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Research Article

In-Vitro Anti-Plasmodial Activities of Ethanolic Extracts of *Adansonia digitata, Morinda citrifolia,* and *Tamarindus indica* Against *Plasmodium falciparum*

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ABSTRACT

Malaria due to *Plasmodium* species is an existential problem and a major disease in many developing countries. The emergence of drug-resistant parasites and the limited availability of effective treatments present major challenges in combating this disease. Thus, searching for more potent antimalarial agents, especially from natural sources, has become urgent and imperative. This study investigates the anti-plasmodial properties of the ethanolic crude extracts of *Adansonia digitata* (stem bark), *Morinda citrifolia* (leaves), and *Tamarindus indica* (leaves) using standard methods. The crude plant extracts were obtained using the maceration technique using 70% ethanol. The *in vitro* anti-plasmodial activities of the crude extracts were assessed using the *Plasmodium* lactate dehydrogenase (*p*LDH) assay against asexual stages of clinical isolates of *P. falciparum* maintained at 5% haematocrit (human type O^+ red blood cells) in complete RPMI-1640 medium. The crude ethanolic extracts of *T. indica* exhibited a significantly higher ($p < 0.05$) *in vitro* anti-plasmodial activity with an IC_{50} value of 5.86µg/mL, followed by *A. digitata* (IC_{50} = 30.22µg/mL,) and *M. citrifolia* (IC_{50} = 31.85µg/mL). The findings in this study indicated that the crude extracts tested showed potential for anti-plasmodial activities that could be harnessed for developing novel anti-plasmodial agents.

Keywords: *In-vitro*; Anti-Plasmodial; *p*LDH, IC_{50}; *A. digitata; M. citrifolia; T. indica; P. falciparum*

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INTRODUCTION

Malaria is an ancient disease that has existed for thousands of years. It is the most important parasitic disease of man. It is still a major health problem in many tropical countries like Nigeria which has over one-quarter of the global burden. The word "malaria" is named after the Italian term "mal'aria" which means "bad air" to represent the association of the disease with marshy areas (Fikadu & Ashenafi, 2023). Malaria is an endemic vector-borne parasitic disease caused by infection with eukaryotic protists of the genus *Plasmodium*. There are six known causative agents of human malaria: *P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae,* and *P. knowlesi* (Chaniad *et al*., 2023). Among these species, *P. falciparum* poses the most serious threat, potentially leading to severe malaria. Malaria is transmitted to humans primarily through the bites of infected female *Anopheles* mosquitoes. Despite the global fight against the disease, malaria has remained one of the most dangerous diseases in tropical and sub-tropical regions of the world. According to the World Malaria Report by the World Health Organisation (WHO,2023), there were an

estimated 249 million cases and 608,000 deaths due to malaria worldwide in the year 2022, this represents about 5 million more cases when compared to the year 2021. About 94% of these cases occur in Africa, with Nigeria particularly carrying the highest disease burden of about 27% (WHO, 2023). Young children (under five years old), pregnant women, and non-immune individuals are the most vulnerable groups affected by malaria. In recent decades, the global strategy for combating malaria has relied on a two-pronged approach: disease control measures, such as preventing mosquito bites through insecticide-treated nets, indoor residual sprays, and drug treatment using artemisinin-based combination therapies (ACTs). More recently, however, the perspective has changed toward malaria elimination and ultimately eradication.

A major factor hindering malaria elimination strategies is the continuous emergence of *P. falciparum* parasites resistant to all known existing malaria drugs including the artemisinin derivatives (Olasehinde *et al*., 2019). However, malaria can be prevented and even cured, as an estimated 2.1 billion malaria cases and 11.7 million deaths were averted in the period 2000-2022; with 82% of cases and 94% mortality averted in the African region (WHO, 2023). One area that offers promising prospects involves using natural products such as plants. Plants represent a potential source of pharmacologically active compounds since they contain a wide variety of chemical structures (Alkandahri *et al*., 2019). A vast majority of prescribed drugs used in the world today contain compounds that are directly or indirectly, via semi-synthesis, derived from plants (Oksman-Caldentey & Inzé, 2004). Most communities in malaria-endemic areas of the world have plants used to treat malaria symptoms. The World Health Organization estimated that 80% of people in developing countries rely on traditional medicines (WHO, 2002). There is an increasing interest in the potential use of medicinal plants for pharmaceutical applications. These phyto-medical compounds can be isolated and characterized from different plant parts such as roots, stems, bark, leaves, seeds, flowers, and fruits (Chaniad *et al*., 2022). Many studies have investigated the anti-malarial properties of some plants as potential sources of new antimalarial agents. Accordingly, this study was aimed at determining the anti-plasmodial efficacy of the crude extracts of *Adansonia digitata, Morinda citrifolia* and *Tamarindus indica* against clinical isolates of *P. falciparum.*

