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CHEMOTHERAPEUTIC ACTIVITY OF *Azadirachta indica* LEAF EXTRACT AND ITS CYCLODEXTRIN COMPLEX ON *Plasmodium berghei* INFECTED MICE

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ABSTRACT

This study investigated the antimalarial activity of *Azadirachta indica* leaf extract complexed with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) alone and a combination of HP-β-CD and polyvinylpyrrolidone (PVP) with a view to improving the antimalarial activity of the complex. Hot aqueous extract (HAE), hot hydroalcoholic extract (HEE) and cold hydroalcoholic extract (CEE) of *A. indica* were assessed for suppressive and curative *in vivo* antimalarial activity following established procedures. HAE was selected for complexation because aqueous extraction is the most commonly used preparation in ethnomedicine; CEE and HEE did not show superior efficacy in the antimalarial tests. HAE-HP-β-CD complexes (at each of 50, 100, 200 and 400 mg/kg) were prepared by freeze-drying at a weight ratio 1:1. HAE (400 mg/kg) - HP-β-CD-PVP complexes were also prepared with each of 5%, 10%, and 20% w/w PVP. The complexes were subjected to curative tests and data were analyzed. In the suppressive tests, CEE, HAE and HEE produced chemosuppression of 75.0%, 78.2% and 77.7% at 400 mg/kg, respectively. HAE at 400 mg/kg produced the lowest percentage parasitemia on day 5 in the curative tests. In the curative test, HAE (400 mg/kg) - HP-β-CD complex showed superior efficacy, ($F_{[5, 22]} = 5.06$, $P < 0.05$), when compared to complexes of lower doses. HAE (400 mg/kg) alone attained 57.9% chemosuppression on day 6 while HAE (400 mg/kg) – HP-β-CD complex attained 51.5%. HAE-HP-β-CD-PVP complexes gave about 30% chemosuppression. The study concluded that complexation of an aqueous extract of *Azadirachta indica* leaf with HP-β-CD at 400 mg/kg produced significant improvement in the activity of the extract while HP-β-CD and PVP complexes did not significantly improve the curative activity.

Keywords: *Azadirachta indica*, hot aqueous extract, 2-hydroxypropyl-β-cyclodextrin, polyvinylpyrollidone, complexation, *in vivo* curative antimalarial activity.

INTRODUCTION

Azadirachta indica A. Juss (Meliaceae), is particularly well known to the populations of West and East Africa as a reputed folklore antimalarial agent (Iwu, 2014). It is a native of Asia but has now naturalized in West Africa. The neem tree known locally as Dogon Yaro or Darbejiya (Hausa), Ogwu-akom (Igbo), Aforo-Oyinbo (Yoruba) is widely cultivated throughout Nigeria as an ornamental plant. The Natural Products Alert (NAPRALERT) database lists 152 general plant medicines which have been used for the treatment of malaria (Phillipson and Wright, 1999). Neem is regarded as one of the priority plants for ethnomedicine production on this list. It is classically taken as a reference in malaria phytomedicine (Benoit-Vical *et al.,* 2003; Lim, 2014)

Several workers have reported the *in vitro* antimalarial activity of *A. indica* (Khalid *et al.*, 1986; 1989; Bray *et al.* 1990; Udeinya 1993; Dhar *et al.* 1998; Udeinya *et al.* 2003, 2008; Oshikoya *et al.* 2008; Kaur *et al.* 2009; Ogbuewu *et al.* 2011) which was highly active against *Plasmodium falciparum*. Most of the reports of the *in vivo* antimalarial activity evaluation of *A. indica* point to the fact that it exhibits significant prophylactic and suppressive effects *in vivo* but very minimal curative activity (Isah *et al.*, 2003; Oshikoya *et al.*, 2008; Farahna *et al.*, 2010).

Cyclodextrins (CD) are non-toxic macrocyclic biodegradable oligosaccharides which are

obtained biotechnologically in large scale by the enzymatic degradation of starch. They consist of D-(+) glucopyranose units attached by α-(1, 4) glycosidic bonds and contain a relatively lipophilic central cavity and a hydrophilic outer surface. They interact with many compounds by inclusion into the hydrophobic cavity via non-covalent hydrophobic interactions, van der Waals forces and hydrogen bonds (Loftson and Brewster, 1996; Al-Burtomani and Suliman, 2017). Consequently, cyclodextrins can improve the solubility, stability and other physicochemical characteristics of respective guest molecules (Challa *et al.,* 2005; Sá Couto *et al.,* 2014). Cyclodextrins have been used to enhance the solubility of drugs and other substances in aqueous solutions. Drug bioavailability is expected to improve through enhancement of the solubility and dissolution rate (Sharma and Baldi, 2016). The enhanced drug bioavailability is expected to bring about an increase in the pharmacological activity of the drug.

Studies have shown that the use of pharmaceutical polymers enhances the complexation efficacy of the cyclodextrins (Ansari, 2015; Pacheco *et al*., 2018). The use of polymers enhances the capacity of cyclodextrins to include the therapeutically active compound and induces as well as enhances a more rapid release of the active compound out of the pharmaceutical formulation (Challa *et al.,* 2005). Examples of such polymers include hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose and polyvinylpyrrolidone (PVP). PVP is a water-soluble polymer which has been used to enhance the capacity of cyclodextrins to include therapeutically active compounds in their hydrophobic core. Water-soluble polymers participate directly in the complexation process thereby improving both pharmaceutical and biological properties of guest–cyclodextrin complexes, independent of guest's physiochemical properties (Hirlekar *et al.,* 2009).

