Original Research Report

PRELIMINARY PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIPLASMODIAL ACTIVITY OF *Terminalia mantaly* AGAINST *Plasmodium falciparum*

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ABSTRACT

Malaria has been one of the world's worst killer diseases throughout recorded human history. Despite attempts to eradicate the disease, it remains a global burden. This could be a result of parasite resistance to current therapy. However, this research aimed at evaluating the in vitro antimalarial activity of ethanolic extracts of Terminalia mantaly *on* Plasmodium falciparum. *The plant extracts were prepared by cold maceration in 70% ethanol and air-dried by a rotary evaporator. The phytochemical analysis was carried out using standard procedures outlined in the Analytical methods of the Association of Official Analytical Chemists (AOAC, 1990) which indicates the presence of tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids, and balsam. The in vitro antimalarial assay was carried out according to the method described by WHO (2001). All data were represented as Mean ± Standard deviation. Ethanolic extracts of the three parts of the plant were subjected to in vitro antimalarial activity at three concentrations (300 mg, 150 mg, and 75 mg) in four replicates with artemether (standard drug) as a positive control. Stem bark at 300 mg/kg completely cleared the parasites with a 0.00% parasitaemia rate and there was no significant difference when compared with positive control at p<0.005 value of 1.00. This study affirms the use of the plant for the treatment of malaria.*

Keywords: *Malaria; terminalia mantaly; plasmodium falciparum; tropical disease*

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Highlights:

1. Malaria is prevalent in many populations of communities despite preventive measures.
2. The experimental was screened for bioactive components which could be the reason for the antimalarial effect and the plant shows dose dependent antimalarial activity.

INTRODUCTION

Malaria has been one of the world's worst killer diseases throughout recorded human history. Malaria is a life-threatening disease caused by protozoa of the genus *Plasmodium* transmitted by the female anopheles mosquito (WHO,2019). Five species of *plasmodium* are known to cause malaria in man: *P. vivax*, *P. falciparum*, *P. malariae*, *P. ovale*, and *Plasmodium knowlesi* (Abdulrazaq et al. 2020).

Despite attempts to eradicate malaria, it remains one of the worst diseases in terms of deaths annually and has actually increased in incidence since the 1970s (Taylor et al. 2006). According to the World malaria report released in December 2019, there were 228 million cases of malaria in 2018, and the estimated number of malaria deaths stood at 405,000 (WHO. 2019). In 2017, five countries accounted for nearly half of all malaria cases worldwide: Nigeria (25%), the Democratic Republic of the Congo (11%), Mozambique (5%), India (4%), and Uganda (4%) (WHO, 2019). Children under 5 years of age are the most vulnerable group affected by malaria; in 2017, they accounted for 61% (266,000) of all malaria deaths worldwide and this could be the result of low immunity.

Nigeria is one of the nations with the highest rates of malaria-related morbidity and mortality. A recent analysis of malaria risk and mortality in Nigeria revealed that, despite a global decline in malaria rates, cases there have climbed exponentially, killing many people (Kayode and Godwin, 2017; Samuel B and Adekunle YA, 2021). The World Health Organization

has estimated the malaria mortality rate for children under five in Nigeria at 729 per 100,000. About 300-500 million clinical cases are observed and 1.5–2.7 million deaths occur each year due to malaria, most of whom are children and pregnant women (Otubanjo 2013). Globally, malaria deaths have declined by 10% since 2000 and many African countries have achieved a 50% reduction in malaria interventions (Otubanjo. 2013).

Currently, four artemisinin combination therapies (ACTs) are recommended for the treatment of malaria: Artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine and artesunate–sulfadoxine-pyrimethamine (WHO. 2009). ACTs are often safe and effective, however there are still differences in their performance in areas where malaria is endemic (Hodel et al. 2013). Studies have revealed that the variability and susceptibility of malaria are influenced by human genetic variables (Driss et al. 2011). It similarly influences how medications are metabolized and how readily they are absorbed, which has an impact on how effective pharmacological therapy is (Desta and Flockhart, 2017). However, malaria parasites develop resistance to most of the available and affordable antimalarial drugs (Teka et al. 2020). Antimalarial drug development and discovery is expected to provide new drugs that not only have antimalarial activity in vitro and in vivo, but also have safety mechanisms applicableto humans (Ekasari, W et al. 2021).