MATERIALS AND METHODS

The stem bark of *A. digitata, and* leaves of *M. citrifolia* and *T. indica* were used for the research. Fresh leaves of *T. indica* and *M. citrifolia* were obtained from Dan Bushiya in Chikun Local Government Area, while the stem bark of *A. digitata* was obtained from Rigachikun in Igabi Local Government Area within Kaduna State, Nigeria. The O^+ whole blood and serum were obtained from the National Blood Bank along Constitution Road Kaduna, Nigeria. The *P. falciparum*-infected blood sample was obtained from Barau Dikko Teaching Hospital Kaduna, after getting the ethical clearance and patient informed consent. Chloroquine phosphate, Quinine, and Artemether were used as the control drugs obtained from Sigma Aldrich (USA).

Collection and Identification of Plant Materials

The stem bark of *A. digitata,* leaves of *M. citrifolia,* and *T. indica* collected were adequately identified and authenticated at the Department of Biological Sciences, Kaduna State University, Kaduna. Voucher numbers were obtained and plant samples were deposited in the herbarium unit of the department.

Preparation of Plant Samples

The different plant materials were washed thoroughly under running tap water to remove adhering dirt and air-dried at room temperature away from direct sunlight to avoid possible photo-reactions or denaturation of their putative compounds. The air-dried plant materials were pulverized into a coarse powder using a mortar and pestle and then into fine powder aseptically using an electric grinder (Labaran *et al.*, 2021). The powdered plant materials were then stored in air-tight containers until further use.

Extraction of Plant Materials

Extraction was done via the maceration method using 70% ethanol as a solvent. The powdered plant materials were placed inside a container; the solvent was poured on top until it completely covered the plant material. The container (with the plant material and solvent) was then closed and kept for at least three (3) days with periodic stirring and shaking to ensure complete extraction. At the end of the extraction, the macerates were filtered using a Büchner funnel lined with filter paper. The residue after the filtration was discarded and the filtrates were concentrated using a rotary evaporator at 40^0C. The concentrates were subjected to steam evaporation using a water bath to obtain a solvent-free crude extract, and further dried into a powder in a desiccator, weighed, labeled, and refrigerated until further use. The physical properties were obtained and the percentage (%) yield of each of the extracts was calculated using the formula:

$$\% \text{ yield of extracts} = \frac{W_2 - W_1}{W_0} \times 100$$

Where;

W_2 = weight of crude extract and container,

W_1 = weight of container alone,

W_0 = weight of dried plant material
(Dagne *et al*., 2021).

***In vitro* Anti-plasmodial Activity**

Preparation of test extracts

About 0.01g of the crude extracts were weighed and dissolved in 0.1% dimethyl sulfoxide (DMSO) and diluted with phosphate buffer saline to obtain a stock solution of 1 mg/mL. The stock solutions were filter-sterilized through a 0.1µm Millipore filter. A double-fold serial dilution of the extracts was performed to arrive at concentrations of 100, 50, 25, 12.5, 6.25, 3.13, and 1.56 µg/mL for treatment of the parasitized cells *in vitro.* The standard drugs were also prepared and tested at the same concentrations as the plant extracts (Dantata & Hotoro, 2020).

Collection of Blood Samples

The inclusion criteria for collection of blood samples were children (aged 6 months and above) and adults who have had fever in the last 24 hours and have not taken any anti-malarial drug in the previous two (2) weeks. Blood samples were collected in Ethylenediamine tetraacetic acid (EDTA) bottles from patients who fulfilled the inclusion criteria after obtaining oral consent. Safety procedures were adopted in collecting samples by swabbing the area to be sampled with 70% alcohol and allowing it to dry before collection. The samples were obtained by experienced clinicians and medical laboratory scientists. All samples collected were adequately preserved until further use.