In this study, *A. indica* leaf extract was complexed with 2-hydroxypropyl-β-cyclodextrin alone and with a combination of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and polyvinylpyrrolidone (PVP). This was done with the aim of evaluating the effect of complexation on the chemotherapeutic activity of *A. indica* leaf extract on established malaria parasite infection in mice.

MATERIALS AND METHODS

Plant material

The leaves of *Azadirachta indica* were collected by noon at Obafemi Awolowo University, Ile- Ife, Nigeria, and identified in the Faculty of Pharmacy Herbarium. A voucher specimen was deposited at Forestry Research Institute of Nigeria for reference (FHI-103435). The leaves were dried in the oven at 40 °C for one week, powdered and stored at room temperature for extraction.

Extraction Procedures

Three different extraction procedures were carried out and 100 g of powdered leaves of *Azadirachta indica* were used in each procedure.

(i) Aqueous Soxhlet Extraction: Powdered leaf (100 g) was extracted with 100 mL of distilled water for 48 h, using the Soxhlet apparatus. The aqueous-derived extract was concentrated under reduced pressure in vacuo at 60°C using a rotary evaporator (Buchi Rotavapor, Switzerland) and lyophilized to give a solid paste. This extraction gave hot aqueous extract (HAE).

(ii) Ethanol - water Soxhlet Extraction: Powdered leaf (100 g) was extracted with 100 mL of 50% ethanol – water for 48 h, using the Soxhlet apparatus. The solvent - derived extract was concentrated and lyophilized as described in (i) above. This extraction produced hot hydroalcoholic extract (HEE).

(iii) Ethanol – water (cold maceration method): Powdered leaf (100 g) was soaked in 500 mL of 50% ethanol - water for 24 h with constant shaking. The residue after filtration was soaked in another 500 mL of 50% ethanol - water for 24 h. The filtrate was pooled, concentrated and lyophilized as described above. This extraction produced cold hydroalcoholic extract (CEE).

Extracts Partitioning

Five grams (5 g) of each of the extracts (HAE, HEE and CEE) was dissolved in 55 mL of distilled water. The solution was poured into a 250 mL separating funnel and partitioned with n-hexane (8 × 10 mL portions). The n-hexane fractions were pooled and concentrated at 40 °C.

The aqueous layer was further partitioned with chloroform (8 × 10 mL portions), the chloroform fractions were pooled and concentrated at 40 °C. Lastly, the aqueous layer was further partitioned with ethyl acetate (8 × 10 mL portions) and the fractions were pooled and concentrated at 40 °C. This procedure was repeated with HAE and HEE.

Thin Layer Chromatography of the fractions

Silica gel pre-coated aluminium plates (Alugram sil G/UV 254) and ultra-violet lamp (254 and 365nm) were used for thin layer chromatography. The three partitioned fractions (n-hexane, chloroform and ethyl-acetate) were spotted on the pre-coated TLC plates after the appropriate solvent system for gedunin (reference compound) was determined to be n-hexane - ethyl acetate 67%:33%. Gedunin (10 mg) was dissolved in 1 mL of chloroform before spotting. About three drops each of gedunin and the extract fractions were spotted separately on the TLC plates and developed with the appropriate solvent system (n-hexane - ethyl acetate 67%:33%) in a shandon tank. The plates were viewed under daylight and under ultra violet light at 254nm and 365nm. The plates were then sprayed with Anisaldehyde solution and scanned immediately after spraying.

Experimental Animals

Female and male adult Swiss albino mice (Vom strain), weighing between 18 and 24 g were used for the experiments. The animals were maintained at 25 ± 2 ºC under natural 12 h daylight/night conditions for at least 5 days before the treatment. All the animals were fed with standard diet and water *ad libitum* in the Department of Pharmacology, Obafemi Awolowo University, Animal House. The "principle of laboratory animal care" (NIH publication No. 85-23) guidelines and procedures were followed in this study. Approval for this research was given by University Research Council (URC) of Obafemi Awolowo University with the approval number URC/05032010/PHP05/06/R/0826. The chloroquine-sensitive strains of *Plasmodium berghei* (NK 65) used in this study was a donation to Professor O. G. Ademowo, Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Nigeria by Malaria Research and Reference Reagent Resource Centre (MR4) U.S.A. The parasites were maintained in animals by serial passages of blood collected from a patent donor mouse to a naive recipient.

Evaluation of antimalarial activity

Three different extracts of *Azadirachta indica;* (HAE, HEE and CEE) were prepared and assessed for their *in vivo* suppressive and curative antimalarial activity, following established procedures described by Peters (1980) and Ryley and Peters (1970), respectively. The hot aqueous extract (HAE) was then complexed with HP-β-CD. The extract at doses of 50, 100, 200 and 400 mg/kg body weight, were dissolved in 5 mL of 50% ethanol. Each of these was mixed (at a weight ratio 1:1) with HP-β-CD dissolved in 5 mL of distilled water. The mixture was agitated with magnetic stirrer (Gallenkamp, England) for 24 h. Separately, HAE (400 mg/kg) – HP-β-CD complex was also complexed with 5%, 10%, and 20% w/w of PVP. The resulting solution was kept in a freezer at -20 °C and lyophilized in a freeze-dryer for 24 h to produce the dried complexes which were stored in airtight containers. The complexes were dissolved in distilled water and assessed for their *in vivo* curative antimalarial activity.

