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The Anti-malarial Potential of the Alkaloids Present in *Mangifera indica Linn* Leaves Extract

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Abstract

With the continuous emergence of malaria infected cases as a result of rise in resistance to antimalarial drugs, the search for alternative anti-malarial therapy in the management of these malaria parasites using plant extracts has been on the rise. In this research the extract of *Mangifera indica* Linn plant leaves was investigated for the presence of alkaloids and their use as antimalarial agents. The *M. indica* L. leaves tested positive for the presence of alkaloids. The antimalarial test was carried out at 24 and 48 hours incubation using a blood sample containing 5 % parasitaemia and Artemether as a standard control was used to determine the antiplasmodial activity of the plant leaves extract. All the different concentrations of the plant extracts; 10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$ and 1.25 $mgml^{-1}$ tested positive for antimalarial activity when screened. The highest concentration 10 $mgml^{-1}$ showed the most elimination of the malaria parasite at both 24 and 48 hours. The leaves extracts of *Mangifera indica* Linn showed a 56 % elimination of *Plasmodium falciparum*. The investigation results of the antimalarial activity are promising and show that *Mangofera indica* Linn could be used in the management of malaria.

Keywords: Antimalarial, Alkaloids, *Mangifera indica* Linn leaves, Antimalarial agents.

Introduction

The World Health Organisation (WHO) reported that in Africa, malaria is attributed to 20-30 % mortality of children under the age of five [1]. Malaria is commonly caused by four main species of Plasmodium parasite, amongst which *Plasmodium falciparum* causes the most severe malaria effect especially in most African countries [1-3]. Malaria is a disease treated with a variety of anti-malaria medications either as single therapy (Chloroquine) or as a combination therapy (ACT (Artemisinin)) [4-6]. Though these medications are readily available, reported malaria cases continue to rise due to misuse of prescriptions which has inevitably led to rise in anti-malarial drug resistance [7]. Natural products have been used traditionally to manage and treat malaria disease with great success. Some antimalarial agents were isolated from plants and majority of these agents belong to alkaloids class of compounds [8].

Quinine an aminoquinoline alkaloid originally extracted from *Cinchona* tree is an antimalarial agent which has been used since the early 1800s [9,10]. In 2008, a review published by Frederich and collaborators reported 31 indole alkaloids with antiplasmodial activity and by 2009 several crude extracts with antiplasmodial activity were published [11]. Other review works on antiplasmodial agents from plant sources have subsequently highlighted the likely presence of alkaloid in the various plant species amongst the reported plants was *Mangifera indica* Linn. *Mangifera indica* Linn popularly known as mango tree is a tropical plant found mainly in Asia and Africa produces mango fruit that is widely consumed and enjoyed by most people around the world [12]. Mango tree leaves and stem bark have been traditionally used to cure several ailments such as hypertension and malaria [13-14].

Materials and Method

Plant material Collection

Mangifera indica Linn leaves were collected from the Umaru Musa Yar'adua University garden, Katsina state.

Plant Preparation and Extraction

The leaves of *M. indica* L. were washed with water, air-dried then pulverized to a powdered form stored before storing in an air-tight container. About 40 g of ground mango leaves was macerated in 200 mL of ethanol for 48 hrs. The mixture was then filtered and evaporated under reduced pressure to yield the crude extract. Distilled water (100 mL) was added to the crude extract to make a mixture. The pH of the resulting mixture was adjusted from 3.4 to 10.50 by the addition of a few drops of concentrated sodium hydroxide. Liquid-liquid partitioning was carried out on the resulting mixture using 3 x 100 mL of dichloromethane (DCM) both layers were collected, the DCM solution was concentrated under reduced pressure to afford a brownish residue.

Alkaloid screening of DCM extract

Qualitative phytochemical screening for the presence of alkaloids was carried out on the DCM extract. All procedures were developed at room temperature. The extract was used for the subsequent qualitative analysis of metabolites using the method described by Junaid and Patil in 2020 [15].