Estimation of Level of Percentage Parasitaemia

A few drops of blood samples were used to prepare thick and thin films on clean slides for each patient. The thin blood films were dried, fixed in methanol, and then stained with 10% Giemsa stain before microscopic examination using the 100× objective lens (with a drop of oil immersion applied). Approximately, 1000 red blood cells (RBC) were counted and examined for parasites (Wahyuni *et al.*, 2023). The mean percentage of the infected RBCs was determined relative to the total RBCs. The percentage parasitaemia was calculated using the formula as follows;

$$\% \text{ Parasitaemia} = \frac{\text{No of infected RBCs}}{\text{Total no of RBCs}} \times 100$$

(Oladejo *et al.*, 2024).

Serum and Erythrocyte Preparation for Culture

Serum was obtained from fresh human blood of O^+ donors. The blood was collected in a plain centrifuge tube without an anti-coagulant and left to stand for an hour to allow the blood to clot. The blood was then centrifuged at 1500rpm for 10 minutes at room temperature to separate the serum from the red blood cells. The serum was then aseptically collected with a Pasteur pipette into sterile tubes, deactivated at 56^0C, and frozen at -20^0C. Fresh uninfected blood from donors with the O^+ blood group was also collected in EDTA bottles and centrifuged at 1500 rpm for 10 minutes at room temperature. The plasma and buffy coat were aseptically removed with a Pasteur pipette, after which the blood was washed twice with phosphate buffer saline (PBS), and lastly with Roswell Park Memorial Institute 1640 (RPMI-1640) by centrifuging at 1500 rpm for 10 minutes in each wash cycle. The washed blood was then resuspended in an equal volume of RPMI-1640 to make up 50% haematocrit and stored in a refrigerator at 4^0C (Basco, 2023).

Initiation of *In vitro* Cultivation of *P. falciparum*

The clinical isolates of *P. falciparum* were cultivated and maintained in fresh human O^+ red blood cells suspended in RPMI-1640 medium according to the method of Trager and Jensen, (1976). The medium was supplemented with 2 mg/mL sodium bicarbonate, 10 µg/mL hypoxanthine, 4.8 mg/mL N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), L-glutamine, 15% human serum and 2.5 µg/mL gentamicin. The culture was maintained at 37^0C in a CO_2 incubator. After every 24 hours, the used medium was replaced with a fresh medium, and Giemsa-stained slides were prepared daily to monitor the level of parasitemia (Chaniad *et al*., 2022).

Determination of *in vitro* Anti-plasmodial Activity of the Crude Plant Extracts

The anti-plasmodial screening of the plant extracts was carried out against clinical isolates of *P. falciparum* by using the *Plasmodium* lactate dehydrogenase (*p*LDH) assay as described by Makler and Hinrichs, (1993). Initially, the asynchronous *P. falciparum* parasites were synchronized to obtain about 90-95% ring-stage parasitized cells by treating them with 5% D-sorbitol. The initial ring stage parasitaemia was maintained at 0.5% in 4% haematocrit using complete medium and fresh O^+ erythrocytes for the screening activity. The parasitized blood was added to the wells of a 96-well microtiter plate containing 100µL of each of the test extracts and the standard drugs. The plate was incubated at 37^0C in 5% CO_2 for 36-40 hours in a CO_2 incubator. All tests were done in triplicates. Chloroquine, Quinine, and Artemether were used as the standard drugs (positive control), and wells containing no drug but parasitized blood only were included in each plate and used as negative control (Gogoi *et al*., 2021). After incubation, the plates were subjected to three freeze/thaw cycles (frozen at -20^0C and thawed at 37^0C) for complete haemolysis. The lysed cells were then transferred to a new 96-well plate containing a mixture of 100µL of Malstat reagent and 20µL of nitro blue tetrazolium/phenazine ethosulfate solution and

incubated for 1 hour in the dark. These solutions were used to determine the parasite's lactate dehydrogenase (*p*LDH) enzyme activity in the cultures. The formation of a purple product indicated the presence of *p*LDH. The optical density was measured using a microplate reader at a wavelength of 650nm. The percentage inhibition and half maximal inhibitory concentration (IC_{50}) were calculated using a non-linear dose-response curve (Chaniad *et al*., 2022). Based on the IC_{50} values, the extracts were categorized into active (<10µg/mL), intermediate (10-25µg/mL) or inactive (>25µg/mL) (Gogoi *et al*., 2021).