Suppressive tests

This assay protocol was based on Peters (1980) using *P. berghei* NK65 - chloroquine sensitive strain. Suppressive (4-day schizonticidal) tests were carried out on the extracts (CEE, HEE and HAE) in order to determine the one with the highest level of suppressive activity. A diluted inoculum (0.2 mL) containing 1×10^7 parasitized erythrocytes was injected intraperitoneally into four groups of 5 mice per group for each extract. The extracts were administered at 4 dose levels (50, 100, 200 and 400 mg/kg) using a stainless steel feeding needle-oral cannula from day of infection (day 1) up to day 4. A negative control group of 5 mice was administered 10ml/kg of normal saline solution while Chloroquine base (10 mg/kg) was administered to another group of 5 mice (positive control group).

On day 4 (96 h post-infection), thin blood films from the tail vein of the mice were prepared and stained with 10% Giemsa for 10 min, rinsed with

distilled water and allowed to dry. The smears were dried and by the aid of immersion oil using a light microscope. The average percentage chemosuppression expressed in relation to the negative control result were determined using the formula:

$$\text{Average \% chemosuppression} = \frac{\text{average parasitaemia in test group}}{\text{average parasitaemia in control}} \times \frac{100}{1}$$

Curative tests

This mode of malaria treatment in experimental mice was carried out according to the method of Ryley and Peters (1970). The mice inoculated with the parasite were left until parasitemia was established before commencement of treatment. Thereafter, the three extracts (CEE, HAE and HEE) were administered at four dose levels (50, 100, 200 and 400 mg/kg) as previously described for five consecutive days. Thin blood smears of each animal were made daily for 5 days. The survival time was determined after observing the animals for 28 days in each group noting and recording the number of deaths per day throughout the 28 days period of conducting the experiment. The survival time per group is the average of number of days the animals in each group were alive.

For the HAE-HP-β-CD complexes; 2, 4, 8 and 16 mg of complex were administered to each mouse at 50, 100, 200 and 400 mg/kg dose respectively for five consecutive days after parasitemia was established. This was because the complexes contained equivalent weights of HAE and HP-β-CD. A negative control group was administered 8mg of HP-β-CD in 0.2 mL of distilled (this is the quantity of HP-β-CD in 400 mg/kg HAE - HP-β-CD complex while Chloroquine base (10 mg/kg) was administered to positive control group.

For the HAE (400mg/kg)-HP-β-CD-PVP complex; 16.8, 17.6 and 19.2 mg was administered to each mouse in HAE-HP-β-CD-5% PVP, HAE-HP-β-CD-10% PVP and HAE-HP-β-CD- 20% PVP groups respectively. Normal saline (10 mL/kg), HP-β-CD + PVP (8 mg+3.2 mg) were given separately to two negative control groups, while Chloroquine base (10 mg/kg) was administered to positive control group. The animals were treated for five consecutive days after parasitemia was established.

Thin blood smears of each animal were made daily for 5 days and survival time was determined as previously described.

Temperature readings

Rectal temperature was measured daily using a digital thermometer, after infection was established, to observe the effect of HAE and the complexes on body temperature in the curative test.

Statistical Analysis

The data from this study were subjected to ANOVA statistical analysis with significant level taken at $P < 0.05$ followed by Student–Newman–Keuls post hoc test.

RESULTS

The yields of HAE, HEE and CEE were 11.38%, 16.43% and 22.94% respectively. The result of the TLC shown in Table 1 indicates that three fractions (n-hexane fractions of CEE and HEE and chloroform fraction of CEE) had brick red spots with R_f values of 0.85 which is similar to that of gedunin. The n-hexane fraction of CEE had the same color intensity as gedunin after spraying with anisaldehyde solution while that of HEE was not as deep. Trace amounts are probably present in the chloroform fraction of CEE since the spot had a faint brick reddish tint. HAE fractions did not show evidence of gedunin in this chromatogram.

Table 1: Thin Layer Chromatography characteristics of Gedunin and fractions of CEE, HAE and HEE

R_f values	SPOTS		
	DAYLIGHT	UV Light (254nm)	After spraying with anisaldehyde
0.85	Gedunin (colorless)	Purple	Deep brick red
0.85	Hexane HEE(colorless with a greenish tint)	Purple	Brick reddish (not as deep as Gedunin)
0.85	Hexane CEE (greenish)	Purple	Deep brick red close to Gedunin
0.85	Chloroform fraction HAE (colorless)	Purplish (faint)	Did not pick up the brick red color

HAE-Hot aqueous extract, HEE-Hot ethanolic extract, CEE-Cold ethanolic extract

The results of the 4-day schizonticidal (suppressive) test for the different extracts of *A. indica* leaves are shown in Table 2. The three extracts produced significant ($P<0.05$) dose-dependent chemosuppression. There was no difference in activity between the 200 and 400 mg/kg doses for the three extracts. Chloroquine produced 68.1% chemosuppression. This is lower than the chemosuppression produced at 100, 200 and 400 mg/kg doses of the three extracts. The results of the established infection test for the extracts are as presented in Table 2.