Parasite resistance has caused some of the least expensive traditional antimalarial drugs to be ineffective. As such, there is an urgent need for new antimalarial therapies (Bekono et al. 2020). With an estimated protective efficacy for routine use, effective management of severe malaria patients has a great potential to reduce case fatality from malaria (Thwing J et al. 2011). Effective therapy can also stop consequences including developmental impairment (Carter JA et al. 2005), infection recurrence, and eventually, the establishment and spread of parasites that are resistant to treatment (WHO. 2021).

In the sub-Saharan African region, hundreds of plants are traditionally used for the treatment of malaria (Teka et al. 2020). In order to identify the bioactive components present in medicinal plants used in conventional medicine, phytochemical screening is a crucial step (Afnan Alqethami et al. 2021). Therefore, phytochemical screenings of medicinal plants for bioactive antimalarial compounds will help in the development of new antimalarial drugs to which the parasites are not resistant. Some studies had been conducted to show the efficacies of plants in the treatment of malaria through phytochemical screening of their constituents globally (Ekasari et al. 2021, Kurniawan et al. 2020). A variety of diseases are treated using plants because they have antibacterial properties. The natural ingredients used in these early attempts—typically native plants or their extracts—were successful in many cases. Green plants have the widest range of synthetic activity and are the source of numerous beneficial chemicals. Coincidentally, over the past ten years, there have also been an increasing number of in-depth research on extracts and biologically active chemicals obtained from plant species used in herbal medicine or natural therapies. *Terminalia mantaly* is a plant used in herbal medicine. (Ebele OP et al. 2021).

Terminalia mantaly is also called umbrella tree. It is a plant of the family of Combretaceae used in traditional medicinal practice in Madagascar, where its stem bark and leaves are used for the treatment of dysentery, mouth candidiasis, and postpartum care. There are almost 300 species of huge trees in the genus *Terminalia*, which is found across tropical areas of the world and belongs to the flowering plant family Combretaceae. Malaria is only one of the many ailments or symptoms that *Terminalia mantaly* are frequently used to treat by traditional healers (Titanji et al. 2008; Ngouana et al. 2015). In Côte d'Ivoire, the leaves are used in the treatment of malaria. *Terminalia mantaly* is a plant of the Cameroonian pharmacopeia used for malaria and/or related symptoms (Tali et al. 2020).

MATERIALS AND METHODS

Fresh plant of *Terminalia mantaly* H. Perrier was collected from the premises of Kebbi State University of Science and Technology Aliero around March 2019 and was authenticated at the herbarium in the Department of Plant Science and Biotechnology in Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria with voucher No-315.

The fresh leaves, roots, and stem bark of *Terminalia mantaly* were shade dried in the laboratory at room temperature separately. The dried leaves, roots, and stem bark were pounded to powder using mortar separately. Each sample was cold macerated in 70% ethanol at room temperature for 72 hours and then filtered using a muslin cloth. The filtrates were dried in a rotary evaporator at 70^{o}C. The extracts were stored in the freezer until required for use.

Plasmodium falciparum was obtained from the General laboratory in Sir Yahaya Memorial Hospital Birnin Kebbi, Kebbi State, Nigeria, and was taken to Zoology laboratory in Kebbi State University of Science and Technology Aliero, Kebbi State, Nigeria.

The phytochemical analysis was carried out using standard procedures outlined in the Analytical methods of the Association of Official Analytical Chemists (AOAC 1990) to screen the presence of bioactive compounds: tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids, and balsam.