Data Analysis

All experiments were conducted in triplicates and the data are presented as mean ± standard error of mean. The 50% inhibitory concentration (IC_{50}) was calculated based on non-linear regression analysis using GraphPad Prism 9 Software (GraphPad Inc., San Diego, CA). The one-way ANOVA followed by Tukey's honest significant difference post hoc test was used to analyze and compare results. Values of $p < 0.05$ were considered significant.

RESULTS

Extraction of Plant Material

Extraction was successfully carried out using 70% ethanol as solvent via the maceration method. The percentage yield and physical properties of each of the ethanol crude extracts of *A. digitata, M.citrifolia,* and *T. indica* are presented in Table 1**.** The results indicated that *M. citrifolia* crude extract had the highest percentage yield of 23.6%, followed by *T. indica* with 15.8%, while *A. digitata* had the least with 5.6%. This indicates that the phyto-constituents of *M. citrifolia* are better extracted with 70% ethanol.

***In vitro* Anti-plasmodial Activity**

The percentage inhibitory activity of the extracts on the asexual stages of *P. falciparum* at varying concentrations (1.56 to 100µg/mL) is shown in Table 2. At concentrations between 1.56 and 3.13 µg/mL, *A. digitata* was observed to have higher significant inhibitory activity. At concentrations ranging from 6.25 to 100 µg/mL, *T. indica* had higher inhibitory activity and was dose-dependent. Comparatively, the plant extracts were found to be more active than the standard drugs at 1.56 and 3.13 µg/mL concentrations, however at concentrations 6.25 to 100 µg/mL, the standard drugs (Chloroquine, Quinine, and Artemether) were significantly ($p < 0.05$) more active than the plant extracts, with Artemether having higher inhibitory activity followed by Chloroquine and Quinine. In addition, *T. indica* had the lowest calculated half maximum inhibitory concentration (IC_{50}) of 5.9±0.64 µg/mL, followed by *A. digitata* (IC_{50} = 30.2±5.68 µg/mL) and *M. citrifolia* (IC_{50} = 31.8±2.21 µg/mL) (Table 3). This indicates the higher inhibitory activity of *T. indica*. Chloroquine with the lowest IC_{50} of 3.5±0.39 µg/mL was statistically insignificant ($p > 0.05$) with quinine (IC_{50} = 8.2±0.27 µg/mL) and *T. indica*. The lower efficacy portrayed by the IC_{50} and Artemether and Quinine indicates parasite resistance to the drugs while being sensitive to chloroquine (Table 3).

Table 1: Percentage yield and physical properties of the ethanolic extracts of *A. digitata, M. citrifolia* and *T. indica*

Plant Extracts	Part used	Color/appearance	Odour	% Yield	Voucher number
A. digitata	Stem bark	Reddish brown/ powdery	Earthy slightly sweet	5.6	NDA/BIOH/2024/04
M. citrifolia	Leaves	Dark green/ viscous	Pungent slightly unpleasant	15.8	NDA/BIOH/2024/05
T. indica	Leaves	Dark brown/ viscous	Earthy slightly astringent	23.6	NDA/BIOH/2024/06

Table 2: Percentage inhibitory activity of crude ethanolic extracts of *A. digitata and T. indica* against clinical isolates of *P. falciparum*

Treatment	Treatment concentration (µg/mL)						
	1.56	3.13	6.25	12.5	25	50	100
ADE	27.4±0.82^{c}	30.5±0.95^{c}	32.1±1.32^{b}	39.4±1.21^{b}	48.9±1.12^{b}	54.5±0.66^{b}	56.9±0.50^{b}
MCE	14.0±0.86^{a}	18.3±0.60^{a}	23.8±0.62^{a}	27.0±0.77^{a}	31.5±0.79^{a}	34.7±0.60^{a}	37.6±0.33^{a}
TIE	20.3±1.45^{b}	24.6±0.62^{b}	33.1±0.51^{b}	43.8±1.33^{c}	50.5±0.80^{b}	61.7±1.27^{c}	66.4±0.92^{c}
CQ	12.4±0.90^{a}	30.0±1.77^{c}	36.9±0.51^{c}	51.9±0.76^{e}	61.5±0.64^{c}	68.3±0.48^{d}	71.6±0.06^{d}
Q	13.9±0.48^{a}	16.0±0.60^{a}	32.0±0.52^{b}	42.4±0.76^{c}	51.3±1.32^{b}	62.2±1.10^{c}	69.8±0.27^{d}
AR	14.1±0.22^{a}	22.5±0.41^{b}	31.0±1.18^{b}	47.0±0.66^{d}	59.2±0.60^{c}	66.4±0.36^{d}	75.7±1.16^{e}

The result is presented as mean ± SEM and values with different superscripts within the column indicate a statistically significant difference ($p<0.05$) at a 95% confidence interval.