Table 2: Suppressive activity of *A. indica* extracts on *Plasmodium berghei* (NK65)

	Cold ethanol extract (CEE)		Hot aqueous extract (HAE)		Hot ethanol extract (HEE)	
Dose (mg/kg)	Average Parasitemia (%)	Average Chemosup pression (%)	Average Parasitemia (%)	Average Chemosup pression (%)	Average Parasitemia (%)	Average Chemosup pression (%)
0	18.8 ± 2.2	0.0	18.8 ± 2.2	0.0	18.8 ± 2.2	0.0
50	14.2 ± 1.1[a]	24.5	6.4 ± 0.4[a]	66.0	7.5 ± 0.7[a]	60.1
100	5.5 ± 0.5[ab]	70.7	4.2 ± 0.6[a]	77.7	5.2 ± 0.4[a]	72.3
200	4.7 ± 0.5[ab]	75.0	4.0 ± 0.3[a]	78.7	4.3 ± 0.2[a]	77.1
400	4.7 ± 0.3[ab]	75.0	4.1 ± 0.5[a]	78.2	4.2 ± 0.3[a]	77.7
CQ (5)	6.0 ± 0.5[ab]	68.1	6.0 ± 0.5[a]	68.1	6.0 ± 0.5[a]	68.1

*a = $P<0.05$ between control and test dose, b= $P<0.05$ between 50 mg/kg and other doses.
CQ-chloroquine. (Mean ± SEM, n = 5)

Table 3: Curative activity of *A. indica* extracts on *Plasmodium berghei* (NK65)

	Average Parasitemia (%)								
	Cold ethanol extract (CEE)			Hot aqueous extract (HAE)			Hot ethanol extract (HEE)		
	Days after infection								
Dose mg/kg	3	5	7	3	5	7	3	5	7
0	5.9 ±0.9	5.6 ±0.5	3.5± 0.5	5.9± 0.9	5.6±0.5	3.5± 0.5	5.9± 0.9	5.6±0.5	3.5±0.5
50	3.6±0.3[a]	7.6±0.6[a]	5.0± 0.6	4.8± 0.4	4.7±0.6	3.7± 0.4	4.4± 0.4	5.5±0.7	4.1±0.4
100	2.9±0.3[a]	5.6 ±0.6	3.6± 0.5	4.5± 0.5	5.8±0.8	6.5±0.5[a]	6.0± 0.7	4.2±0.5	2.4±1.2
200	4.1±0.4[a]	2.3±0.5[a]	5.6±0.6[a]	4.2± 0.5	4.9±0.6	4.6± 0.6	3.8± 0.4	6.7±0.6	4.4±0.7
400	4.5 ±0.5	5.6 ±0.5	4.2± 0.4	2.8±0.3[a]	3.3±0.3	2.7± 0.4	7.7± 1.6	5.5±0.9	2.2±0.3
CQ(5)	2.8±0.3[a]	4.5 ±0.6	2.8± 0.3	2.8±0.3[a]	4.5±0.6	2.8± 0.3	2.8±0.3[a]	4.5±0.6	2.8±0.3

*a = P<0.05, between control and test dose (Mean ± SEM, n= 5)

The three extracts (CEE, HAE and HEE) did not show any appreciable curative antimalarial effect (Table 3). In addition, CEE and HEE did not exhibit superior activity when compared with HAE. Thus, the complexation study was done with HAE.

Table 4 shows the effect of HAE complexed with HP-β-CD on established infection. Percentage parasitemia decreased for chloroquine and HAE-HP-β-CD test doses on day 4 after infection. Average percentage parasitemia decreased with chloroquine till day 7 after infection compared to controls. Chloroquine and HAE-HP-β-CD at 400 mg/kg gave a significant (P<0.05) increase in chemosuppression, compared to control, throughout the duration of the test although the extent of suppression achieved by the latter is less than that of chloroquine.

The survival period of mice treated with HAE - HP-β-CD complex is shown in Table 5. The chloroquine group had the highest survival period of 28.00 days while 50 mg/kg, 200 mg/kg and 400 mg/kg groups survived for a mean period of 19.00, 20.40 and 22.00 days respectively. These values were higher than the survival period of 18.20 days in the controls although there were no significant differences in the survival period.

Table 4: The effect of HAE complexed with 2-hydroxypropyl-β-cyclodextrin on established infection.

Doses (mg/kg)	Day 3		Day 4		Day 5		Day 6		Day 7	
	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)
Control (CD)	11.36±1.22	0.00	8.92±1.21	0.00	13.40±0.93	0.00	11.99±1.04	0.00	20.41±1.84	0.00
50mg/kg + CD	12.68±1.14	0.00	8.04±0.75[b]	9.87	12.57±1.00[b c]	6.20	13.26±0.88[b]	0.00	21.12±1.70[b c]	0.00
100mg/kg +CD	15.72±0.93	0.00	9.90±0.64[b c]	0.00	15.90±1.21[b c]	0.00	12.79±0.86[b]	0.00	19.70±0.92[b c]	0.00
200mg/kg +CD	9.36±0.55	17.61	6.87±0.53	22.98	11.33±0.90 [b c]	15.41	17.11±1.84[b]	0.00	22.44±1.97[b c]	0.00
400mg/kg +CD	9.15±0.83	19.45	6.25±0.44[a c]	29.93	8.36±0.64 [a b c]	37.61	13.93±1.46[b]	0.00	12.50±0.76[a b c]	38.76
Chloroquine (10)	6.11±0.53	46.21	4.22±0.35 [a]	52.69	1.86±0.22[a]	86.12	1.46±0.25[a]	87.82	2.42±0.30[a]	88.10