Test for alkaloids

The DCM extract was dissolved in dilute hydrochloric acid and filtered. Wagner's reagent was prepared by mixing 2 g of potassium iodide and 1.30 g of iodine crystal in 100 ml of distilled water, and then added to the previously obtained filtrate. The presence of alkaloids was confirmed with the appearance of reddish-brown precipitate.

Antimalarial screening of crude DCM extract

The antimalarial activity of the leaves extract of *M. indica* L was determined using blood sample with 5% parasitaemia collected from Bayero University Care Hospital Kano. Malaria parasite was identified by the ring shape of the immature trophozoites which retained blue colour of the stain.

Parasitaemia levels and species parasites were recorded as described by Chotivanich *et al.* [16].

Separation of the erythrocytes

Blood sample with 5% parasitaemia collected from Bayero University Care Hospital Kano was centrifuged at 2500 rm^{-1} for 15 mins. After centrifugation, the supernatant (plasma) was discarded while the sediments (erythrocytes) were further centrifuged with normal saline at 2500 rm^{-1} for 5 mins. The supernatant was then discarded and the erythrocytes were suspended in normal saline.

***In vitro* anti-malarial assay**

Mangifera indica Linn leaves extract was screened for antimalarial activity against the *P. falciparum* strain. The *P. falciparum* strain was cultivated by a modified method described by Trager and Jensen [18]. The extracts were dissolved in Dimethyl sulfoxide (DMSO). The final concentration of DMSO used did not interfere or interact with the assay. Stock solution was prepared by dissolving 1.0 g of the extract in 1ml of DMSO. Using serial doubling dilution, four different concentrations (10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$ and 1.25 $mgml^{-1}$) of the extract was prepared. The antiparasitic effect of the compounds was measured by growth inhibition percentage as described by Carvalho and Krettli [17]. The culture media of 1 L of RPMI 1640 liquid media was prepared by dissolving 10.4 g of the powdered RPMI 1640 into 1 L of distilled water before autoclaving at 121 ℃ for 15 mins after which the solution was allowed to cool to room temperature. Afterwards, 0.5 mL of the different extract concentrations (10 $mgml^{-1}$, 5 $mgml^{-1}$, 2.5 $mgml^{-1}$, 1.25 $mgml^{-1}$) and 0.5 mL RPMI 1640 media were each transferred into their clearly labelled test-tubes. To each concentration of the extract, 0.1 ml of the malaria positive erythrocytes was added and shaken gently to ensure even distribution of the erythrocytes. The test tubes were transferred into a bell jar containing a burning candle. The cover of the bell jar was then replaced until the flame of the candle stopped burning. This supplied about 95 % Nitrogen, 2 % Oxygen, and 3 % Carbon dioxide as described by Trager *et al.*, 1976 [18]. The whole set up was transferred into a 35 ˚C pre-heated incubator for 24 h to 48 hrs. A control group consisting of culture media and positive erythrocytes (negative control) and culture media positive erythrocytes and anti-malarial agent Artemether (positive control) was also incubated along with the test concentrations. After 24 hrs of incubation, a thin smear from test tube was transferred onto clean glass slides and fixed in absolute methanol (CH_3OH) then stained with Giemsa's stain. Each smear was observed under microscope using oil immersion to count the number of infected erythrocytes [17-19].

Results and Discussion

Results

Phytochemicals of the extracts of *Mangifera indica* Linn leaves

The qualitative phytochemical analysis to test for the presence of alkaloid in the crude DCM extract showed the presence of alkaloids in the leaves of *M. indica* L plant. The pH of the crude extract

which was initially 3.4 was adjusted to 10.5 to enable the efficient extraction of alkaloids.

