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ANTIPLASMODIAL ACTIVITIES OF CRUDE *Moringa oleifera* LEAVES EXTRACTS ON CHLOROQUINE SENSITIVE *Plasmodium falciparum* (3D7)

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ABSTRACT

The antimalarial efficacy of crude hexane, methanol and lyophilized aqueous Moringa oleifera leaf extract was evaluated on chloroquine sensitive (CQS) strain of Plasmodium falciparum (3D7) in vitro, with a view to validate traditional use of M. oleifera as antimalarial. A dose dependent suppression of parasite growth was observed for all extracts, at microgram per mills (µg/mL) concentrations. At the lowest concentration of extract (6.25 µg/mL), the hexane, methanol and lyophilized aqueous extracts showed 63.52%, 71.42% and 60.65% suppression of plasmodium growth respectively. The extracts showed potent biological activity at the highest concentration of extract, with a strong inhibition of plasmodium growth (71.31%, 83.06% and 80.36%) observed for the hexane, methanol and lyophilized aqueous extracts. Although some extracts are observed to be more potent than others, all extracts are observed to be biologically active against the 3D7 strain of P. falciparum (Hexane extract IC_{50} = 3.36 µg/mL; methanol IC_{50} = 3.44 µg/mL and aqueous IC_{50} = 4.09 µg/mL respectively). The antiplasmodial activities observed may well be attributed to the presence of phenols, tannins, alkaloids and flavonoids in all solvent extracts.

Key words: Antiplasmodial, Moringa oleifera, Phytochemical screening, Plasmodium falciparum

INTRODUCTION

Malaria, a common cause of fever in endemic countries, is thought to be the leading cause of low birth weight, neonatal death as well as maternal mortality (Haruna and Daskum, 2018). The disease is caused by one of the four main species of plasmodium parasites; *P. falciparum, P. vivax, P. ovale,* and *P. malariae* (Ortiz-Ruiz *et al*., 2018). Recently, a 5th species known to infect non-human primates, *P. knowlesi*, was reported to cause zoonotic malaria (Sabbatani *et al.,* 2010). Amongst these, *P. falciparum* is the most virulent and causes most of malaria mortality, while *P. vivax* is the most widespread (Flannery *et al.,* 2013). Malaria is often characterized by a vague absence of wellbeing, headache, fatigue, muscle aches, and abdominal discomfort, which are followed by nausea, vomiting, and recurrent high fevers and, neurological impairments such as brain damage and coma, in the case of cerebral malaria (White *et al.,* 2014). An estimated 3.4 billion individuals, almost half of the world's population are at risk of getting infected with the disease, with people living in the poorest countries of the world being the most vulnerable to malaria (Tekwani and Walker 2005). Similarly, immuno compromised individuals such as HIV/AIDS patients and travelers to endemic areas are primarily susceptible to the dangers of infection (Andrews *et al.,* 2014).

A clinically ample resistance to all classes of antimalarial drugs, possibly with the exception of artemisinin combination therapies (Barnes and White, 2005) was reported. However, evidence of resistance to artemisinin derivatives (Dondorp *et al.,* 2009; White *et al.,* 2014), defined as "delayed parasite clearance following treatment with an artesunate monotherapy, or after treatment with an artemisinin-based combination therapy (ACT)" (Ringwald, 2015) had since emerged.

Despite the availability of modern medicines in clinical use, traditional herbal medicines maintain their popularity because of historical and cultural reasons as well as their cheaper costs (Lawal *et al.,* 2015). In many developing countries, one in five of malaria patients use indeginous herbal remedies to treat the disease (Adebayo and Krettli, 2011). In traditional medicinal practice, the use of *M. oleifera* to cure diseases dates back to centuries in many cultures around the world (Mahmood *et al.,* 2010). In many parts of Nigeria for example, a decoction of the fresh leaves of *M. oleifera* is used to treat typhoid and malaria fevers (Stevens *et al.,* 2013).

In this study, the antimalarial efficacy of crude hexane, methanol and aqueous *M. oleifera* leaf extract was assessed against the chloroquine sensitive strain (3D7) of *Plasmodium falciparum, in vitro*.