*a = P<0.05 between control and test dose, b=P<0.05 between chloroquine and other test doses, c=P<0.05 between 400 mg/kg + CD and (50,100,200) mg/kg+CD, ACS - Average chemosuppression, 50+CD, 100+CD, 200+CD, 400+CD – HAE - HP-β-CD complexes, Control - 8mg of HP-β-CD, (Mean ± SEM, n = 6)

Table 5: Mean survival period of mice treated with HAE - HP-β-CD complex in established infection

Dose of HAE-HP-β-CD	Survival (days)
Control	18.20 ± 3.84
CQ (10mg/kg)	28.00 ± 0.00
50 mg/kg + CD	19.00 ± 1.97
100 mg/kg + CD	16.50 ± 3.77
200 mg/kg + CD	20.40 ± 3.49
400 mg/kg + CD	22.00 ± 2.12

50+CD, 100+CD, 200+CD, 400+CD – HAE-HP-β-CD complexes, Control - 8mg of HP-β-CD. (Mean ± SEM, n = 6).

Table 6: The effect of HAE complexed with 2-hydroxypropyl-β-cyclodextrin and polyvinyl pyrollidone on established infection

		Day 3		Day 4		Day 5		Day 6		Day 7	
Drug	Dose (mg/kg)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)	Average Parasitemia (%)	ACS (%)
Normal saline	10 ml/kg	9.00±1.00	0.00	7.19±0.67[b]	0.00	12.76±1.75	0.00	21.45±4.31[b]	0.00	22.89±0.63[b]	0.00
HP-β-CD + PVP	8 mg+3.2 mg	11.36±1.21	0.0	8.85±1.16[b]	0.00	14.50±5.68[b]	0.00	15.79±4.80[b]	0.00	16.83±5.08	0.00
CQ	10	10.91±1.02	0.00	0.81±0.47	88.73	1.02±0.19	92.00	0.45±0.15	97.90	1.89±1.52	91.74
HAE	400	7.00±2.51	22.22	6.11±1.94	15.02	10.08±4.34	21.00	9.04±1.01	57.86	12.05±0.86	47.36
	PVP concentration (%)										
HAE - HP-β-CD	0	7.13±1.81	20.78	7.80±2.98	0.00	9.93±0.91	22.18	10.41±2.09	51.47	23.01±6.54[b]	0.00
	5	10.03±3.93	0.00	7.44±1.54	0.00	16.32±2.52[b]	0.00	15.63±4.83	27.13	24.64±5.74[b]	0.00
	10	6.60±1.38	26.67	6.16±1.17	14.32	10.90±2.38	14.58	14.30±3.76	33.33	21.49±3.85[b]	6.12
	20	9.20±2.13	0.00	7.74±1.53	0.00	10.71±5.93	16.07	14.66±1.00	31.66	16.91±2.40	26.12

*b= P<0.05 between chloroquine group and other groups, ACS - Average chemosuppression CQ – chloroquine, PVP-polyvinyl pyrollidone, HAE - HP-β-CD: 400 mg/kg of HAE + HP-β-CD (Mean ± SEM, n = 6).

The established infection (curative) effect of HAE complexed with HP-β-CD and PVP on established infection is as shown in Table 6. Chloroquine produced a significant ($p < 0.05$) increase in chemosuppression which was 91.74% on day 7. On day 6, suppression of parasitemia produced by HAE (57.86%) was higher than that of HAE- HP-β-CD complex (51.47%).

HAE-HP-β-CD-PVP complexes attained chemosuppression of 27.13%, 33.33% and 31.66% at 5%, 10% and 20% PVP content respectively on day 6. Thus, the HAE showed greater activity than HAE-HP-β-CD and HAE-HP-β-CD-PVP complexes.

The results of the effect of HAE-HP-β-CD-PVP on body temperature of mice in curative test are shown in Table 7. HAE at 400 mg/kg caused a steady decrease in temperature while temperatures decreased and increased on alternate days with HAE-HP-β-CD and HAE-HP-β-CD-PVP 5%. The other PVP complexes did not increase the temperature in the infected mice. Chloroquine caused a steady increase in temperature up till day 6.

The mean survival period of mice treated with extract alone and the complexes in established malaria infection is shown in Table 8. Chloroquine gave the highest survival of 28 days while control 1 (normal saline) and control 2 animals had survival periods of 15.20 and 19.20 respectively. HAE-HP-β-CD-PVP 5% and HAE-HP-β-CD-PVP 10% had slightly increased survival periods compared to controls, but these differences were not significant ($P>0.05$). The HAE-HP-β-CD-PVP 20% group had the lowest mean survival period of 14.60.