In vitro anti-malarial assay

Mangifera indica Linn leaves extract was screened for its *in vitro* antimalarial activity against the *P. falciparum* strain using artemether as control. The anti-malarial activity of the extracts was tested after 24 h and 48 h incubation respectively to determine growth inhibition of the parasite. At 24 hrs, death of some *P. falciparum* were observed at all concentrations of the crude extract with 10 $mgml^{-1}$ showing the highest death of 6 parasites and 1.25 $mgml^{-1}$ showing the lowest death 1 parasite. At 48 h, death rate at each concentration increased with 10 $mgml^{-1}$ still presenting the highest parasite death count of 8 and 1.25 $mgml^{-1}$ presenting the lowest death count of 2 parasites. On average after 48 hrs death of parasites were observed at all concentrations; 10, 5, 2.5 and 1.25 $mgml^{-1}$. Whereas, for the standard control (artemether) after 48 h the death of all the parasites were observed at the different concentrations 10, 5, 2.5 and 1.25 $mgml^{-1}$ (Table 1).

Table 1: Raw data analysis at each concentration activity of the extract and standard control (Artemether) after incubation.

Parasites dead after incubation period	Concentrations of DCM extract $mgmL^{-1}$			
	10	5	2.5	1.25
24 h	6	4	2	1
48 h	8	5	3	2
Artemether (control)- All parasites were dead after 48 h at all concentrations				

The percentage elimination at the end of the incubation was determined for all the concentrations of the crude extract (Table 2). The results showed a 56 % elimination of the *P. falciparum* parasite by the crude leave extract while the standard control (artemeter) showed 2 % elimination at the end of the incubation.

Table 2: Anti-plasmodial activity of the extract and standard control (Artemether) against the malaria parasites

	Crude DCM Extract (at all concentrations)	Artemether (Control)
Average number of parasites before incubation	72	72
Average number of parasites after incubation	41	2
Total number of parasites dead after incubation	31	70
Percentage of elimination at the end of incubation.(%)	56	3
%=N/NxX100		

Where N is the total number of the parasites after incubation and Nx is the total number of the parasites before incubation.

Discussion

The qualitative phytochemical screening for the presence of alkaloids in *M. indica* L leaves extract tested positive for alkaloids. Alkaloids are a class of nitrogen-containing organic compounds which have been used as analgesics, anti-cancer, antibiotics, narcotics and anti-malaria amongst others. The wide range of alkaloid applications and its presence in nature means that alkaloid containing plants can be used as treatment for a variety of ailments including malaria. Based on the positive results observed the pH of aqueous extract was adjusted to 10.5 to ensure efficient and sufficient extract of alkaloids. Alkaloids are basic compounds which based on their polarity and affinity to the aqueous solution needs to have the pH adjusted to ensure alkaloids are extracted into the organic solvent [20].

The DCM extract was then subjected to antimalarial screening against *P. falciparum* and artemether as the positive control (Table 1). The Table shows different concentrations of the extract tested against *P. falciparum* at 24 and 48 h after incubation. At all the concentrations parasitic death was observed suggesting that bioactive compound(s) present in the extract are anti-parasitic components. After 24 h and 48 h incubation, at 1.25 $mgmL^{-1}$ the death rate of the parasites was observed but at a very slow rate. However, from 2.5 - 10 $mgmL^{-1}$ the total number dead parasites gradually increases with their highest death observed after 48 hrs. From literature alkaloids such as quinines are good

antimalarial agents we postulated that an alkaloid was the likely bioactive compound responsible [11].

The percentage elimination observed from the extracts was compared with that of the artemether the positive control (Table 2) and as expected a significant difference in their elimination was observed. Artemether is a potent antimalarial used to treat malaria however, accessibility, rise in resistance to treatment has led to the search for alternatives such as *M. indica* Linn to be used as management of malaria.

Conclusion

The presence of alkaloids was confirmed in *M. indica* Linn leaves after qualitative screening. The antimalarial activity of the extract at different concentrations against *P. falciparum* was carried out. The results showed mortality of the parasite at all concentrations with 1.25 $mgmL^{-1}$ as the minimum inhibitory concentration (MIC) for antimalarial activity against *P. falciparum*.