This was carried out according to the technique described by Trager and Jensen (1976). A packet of Rosewell Memorial pack Institute (RPMI) 1640 medium (containing 25 mM of HEPES buffer, glucose) was dissolved in 960 ml of distilled water, and 40 µg/ml of gentamycin sulfate (1.2 ml of Gentamycin/L) was added. It was passed through a millipore filter of 0.22 µm porosity and was stored at 4ºC in 96 ml aliquots in a glass media bottle. Exactly 4.2 ml of 5% sodium bicarbonate (5 gms of sodium bicarbonate dissolved in 100 ml double distilled water and filtered through a millipore filter of 0.22 µm porosity and stored at 4°C) was added to 96 ml of stock RPMI 1640 media (incomplete media). O+ blood was collected in a centrifuge tube without anticoagulant and kept at 4°C. It was centrifuged at 10000 x g for 20 min at 4°C the next day. Serum collected was separated aseptically and kept in aliquots. The serum was inactivated by using a water bath at 56°C for half an hour. Normal inactivated O+ human serum (10 ml) was added to 90 ml of incomplete media to make complete malaria media (CMM).

Uninfected cells were added to 0.75 % of parasitaemia and diluted with CMM to get 0.5 % cell suspension (5 % hematocrit). The culture was kept in a candle jar in an atmosphere of CO_2 at 37°C for 48 hours. After every 48 hours the media was removed using a sterile Pasteur pipette without disturbing the cells that settled down. Then the cells mixed without frothing and a drop of blood was placed on the slide to make a thin film. The prepared thin film was stained and examined for parasitaemia.

This was done in 96 well plates according to the method of WHO (In-vitro micro test (Mark, III) by assessing the maturation of the schizonts. Rosewell Memorial pack Institute (RPMI) 1640 was incorporated. Dilution was prepared from the extracts of leaves, roots and stem bark of *Terminalia mantaly* at concentrations of 300 mg, 150 mg and 75 mg in four groups each. Positive controls were treated with 1 ml Arthemether injection and negative controls were untreated. 200 microliters from blood mixture with media was added to each well in the plate and was incubated for 48 hours in a CO_2 incubator at 37ºC. The samples were harvested and smeared on slides. The blood films were fixed with methanol, stained with Geimsa at pH 7.2 for 10 minutes and examined under the microscope for the presence of parasites. The parasite density was calculated for each well in the plate by comparing the parasitaemia in each group. The antimalarial activity of the plant extracts at different concentrations were recorded. Percentage parasitaemia was obtained using the formula below. At each concentration of the extract, number of parasites were counted in quadrant, their mean and standard deviation were recorded and percentage parasitaemia was calculated using the formula below (Hagazy et al. 2020).

$$\% \text{ parasitaemia} = \frac{\text{Number of Parasitized cells}}{\text{Total Number of cells}} X100$$

RESULTS

Phytochemical screening of *Terminalia mantaly*

The phytochemical screening revealed the presence of tannins, alkaloids, saponins, flavonoids, glycosides, steroids, phenols and balsam, whereas terpenes are completely absent.

The composition of tannins in stem bark and leaves is much, whereas little in roots. Alkaloids and phenols are little in both three parts of the plant. The composition of saponins is very much in stem bark, whereas much in leaves and little in roots. Flavonoids are much in stem bark but little in leaves and roots. The composition of glycosides, steroids and balsam are much in all the three parts of the plant but terpenes are completely absent in all the parts of the experimental plant.

Table 1. The phytochemical screening of *Terminalia mantaly*

Phytochemicals	Stem bark	Leaves	Roots
Tannins	++	++	+
Alkaloids	+	+	+
Saponins	+++	++	+
Flavonoids	++	+	+
Glycosides	++	++	++
Steroids	++	++	++
Phenols	+	+	+
Balsam	++	++	++
Terpenes	-	-	-

KEY: +++= Very much, ++= Much, += Little, -=Absent

The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation

The result of in vitro antimalarial activity of the ethanolic extract of roots, leaves and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation are presented in Table 2. The stem bark ethanol extracts of the plant were recorded as follows: parasitaemia rate at 300 mg was 2.04% (3.50±4.43), parasitaemia rate at 150 mg was 23.32% (40.00±7.07), and parasitaemia rate at 75 mg was 30.76% (52.75±10.68). For positive control, the parasitaemia rate recorded after 24 hours of incubation was 1.17% (2.00±2.82).