Table 7: Effect of HAE – HP-β-CD – PVP on body temperature of mice in curative test

	Temperature readings on days after infection (°C)				
	3	4	5	6	7
Control 1	35.94±1.03	36.72±0.19	36.37±0.72	34.97±0.50	35.45±0.45
Control 2	36.72±0.50	36.07±0.64	36.18±0.52	35.44±0.20	35.11±0.39
CQ 10mg/kg	35.92±0.47	36.81±0.44	37.16±0.28	37.1±0.25	36.78±0.46
400 mg/kg	36.55±0.57	36±0.81	35.77±0.52	35.43±0.47	35.33±0.43
400 mg/kg + CD	36.4±0.94	35.5±0.93	36.22±0.66	35.05±0.75	35.65±0.77
400+CD+PVP5%	36.38±0.44	34.88±0.54	35.58±0.14	34.7±0.65	35.48±0.21
400+CD+PVP10%	36.34±0.43	36.05±0.32	36.02±0.22	34.56±0.53	35.16±0.45
400+CD+PVP20%	34.86±0.55	35.49±0.63	35.6±0.33	35.2±0.10	35.05±0.95

Control 1 - normal saline, Control 2 - 8 mg of HP-β-CD +3.2mg of PVP, CQ - chloroquine 10 mg/kg 400 mg/kg- 400 mg/kg HAE, 400+CD - 400 mg/kg HAE + HP-β-CD, 400+CD-+5% - 400 mg/kg HAE + HP-β-CD + PVP 5%, 400+CD+10% - 400 mg/kg HAE + HP-β-CD + PVP 10%, 400+CD+20% - 400 mg/kg HAE + HP-β-CD + PVP 20%. (Mean ± SEM, n = 6).

Table 8: Mean survival period of mice treated with HAE-HP-β-CD-PVP complex in established infection

Dose of HAE-HP-β-CD-PVP	Survival (days)
Control 1 (normal saline only)	15.20 ± 1.53
Control 2 (CD + PVP alone)	19.20 ± 3.31
CQ (10mg/kg)	28.00 ± 0.00[a,b]
400mg/kg	17.00 ± 3.26
400mg/kg + CD	16.60 ± 2.14
400mg/kg + CD + PVP5%	19.20 ± 2.63
400mg/kg + CD + PVP10%	19.80 ± 0.80
400mg/kg + CD + PVP20%	14.60 ± 0.81

*a =P<0.05 between control 1 (normal saline) and chloroquine
b = P<0.05 between control 2 (8 mg of HP-β-CD and 3.2 mg of PVP) and chloroquine
400 mg/kg- 400 mg/kg HAE,
400+CD - 400 mg/kg HAE + HP-β-CD
400+CD+5% - 400 mg/kg HAE + HP-β-CD + PVP 5%
400+CD+10% - 400 mg/kg HAE + HP-β-CD + PVP 10%
400+CD+20% - 400 mg/kg HAE + HP-β-CD + PVP 20%
(Mean ± SEM, n = 6).

DISCUSSION

The enormous therapeutic potentials of *A. indica* have been identified (Achi *et al*., 2018) and efforts have been made to improve the *in vivo* antimalarial effect of gedunin, which is believed to be its main antimalarial component (Khalid *et al.,* 1989; Mackinnon *et al*., 1997).

In this study, an attempt was made to increase bioavailability of *A. indica* extract constituents by complexation with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) alone and with the combination of 2-hydroxypropyl-β-cyclodextrin and polyvinyl pyrrolidone (PVP) at different concentrations. Bray *et al.* (1990) indicated *A. indica* has better *in vitro* and poor *in vivo* activity, which could be due to poor absorption from the gastrointestinal (GI) tract. Therefore, appreciable amount of the plant constituents did not reach the site of action (that is the blood) in the mice model of *Plasmodium berghei* infection. Hence, the complexes were prepared to assess the possibility of increasing the absorption of the extract constituents and enhancing pharmacological activity.

The hot aqueous extraction method gave the lowest yield which might be due to the fact that a large proportion of *A. indica* constituents are limonoids. Limonoids are moderately polar

substances which are insoluble in water and hexane but are soluble in ethanol (Aliero, 2003). It is therefore expected that 50% ethanol will capture more of these constituents as seen in the higher yield obtained from the hot hydroalcohol extraction process. The highest yield was obtained with the cold extraction with 50% ethanol. The low yield of both HAE and HEE might be due to the fact that some of the volatile constituents might have evaporated with the application of heat.

The presence of gedunin in the extract fractions was confirmed using chromatographic technique. The detection of a minimal quantity of gedunin in the chloroform fraction of HAE may be due to its low expression in *A. indica* leaves. This is likely to have contributed to the little activity observed with the HAE and HAE-HP-β-CD.

CEE, HAE and HEE were subjected to suppressive and curative antimalarial activity tests but HAE was selected for complexation for two reasons. Aqueous extraction is the most commonly used preparation in ethnomedical practice and the other extracts (CEE and HEE) did not show superior efficacy in the suppressive and curative tests in this study. Moreover, it has been suggested that for the purposes of production of antimalarials, priority should be given to water extracts rather than organic solvents since water is the solvent commonly used in ethno-medicine (Rukunga and Simons, 2006).