MATERIALS AND METHODS

Identification and collection: Identification and collection of *M. oleifera* was perforemed in the botanical garden of the Department of Biological Sciences, Yobe State University, Damaturu, Nigeria. Following identification, fresh leaves were hand-picked, washed and air dried under shade. Dried samples were treated in accordance with standard procedures (Bukar *et al.,* 2009; Yusuf *et al.,* 2014). Phytochemical extraction of secondary metabolites was performed as per Mojarrab *et al.* (2014). Subsequent to extraction, qualitative screening of crude extracts was performed in accordance with Kumar *et al.* (2013).

Parasite culture: Chloroquine sensitive *Plasmodium falciparum* (3D7) was propagated in blood group O+ (2-5% hematocrit) and maintained in continous culture. RPMI 1640 (gibco) media supplemented with Na_2CO_3, glucose, Hypoxanthine, gentamicin, L-glutamine and Albumax was prepared in line with the protocol of Moon *et al.* (2013). Parasite culture was gassed (88% Nitrogen, 7% Carbon dioxide and 5% Oxygen) and incubated at 37^{o}C as previously described (Trager and Jensen, 1976). Culture was monitored on daily basis and medium replenished (D'Alessandro *et al.,* 2013).

Parasite synchronization: Following culture, synchronization was performed by density gradient method with histodenz as described (Amir, 2016). Briefly, parasite culture was pelleted by centrifugation and infected red blood cells (iRBCs) (2mL) were gently layered over 5 mL of histodenz working solution (Plate 1A). The preparation was thereafter, centrifuged at 2000 rpm for 10 minutes with low brakes and acceleration. Three (3) layers (Plate 1B); a brown interphase at the top, layer of histodenz and pelleted cells were formed. The brown interphase containing mainly schizonts was collected and washed once (1800 rpm for 5 min) in culture medium. To confirm stage, a thin smear was prepared and observed microscopically.

Seeding: For *in vitro* antimalarial activity assay, rings and trophozoites stages of synchronized *P. falciparum* culture (1% parasitaemia, 2% hematocrit) were used. Into duplicate wells of a sterile 96 wells flat bottom plate, an aliquot (180 µL) of sychronized culutre was added, following which, 20 µL of various concentrations (500, 250, 125, 62.5 µg/ml) respectively of crude plant extracts was added to columns 1-8 to yield a final concentration (50, 25, 12.5 and 6.25µg/mL) of each extract in the respective wells (Donkor *et al.*, 2015). Columns 9 and 10 received 20 µL of the culture medium to serve as negative controls, while columns 11 and 12 received 20 µL different concentration (1000, 500, 250, 125, 62.50, 31.25 and 15.625 nM/mL) of chloroquine diphosphate ($CQdiPO_4$; Sigma aldrich) to serve as positive control. Microplates were covered, placed in a gas chamber, gassed (88% Nitrogen, 7% Carbon dioxide and 5% Oxygen) and incubated at 37^{o}C for 48 hours. Gassing was repeated after the first 24 hours and gas chamber placed back in the incubator for another 24 hours.

Harvesting: This was performed in line with (Basco, 2007) and percent parasitaemia as well as growth suppression was calculated as per the following formulae;

$$\text{Percentage parasitaemia} = \frac{\text{Number of infected Red Blood Cells (iRBCs)}}{\text{Total number of Red Blood Cells (RBCs)}} \times 100$$

$$\text{Growth suppression (\%)} = \frac{\text{Mean parasitaemia}_{\text{(Negative control)}} - \text{Mean parasitaemia}_{\text{(Treated group)}}}{\text{Mean parasitaemia}_{\text{(Negative control)}}} \times 100$$

RESULTS AND DISCUSSION

Results of phytochemical analysis of three solvent extracts of *M. oleifera* are presented in Table 1. Findings revealed the presence of phenols, tannins, alkaloid and flavonoids in all solvent extracts. However, anthraquinones was not identified in each of the extracts. Biological activities were related to bioactive metabolites in the extracts. This finding corroborate with those of similar studies (Dondee *et al.,* 2016; Somsak *et al.,* 2016; Mulisa *et al.,* 2018) who reported the presence of secondary metabolites, such as alkaloids, polyphenols, flavonoids, terpenoids, quercetin, and kaempferol in the leaf extract of *M. oleifera.*