The leaves ethanol extracts of the plant recorded after 24 hours were as follows: parasitaemia rate at 300 mg was 16.67% (53.75±65.04), parasitaemia rate at 150 mg was 26.12% (84.25±60.84), and parasitaemia rate at 75 mg was 47.36% (1.52±66.30), while the parasitaemia rate for positive control was 4.34% (14.00±23.33). The roots ethanol extracts of the plant recorded after 24 hours were as follows: parasitaemia rate at 300 mg was 23.67% (60.20±62.82), parasitaemia rate at 150 mg was 35.46% (90.75±49.21), parasitaemia rate at 75 mg was 64.44% (1.64±65.09), and parasitaemia rate for positive control was 1.57% (4.00 ±2.00) (Table 2).

Table 2. The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 24 hours of incubation

Concentration	(Mean ± SD)					
	Stem bark		Leaves		Roots	
	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %
Positive Ctrl	2.00 ± 2.82[a]	1.17%	14.00 ± 23.33[a]	4.34%	4.00 ± 2.00[a]	1.57%
300 mg	3.50 ± 4.43[a]	2.04%	53.75 ± 65.04[a]	16.67%	60.25 ± 62.82[ab]	23.67%
150 mg	40.00 ± 7.07[b]	23.32%	84.25 ± 60.84[ab]	26.12%	90.75 ± 49.21[bc]	35.46%
75 mg	52.75 ± 10.68[c]	30.76%	1.52 ± 66.30[b]	47.36%	1.64 ± 65.09[c]	64.44%

KEY: SD= standard deviation, Superscripts= means followed by the same superscripts are statistically the same with the control using one-way ANOVA post hoc (Duncan) at p< 0.05, others had statistically significant lower antimalarial activity with the control (Positive).

Table 3. The *In-vitro* antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation

Concentration	(Mean ± SD)					
	Stem bark		Leaves		Roots	
	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %	Parasite count	Parasitaemia %
Positive Ctrl	0.00 ± 0.00[a]	0.00%	2.75 ± 4.85[a]	0.57%	0.75 ± 0.95[a]	0.08%
300 mg	0.00 ± 0.00[a]	0.00%	5.50 ± 7.14[a]	1.15%	50.25 ± 62.99[ab]	16.48%
150 mg	1.50 ± 1.29[c]	0.87%	24.00 ± 18.18[a]	5.00%	74.50 ± 52.00[ab]	24.45%
75 mg	2.50 ± 1.29[c]	1.45%	57.0 ± 35.18[b]	11.88%	1.10 ± 48.09[c]	36.23%

KEY: SD= standard deviation, Superscripts= means followed by the same superscripts are statistically the same with the control using one-way ANOVA post hoc (Duncan) at p< 0.05, others had statistically significant lower antimalarial activity with the control (Positive).

The in vitro antimalarial potential of the ethanolic extracts of roots, leaves, and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation.

The result of in vitro antimalarial activity of the ethanolic extract of roots, leaves and stem bark of *Terminalia mantaly* on *Plasmodium falciparum* after 48 hours of incubation are presented in Table 3.

The stem bark ethanol extracts of the plant after 48 hours of incubation at 300 mg were recorded: parasitaemia rate at 300 mg was 0.00% (0.00±0.00), parasitaemia rate at 150 mg was 0.87% (1.50±1.29), parasitaemia rate at 75 mg was 1.45% (2.50±1.29), and parasitaemia rate for positive control was 0.00% (0.00±0.00).

The leaves ethanol extracts of the plant at 300 mg were recorded 1.15% (5.50±7.14) for parasitaemia rate after 48 hours of incubation, 5.00% (24.04±18.18) for parasitaemia rate at 150 mg, 11.88% (57.00±35.18) for parasitaemia rate at 75 mg, and 0.57% (2.75±4.85) for positive control. The roots ethanol extracts of the plant at 300 mg were recorded 16.48% (50.25±62.99) for parasitaemia rate after 48 hours of incubation, 24.45% (74.50±52.00) for parasitaemia rate at 150 mg, 36.23% (1.10±48.09) for parasitaemia rate at 75 mg, and 0.08% (0.75±0.95) for positive control.