The results of the present study showed that *A. indica* produced mild *in vivo* curative antimalarial activity at 400 mg/kg. The poor *in vivo* activity could be due to poor absorption of its bioactive constituents from the GIT and first-pass metabolism effects like previously reported (Bray *et al*., 1990; Omar *et al*; 2003). Furthermore, gedunin had been identified as the most active antimalarial component of *A. indica* and *Melia azedarach* (Mackinnon *et al*., 1997), but the amount of gedunin in *A. indica* leaf is low (Omar *et al.,* 2003) and variable (Bray *et al*., 1990; Mackinnon *et al*., 1997). The curative activity of HAE at 400 mg/kg complexed with HP-β-CD showed superior efficacy when compared to complexes of the lower doses. Also, the survival period of mice treated with HAE-HP-β-CD complex was highest at the 400 mg/kg dose. However, HAE-HP-β-CD-PVP complexes produced lower chemosuppression than HAE-HP-β-CD complex while the HAE-HP-β-CD attained comparable chemosuppression with the extract alone. The complexes did not reverse hypothermia when compared with the positive control (chloroquine). Malaria parasitemia in rodents is characterized by a decrease in temperature (hypothermia) thus an antipyretic agent, like chloroquine, will increase the temperature in infected rodents (Farahna *et al*., 2010).

The results of this study showed that HAE-HP-β-CD complex improved the *in vivo* curative antiplasmodial activity of *A. indica* aqueous leaf extract while combination of HAE-HP-β-CD with PVP did not increase the *in vivo* curative efficacy. Cyclodextrins encapsulate compounds/drugs with low aqueous solubility in their hydrophobic cavity to improve transport across biological membranes (Muankaew and Loftsson, 2018). In addition, previous studies have revealed that cyclodextrin complexation enhanced bioavailability and pharmacological efficacy of medicinal agents, crude extracts and natural products. The efficacy of Resveratrol (a plant-based agent), in prevention of dimethylbenz[a]anthracene-induced oral carcinogenesis, was significantly improved when it was complexed with HP-β-CD (Bertaa *et al*., 2010). Dima *et al.* (2014) showed that HP-β-CD encapsulated coriander essential oil had a comparable free radical scavenging activity (79.77%) with the free coriander essential oil (87.89%). The encapsulated coriander oil retained about 41-46 % antifungal activity of the coriander essential oil against *Aspergillus niger* and *Penicillium glaucum* MIUG M5. *Berberis vulgaris* extract-β cyclodextrin formulation presented better hepatoprotective effects, against carbon tetrachloride–induced acute toxicity in mice, than free extract on oral administration probably due to improved bioavailability (Hermenean, *et al*., 2012). The findings of these studies corroborate the improved *in vivo* curative antimalarial activity seen with HAE-HP-β-CD complexation.

A large number of compounds have been isolated from different parts of *A. indica* thus the extract (HAE) contained several bioactive constituents

(Biswas *et al*., 2002; Alzohairy, 2016). Some of these phytoconstituents, especially those with high molecular weights, were not likely to have been tightly entrapped in the cavity of the cyclodextrins. The constituents with smaller molecular weights might have been entrapped in the cyclodextrin cavity thus reducing the efficacy of the complex formed. An example of this is the effect of encapsulation in β-cyclodextrin on the water solubility and *in vitro* releasing properties of constituents of an ethanol extract of Greek propolis (Kalogeropoulos *et al.,* 2009). Propolis extract is a multi-component matrix containing compounds with diverse sizes and functional groups which differentiate their ability for interactions with the β-CD cavity. The authors concluded that complex formation with β-CD resulted in an increase of water solubility for several propolis extract bioactive constituents. The more effectively encapsulated small phenolic molecules were released from the β-CD cavity with more difficulty, while the opposite was true for flavonoids, anthraquinones and terpenes. The net release of specific compounds from the encapsulated extract depended not only on their chemical properties but also on their relative abundance in the propolis extract sample (Kalogeropoulos *et al.,* 2009). An extrapolation of this previously reported finding to our study implies that a bioactive constituent, like gedunin, which is not abundant in our extract and has a relatively higher molecular weight, may have been readily released from the complex into solution but it was not likely to be in abundance in the solution. This could be one of the reasons why HAE and HAE-HP-β-CD complex exhibited lower antimalarial and little or no antipyretic activity compared to chloroquine.

Apart from this assertion, some other reasons which include the ratio factor for complexation may be attributable. The ratio of HP-β-CD to guest molecule has been shown to affect the degree of encapsulation. In this study, HAE –HP-β-CD complex was prepared at a ratio 1:1 based on the assumption that most inclusion complexes formed by interaction of guest molecules with host cyclodextrin molecules occur at a ratio 1:1 (Wankar *et al.,* 2020). However, some studies have shown that use of ratio 1:1 may not achieve optimal encapsulation and solubility. Lopez and Pascual-Villalobos (2010) investigated the effect of using different ratios of β-cyclodextrin to monoterpenoids during the complexation process on the efficiency of encapsulation of 6 monoterpenoids (linalool, camphor, geraniol, S-carvone, γ-terpinene, and fenchone) and 2 phenylpropanoids (E-anethole and estragole). They found that encapsulation, at the ratio of 3.33:1 of β-CD to linalool and γ-terpinene, was higher whereas S-carvone, camphor, E-anethole, geraniol, estragole and fenchone showed the greatest encapsulation when the ratio was 6.66:1. In another study, the effects of complexing ethanolic extract of *Angelina sinensis* (AS) extract with HP-β-CD on human hepatoma cells Hep3B cell growth was determined by analyzing cytotoxicity and cellular uptake. The complex with a weight ratio of AS extract: HP-β-CD (1: 5) had the best solubility. It produced cytotoxicity inhibition of the hepatoma cells by 94% while AS produced about 68% (Hu *et al*., 2014). The inclusion of anthraquinones from rhubarb extract in HP-β-CD was achieved at a weight ratio of rhubarb extract: HP-β-CD of 1:9. The inclusion complex increased the aqueous solubility and bioavailability of rhubarb and thus enhanced its effect on hepatoma cells (Hu *et al.,* 2013). In the case of olive leaf extract, an inclusion complex was formed with β-cyclodextrin at a molar ratio of 1:1. The molar ratio calculations were based on the molecular weight of oleuropein, since this compound was the main constituent of the olive leaf extract. The NMR data established the formation of a 1:1 complex with β-CD (Mourtzinos *et al.,* 2007). It can be deduced that ratio 1:1 was optimal for inclusion complex formation because oleuropein is the major constituent of olive leaf extract.