Table 2 summarizes the percentage parasitaemia, percentage suppression of parasite growth and the IC_{50} of extracts investigated. Results are presented as Mean ± Standard error of mean (M±SEM) except for the IC50. Briefly, a dose dependent suppression of parasite growth was observed for all extracts, at microgram per mills (µg/mL) concentration (Table 2). In line with the recommendations of Tona *et al.* (1999), compounds/extracts are

considered more active when such compound However, suppression of parasite growth by 50% but <70% is considered active while suppression <50% inactive. As per Tona *et al.* (1999), the hexane, methanol and lyophilized aqueous extracts studied herein could be classified as active (≥60% suppression of parasite growth) at the lowest concentration (Table 2). Although some extracts are more potent than others all extracts are observed to be biologically active against the 3D7 strain of *P. falciparum* (Hexane extract IC50 = 3.36 µg/mL; methanol IC50 = 3.44 µg/mL and aqueous IC50 = 4.09 µg/mL respectively). At the lowest concentration of extract, the methanol extract showed potent biological activity (71.42%). At the highest concentration of extract, a strong inhibition (83.06%) of plasmodium growth was observed. This finding validate those of Dondee *et al.* (2016). The possible mechanisms for which the biological activities observed in the leaf extract of *M. oleifera*, in this study, might likely be attributed to; antioxidant effect of the extract (Vergara-Jimenez *et al.,* 2017), free radical scavenging property (Somsak *et al.,* 2016), inhibition of protein synthesis (Dondee *et al.,* 2016) or by other mechanism not known/reported in the literature.

Although, the antiplasmodial activities of each solvent extracts reported in here are dependent on concentration, Donkor *et al.* (2015) reported the contrary. In their work, inhibition of parasite growth by the extracts was observed to increase as the concentration of extracts decreased. Additionally, Donkor and co-workers reported IC50 values that contradicts the findings of this research. The ethanol and aqueous leaf extract reported therein had IC50 values of 15.18 could suppress parasite growth by ≥ 70%. µg/mL and 43.65 µg/mL respectively, in the same parasite strain (3D7) *in vitro*. This variation in antiplasmodial activity was attributed to the activities of some metabolites. Donkor *et al.* (2015) suspected that some metabolites present in the leaf extracts of *M. oleifera*, promote plasmodial growth at high enough concentrations, while others are believed to cause inhibition even at concentrations where the former has no significant activity.

At nM concentrations, ≥60% suppression of plasmodial growth was observed for the lowest concentration (15.63 nM/mL) of the reference antimalarial, chloroquine diphosphate (Table 2). Suppression of plasmodial growth was observed to be directly proportional to drug concentration. As the concentration increases, so does the inhibition of parasite growth. At 125 nM/mL ~90% growth suppression was observed while total clearance was observed at the highest dose 1000nM/mL. This finding further suggest that a much lower concentration comparable to the lowest concentration used, is required to prevent the growth of parasites by 50% (IC50 = 11.79 nM/mL). It was not surprising that the reference drug showed potent antimalarial activity. This was the exact reason for its use as the positive control. Although, the use of chloroquine was officially banned in Nigeria since 2004, the policy was not effected until 2010 (Soniran *et al.,* 2012). However, the drug could still be obtained over the counter in pharmaceutical stores. For safety reasons, availability and affordability by locals, medicinal plants such as *M. oleifera* stand out as good source of antimalarials in local communities.

Table 1: Phytochemical constituents of Hexane, Methanol and Aqueous crude leaves extracts of *Moringa oleifera*

Plant	**Phytochemicals**	**Type of Extract**		
		Hexane	Methanol	Aqueous
M. oleifera	Phenols	+	+	+
	Tannins	+	+	+
	Anthraquinones	-	-	-
	Alkaloids	+	+	+
	Flavonoids	+	+	+

Key: + = present; - = absent

Table 2: *In vitro* antimalarial effect of *M. oleifera* leaves extract against *P. falciparum* (3D7).

