimination of the extract justifies it antimalaria activity. While that of 1000mg/ml and 500mg/ml may also show growth elimination. Currently, no data is available from literature regarding the antimalarial activity carried out *Mimosa pigra* plant.

**Table 2:** Antimalarial Test Result

Concentration of extract used µg/ml	Final parasitemia count of red blood cell (per field) at end of incubation after 48 hours	Percentage elimination of sample	Percentage elimination of the control
5000	28	84.8%	88.6%
2000	35	80.9%	84.2%
1000	48	73.9%	
500	69	62.5%	

Total counts of infected and non-infected red blood cell = 218

Initial count of infected red blood cell before use = 184

Control: Artemether (20mg) Lumefantrine(120mg)

**Calculation of Percentage Elimination at End of Incubation**

The percentage elimination at end of incubation can be calculated using equations below

$$\% = \frac{N}{Nx} \times 100$$

Where % = percentage activity of the extracts  
 N = Total number of cleared Red Blood Cells (RBC)  
 Nx = Total number of parasitized RBC  
 At 5000 µg/ml concentration

$$N = 184 - 28 = 156$$

$$\% = \frac{156}{184} \times 100 = 84.8\%$$

At 2000 µg/ml concentration

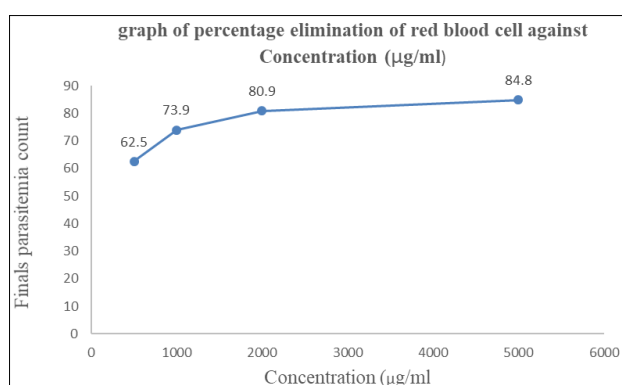
$$N = 184 - 35 = 149$$

$$\% = \frac{149}{184} \times 100 = 80.9\%$$

However, the ethanol crude fraction exhibits a percentage elimination of 73.9% and 62.5% for 1000µg/ml and 500µg/ml respectively.

### Relationship between the Percentage Elimination and the Concentration of Ethanol Extract Used

The results show that percentage elimination of infected red blood cell increases with an increase in the concentration of ethanol extract of the plant sample. This is illustrated by the graphical representation (fig 2) as shown below.



**Fig 1:** Graph of Percentage Elimination against Concentration of crude ethanol extract

### Conclusion

The qualitative phytochemicals analysis of ethanol extract reveals the presence of Carbohydrate, Reducing sugar, Alkaloids, Saponins, Tannins, Flavonoids and Terpenes. Most of these phytochemicals are responsible for activity of the plant.

Due to its high potency against *Plasmodium falciparum* strain, this research work is significantly supporting the use of leaves of *M. pigra* as an alternative source of antimalaria. And also suggesting its use as an alternate source of antimalarial drugs.

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