DISCUSSION

The phytochemical screening of the extracts showed the presence of tannins, alkaloids, saponins, flavonoids, glycosides, phenol, steroids and balsam. It agreed with the findings of (Tali et al. 2020) and (Mbouna et al. 2018). However, glycosides and balsam were not detected. It could be a result of genetic and environmental factors as they influenced the content and composition of secondary metabolites in plants. Similarly, these findings were in line with the findings of Mudi and Muhammad (2009). However, phenols were not detected and this could be a result of species differences as they worked with *Terminalia catappa*. Similarly, this work was in agreement with the work of Emilie et al. (2015), but tannins were not detected. Biological activity is attributed to the presence of various secondary metabolites in plants. Alkaloids, flavonoids, and saponins are the active constituents with an antimalarial activity which reduced parasitaemia in extract-treated groups in vitro antiplasmodial activity and *Plasmodium berghei* infected rats, hence prolonged the life span of the rats in the experiment. The quantity of phytochemical constituents of the plant determines the extent of its bioactivity. Likewise, the presence of more than one secondary metabolite in a plant determines the extent of its bioactivity as reported by (Musila *et al*. 2013). Alkaloids are the major classes of compounds with antimalarial activity and are detected in three parts of the plant. Alkaloids, steroids, and

saponins have been reported to be detrimental to several infectious protozoans such as *Plasmodium falciparum* and these bioactive compounds have been reported to have many medicinal purposes and play a vital role in the antimalarial activity.

The result of in vitro antimalarial activity showed that the stem bark ethanolic extract of *Terminalia mantaly* had the highest antimalarial activity on *Plasmodium falciparum*. Stem bark completely cleared the parasites when treated with 300 mg of the extract after 48 hours of incubation and there was no statistically significant difference of $p<0.05$ in their result when compared with the positive control. The antimalarial activity of stem bark aqueous extract was reported by (Tali et al. 2020). It was in agreement with the findings in this study as they found out that the stem bark of *Terminalia mantaly* presented the highest antiplasmodial activities in vitro on both resistant and sensitive strains of *P. falciparum* with IC50*Pf*W2 = 0.809 µg/ml. Similarly, Anti-*Plasmodium falciparum* activity of extracts from 10 Cameroonian medicinal plants was reported by Marie et al. (2018). It agreed with these findings as they reported that the highest SI values were obtained for the decoction extract of leaves and stem bark of *Terminalia mantaly* (SI>80.32). Decoction extracts of leaves of *Terminalia catappa* and *Terminalia mantaly* were considered of interest since they displayed high antiplasmodial activity (IC50 = 1.90–8.10 µg/mL) with high selectivity indices (SI > 31.20) against both *Plasmodium falciparum* 3D7 and INDO strains. The findings confirmed the use of many of these plants in the treatment of malaria and related symptoms.

Strength and limitation

This study proposes a treatment strategy that utilizes locally available and affordable materials. It emphasizes the significance of inexpensive and readily available malaria treatment, as this disease is frequent in low- and middle-income countries. Clinical trial are still necessary to demonstrate the efficacy of Terminalia mantaly ethanolic extract as a malaria treatment.

CONCLUSION

Phytochemical screening revealed the presence of some secondary metabolites at different concentrations in the three parts of the plant. Alkaloids, flavonoids, and saponins were the active constituents that could be the reason for the antimalarial activity on *Plasmodium falciparum* as they were reported to be the secondary metabolites responsible for antimalarial activity.

All the plant part extracts in vitro analysis showed antimalarial activity on *Plasmodium falciparum* on different doses of the plant extract and this study showed that the extract from the stem bark has great potential to cure malaria as it clears the malaria parasites after 48 hours of incubation in a CO_2 incubator. More research is recommended on the use of *Terminalia mantaly* to treat other diseases in order to develop new drugs for the treatment of a variety of illnesses to which the parasites are not resistant in order to reduce the global burden.

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Conflict of interest

We declare no conflicts of interest.

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Author contribution

BMU wrote, verified the analytical methods, and revise the manuscript from reviewer, DDA and DYK checked the sources and conceived the original manuscript.

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