Key: ADE = *Adansonia digitata* ethanolic extract**;** MCE = *Morinda citrifolia* ethanolic extract**;** TIE = *Tamarindus indica* ethanolic extract; CQ = Chloroquine; Q = Quinine; AR = Artemether

Table 3: **IC_{50} of crude ethanolic extracts of *A. digitata, M. citrifolia,* and *T. indica* against clinical isolates of *P. falciparum***

Treatment Group	IC_{50} (µg/mL)
ADE	30.2 ± 5.68^{c}
MCE	31.8 ± 2.21^{c}
TIE	5.9 ± 0.64^{a}
CQ	3.5 ± 0.39^{a}
Q	8.2 ± 0.27^{a}
AR	21.6 ± 0.66^{b}

The result is presented as mean ± SEM and values with different superscripts within the column indicate a statistically significant difference ($p<0.05$) at a 95% confidence interval.

Key: ADE = *Adansonia digitata* ethanolic extract**;** MCE = *Morinda citrifolia* ethanolic extract**;** TIE = *Tamarindus indica* ethanolic extract; CQ = Chloroquine; Q = Quinine; AR = Artemether

DISCUSSION

Plants have been explored for their medicinal potential since ancient times. Herbal medicinal plants can synthesize numerous secondary bioactive phytochemicals with therapeutic potential, such as antimalarial, antimicrobial, antioxidant, and anti-inflammatory, thus improving health and limiting the occurrence of diseases (Basit *et al*., 2023). In this study, three plants from three different plant species namely; *A. digitata* from the Malvaceae family, *M. citrifolia* from the Rubiaceae family, and *T. indica* from the Fabaceae family were investigated to determine their anti-plasmodial activity against clinical isolates of *P. falciparum* using *p*LDH *in vitro* assay protocol. Ethanol was chosen as the solvent of extraction in line with the traditional practices of some herbalist using both aqueous and gin in their preparation and administration of their herbal decoction. In addition to the fact that ethanol consists of both polar (-OH) and nonpolar (CH_2CH_3) groups, making it an ideal solvent to dissolve both polar and non-polar secondary metabolites. Also, extraction using polar solvents like ethanol results in a higher yield of extracts (Roka Aji *et al.*, 2020). The *M. citrifolia* crude ethanol leaf extract yielded the highest percentage of 23.6% making it a better solvent for its extraction. However, the low performance of the extract indicated that the solvent is a better choice for the extraction of the active ingredient or the lead anti-plasmodial compound. Moreover, the bulky chlorophyll content in the leaf and its high solubility in alcohol may factor in the higher yield. In a similar study conducted by Sam-ang *et al*. (2020), *M. citrifolia* crude leaf extract yielded a high percentage of 10.92% compared to the crude root (7.96%) and crude stem (3.30%) extract. In addition, Roka Aji *et al*. (2020) and Chaniad *et al.* (2022) reported a percentage yield of 9.3% and 11.3% respectively. The *T. indica* crude leaf extracts in this study had a percentage yield of 15.8%. This is similar to the findings of Ayat *et al*. (2019) where the percentage yield of *T. indica* was 14.5%. *A. digitata* stem bark extract had a percentage yield of 5.6% similar to the 4.2% yield reported by Gaffo *et al.* (2022). However, Ayat *et al*. (2019) reported a lower yield of 1.9%. The variations seen in the percentage yield in the studied plants may be due to several reasons such as; the quality of plant material used, method of extraction, choice of solvent, duration of extraction, environmental conditions, plant variability, and storage conditions (Zeroual *et al*., 2021). However, the solvent is a good choice for the extraction of active ingredients and the lead anti-malarial compounds, following the performance of the extract in the anti-plasmodial activity.