Type of extract	Dose (µg/mL)	Parasitaemia (%)	Growth suppression (%)	IC_{50} (µg/mL)
		Mean ± SEM		
Hexane	6.25	2.58 ± 0.17	63.52	3.36
	12.5	2.44 ± 0.13	65.60	
	25	2.28 ± 0.13	67.88	
	50	2.03 ± 0.13	71.31	
Methanol	6.25	2.02 ± 0.04	71.42	3.44
	12.5	1.91 ± 0.12	73.01	
	25	1.60 ± 0.16	77.37	
	50	1.20 ± 0.14	83.06	
Aqueous	6.25	2.79 ± 0.26	60.65	4.09
	12.5	2.47 ± 0.25	65.18	
	25	2.23 ± 0.25	68.52	
	50	1.39 ± 0.09	80.36	
Chloroquine	15.625	2.85 ± 0.59	59.84	11.79
	31.25	1.55 ± 0.03	78.12	
	62.50	0.74 ± 0.06	89.63	
	125	0.68 ± 0.1	90.40	
	250	0.41 ± 0.09	94.28	
	500	0.21 ± 0.05	97.03	
	1000	0 ± 0	100	
Negative control	**CM only**	7.08±1.39	0.00	-

Key: µg/mL = Microgram per mills; SEM = Standard error of mean

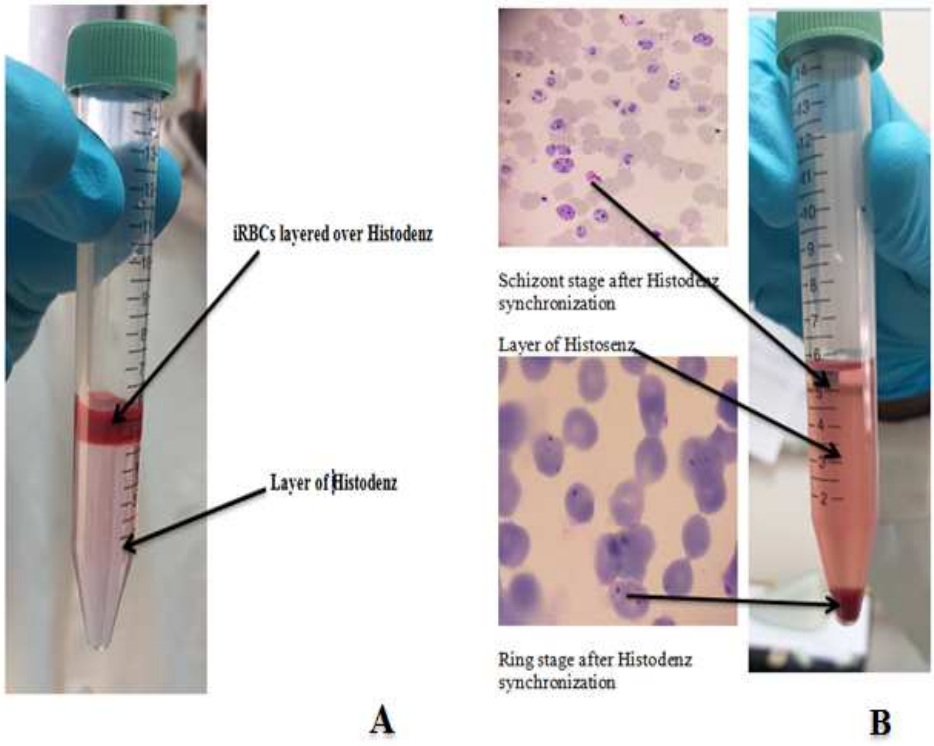

Plate 1: Density gradient synchronization of parasites using Histodenz (A) parasitized red blood cells (iRBCs) layered over histodenz and (B) Formed layers following centrifugation, showing a brown ring at the interphase containing schizonts stages, the layer of histodenz and the pelleted red blood cells containing mainly rings and few trophozoites

CONCLUSION

Based on the findings of this study, it could be concluded that the medicinal plant *M. oleifera* can be a good source of antimalarials. The presence of certain phytochemicals such as phenols, tannins, alkaloid and flavonoids in crude hexane, methanol and lyophilized aqueous extracts may perhaps make this plant a good candidate source for antimalarial formulations. Similarly, the potent antimalarial activities observed for both extracts could be attributed to the presence of the secondary metabolites in *M. oleifera* leaves extracts.

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