References

1. WHO guidelines for malaria, 16 October 2023. Geneva: World Health Organization; 2023 (WHO/UCN/GMP/ 2023.01 Rev.1). License: CC BY-NC-SA 3.0 IGO.
2. Snow RW. (2015) Global malaria eradication and the importance of Plasmodium falciparum epidemiology in Africa. BMC Med 2015 Feb 03;13:23
3. Garcia LS (2010). Malaria. Clin Lab Med. (1):93-129.
4. Zekar L and Sharman T. Plasmodium falciparum Malaria. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treaseure Island (FL): StatPearls Publishing; 2025 Jan.
5. Goel P & Gerriets V. (2023). Chloroquine. StatPearls. Treasure Island.
6. Nosten F & White NJ. (2007). Artemisinin-Based Combination Treatment of Falciparum Malaria. In: Breman JG, Alilio MS, White NJ. Defining and Defeating the Intolerable Burden of Malaria III: Progress and Perspectives: *Am. J. Trop. Med.* Hyg. 77(6) 181-92 Available from: https://www.ncbi.nlm.nih.gov/books/NBK1713/.
7. Plowe CV. (2022). Malaria chemoprevention and Drug Resistance: a review of the literature and policy implications. *Malar J* 21, 104. https://doi.org/10.186/s12936-022-04115-8.
8. Uzor PF, Prasasty VD & Agubata CO. (2020). Natural Products as Sources of Antimalarial Drugs. Evidence-Based Complementary and Alternative Medicine 1. https://doi.org/10.1155/2020/9385125.
9. Brown WC. (1911) The present position of the quinine prophylaxis of malaria. *Trans R Soc Trop Med Hyg*. 4:193-209.
10. Thomson J. (1917) Quinine in malaria: its limitation and possibilities. Trans R Soc Trop Med Hyg. 11:226-231.
11. Frederich M, Tits M and Angenot L. (2008). Potential antimalarial activity of indole alkaloids. *Trans R Soc Trop Med Hyg*. 102;1:11-19.
12. Igoli JO, Ogaji OG, Tor-Anyiin TA and Igoli NP. (2005) Traditional medicine practice amongst the Igede people of Nigeria. *Part II African Journal of Traditional Complementary and Alternative Medicine*, 2:134-152.

13. Ajaiyeoba EO, Abiodun OO, Falade MO, Ogbole NO, Ashidi JS, Happi CT and Akinboye DO. (2006). In vitro cytotoxicity studies of 20 plants used in Nigerian antimalarial ethnomedicine. *Phytomedicine*, 13:295-298
14. Awe SO, Olajide OA, Oladiran OO and Makinde JM. (1998). Antiplasmodial and antipyretic screening of Mangifera indica extract Phytotherapy Research, 12:437-438.
15. Junaid S & Patil M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. Int J. Chem. studies.; 8:603-608. DOI-10.22271/chemi.2020.v8.i2i.8834.
16. Chotivanich, K., Silamut, K., & Day, N. P. (2006). Laboratory diagnosis of malaria infection. Aust. J. Med. Sci., 27(1), 11-15.
17. Carvalho LH & Krettli AU. (1991). Antimalarial chemotherapy with natural products and chemically defined molecules. Mem. Inst. Oswaldo. Cruz.; 86: 181–184.
18. Trager, W. & Jensen, J.B. (1976). Human Malaria Parasites in Continuous Culture. Sci., 193, 673-675. http://dx.doi.org/10.1126/science.781840.
19. Makler MT, Ries JM, Williams JA, Bancroft JE, Piper RC, Gibbins BL & Hinrichs DJ. (1993). Parasite lactate dehydrogenase as an assay for Plasmodium falciparum drug sensitivity. Am J Trop Med Hyg., 48(6):739-41. doi: 10.4269/ajtmh.1993.48.739.
20. Qiu S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y & Wang XJ. (2014). Natural alkaloids: basic aspects, biological roles, and future perspectives. Chin. J. Nat. Med. 12(6):401-6. doi: 10.1016/S1875-5364(14)60063-7.