The anti-plasmodial activity of the extracts was tested *in vitro* against clinical isolates of *P. falciparum* using the (*p*LDH) assay protocol. Lactate dehydrogenase (LDH) is an important enzyme in the glycolytic pathway in malaria parasites that is produced during the sexual and asexual stages of the parasite. The production and accumulation of LDH are used as reliable markers to determine parasite viability (Chaniad *et al*., 2022). Hence, the detection of LDH is specific to the parasitic enzyme and has been used for antimalarial drug screening. The percentage inhibition of parasitemia of each treatment and standard drug at different concentrations against the clinical isolates of *P. falciparum* was determined using the optical density produced using a microplate reader. These readings were also used to determine the IC_{50}. The crude extracts and standard drugs used exhibited an inhibitory effect in a dose-dependent manner. At a concentration of 100µg/mL, *T. indica* had the highest ($p < 0.05$) percentage of parasitemia inhibition, followed by *A. digitata* and *M. citrifolia*. The standard drugs used also

had a significantly higher ($p < 0.05$) inhibitory effect than the crude extracts of the tested plants, with Artemether having the highest percentage inhibitory effect, followed by Chloroquine and Quinine which had a similar inhibitory effect. Plant extracts with *in vitro* anti-plasmodial activity are categorized based on their IC_{50} values: extracts exhibiting high activity have IC_{50} values less than 10µg/mL, moderate or intermediate activity between 11-50µg/mL, mild activity between 51-100µg/mL, and inactivity above 100µg/mL (Chaniad *et al*., 2023). Based on these criteria, *T. indica* demonstrated potent anti-plasmodial activity against the tested clinical isolate of *P. falciparum* with an IC_{50} value of 5.9µg/mL ($P < 0.05$). This finding aligns with the 4.8µg/mL IC_{50} reported by Tajbakhsh *et al*., (2021a). However, a significantly higher IC_{50} value of 186.9µg/mL was reported by Osama *et al*., (2015). Similarly, *A. digitata* and *M. citrifolia* both exhibited moderate anti-plasmodial activity with IC_{50} values of 30.2µg/mL and 31.8µg/mL, respectively. This contrasts with Tajbakhsh *et al*. (2021a) who reported a high anti-plasmodial activity for *A. digitata* with an IC_{50} of 8.2µg/mL. Conversely, Muthaura *et al.* (2015) and Manuel *et al*. (2020) reported a low anti-plasmodial activity of *A. digitata* with IC_{50} values of 67.3µg/mL and 423.9µg/mL, respectively. For *M. citrifolia*, Naing *et al*. (2019) observed high anti-plasmodial activity with an IC_{50} of 9.3µg/mL, while da Silva *et al*. (2021) reported an IC_{50} of 175.3µg/mL, indicating inactivity based on the aforementioned criteria. Despite having the highest percentage yield of extract in comparison to *A. digitata* and *T. indica*, *M. citrifolia* exhibited the least *in vitro* anti-plasmodial activity. This may be attributed to the fact that different plant species have varying amounts of phytochemicals. Some plants naturally have high concentrations of phytochemicals, which may lead to a lower yield of extract but a higher concentration of the desired compounds. Biologically active compounds usually occur in low concentrations in plants (Tajbakhsh et al., 2021a). The difference in IC_{50} values across different studies, despite using the same plant species under similar laboratory conditions, might be attributed to factors such as differences in the geographical origins of the plants, plant parts used, the solvent of extraction, extraction methodologies, and the specific strain of *P. falciparum* used in the assays. It is worth noting that the lower efficacy portrayed by the IC_{50} values of Artemether and Quinine is an indication of parasite resistance to the drugs, while being sensitive to Chloroquine.

CONCLUSION

The crude ethanolic extracts of the leaves of *T. indica* exhibited a high *in vitro* anti-plasmodial activity, while the stem bark of *A. digitata* and leaves of *M. citrifolia* exhibited moderate anti-plasmodial activity, against the clinical isolates of *P. falciparum* tested. The parasite displayed sensitivity to Chloroquine but showed resistance to both Quinine and Artemether. This highlights the importance of exploring new anti-plasmodial agents. Therefore, a bio-assay-guided fractionation of compounds from these plants could lead to the identification and purification of novel compounds with potential anti-plasmodial properties.

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