For complex multicomponent extracts, whose ratio calculations are based on weight of the extract not on molar value of one constituent, a weight ratio 1:1 of extract: cyclodextrin might not be the most ideal for inclusion complex. The ratio of extract to HP-β-CD used in this present study was 1:1. This ratio might not have been optimal for complexation and enhancement of biological activity, a higher weight of HP-β-CD might have produced a more effective HAE-HP-β-CD complex. This is also a probable reason for the inability of PVP to improve the activity of HAE -

HP-β-CD complex since the complexation achieved between extract and HP-β-CD might not have been at its best.

CONCLUSION

In conclusion, this study revealed that the complexation of an aqueous extract of *A. indica* leaf with HP-β-CD produced significant improvement in the activity of the extract at 400 mg/kg only while HP-β-CD plus PVP complexes with the extract did not improve the curative activity. These results suggest that HP-β-CD is useful for the enhancement of *A. indica* leaf extract antimalarial activity and *A. indica* extract-HP-β-CD complexation should be further investigated for clinical applications.

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REFERENCES

Achi, N.K., Onyeabo, C., Nnate, D.A., Ekeleme-Egedigwe, C.A., Kalu, I.K., Chibundu, I.C., and Wokoma, G.C. 2018. Therapeutic effects of *Azadirachta indica* A. Juss. leaves in malaria-induced male Wistar rats. *Journal of Pharmacy and Pharmacognosy Research,* 6(3): 191-204.

Al-Burtomani, S.K.S and Suliman, F.O. 2017. Inclusion complexes of norepinephrine with β-cyclodextrin, 18-crown-6 and cucurbit[7]uril: experimental and molecular dynamics study. *Royal Society of Chemistry Advances,* 7: 9888-9901.

Aliero, B.L. 2003. Larvaecidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of Anopheles mosquito. *African Journal of Biotechnology*, 2**:** 325-327.

Alzohairy, M.A. 2016. Therapeutics Role of *Azadirachta indica* (Neem) and Their Active Constituents in Diseases Prevention and Treatment. *Evidence Based Complementary and Alternative Medicine,* 2016: 7382506.

Ansari, M. 2015. Investigations of polyethylene glycol mediated ternary molecular inclusion complexes of silymarin with beta cyclodextrins. *Journal of Applied Pharmaceutical Science*, 5(09): 26-31.

Benoit-Vical, F., Imbert, C., Bonfils, J.P., and Sauvaire, Y. 2003. Antiplasmodial and antifungal activities of Iridal, a plant triterpenoid. *Phytochemistry*, 62(5): 747-751.

Bertaa, G.N., Salamonea, I.P., Sprioa, A.E., Scipioa, F.D., Marinosa, L.M., Sapinob, S., Carlottib, M. E., Cavallib, R., and Carloa, F. D. 2010. Chemoprevention of 7, 12-dimethylbenz[a]anthracene (DMBA)-induced oral carcinogenesis in hamster cheek pouch by topical application of resveratrol complexed with 2-hydroxypropyl-β-cyclodextrin. *Oral Oncology*, 46(1): 42-48.

Biswas, K., Chattopadhyay, I., Ranajit, K., Banerjee, R.K. and Bandyopadhyay, U. 2002. Biological activities and medicinal properties of Neem (*Azadirachta indica*). *Current science*, 82(11): 1336-1345.

Bray, D.H., Warhust, D.C., Connolly, J.D., O'Neill, M.J. and Phillipson, J.D. 1990. Plants as sources of Antimalarial Drugs. Part 7: Activity of some species of Meliaceae plants and their constituent limonoids. *Phytotherapy Research*, 4(1): 29-35.

Challa, R., Ahuja, A., Ali, J., and Khar, R. 2005. Cyclodextrins in Drug Delivery: An Updated Review. *AAPS PharmSciTech*, 06(02): E329-E357.

Dhar, R., Zharg, K., Talwar, G.P., Garg, S. and Kumar, N. 1998. Inhibition of the growth and development of asexual and sexual stages of drug-sensitive and resistant strains of the human malaria parasite *Plasmodium falciparum* by Neem (*Azadirachta indica*) fractions. *Journal of Ethnopharmacology*, 61(1): 31-39.

Dima, C., Cotarlet, M., Tiberius, B., Bahrim, G., Alexe, P. and Dima S. 2014. Encapsulation of Coriander Essential Oil in Beta-Cyclodextrin: Antioxidant and Antimicrobial Properties Evaluation. *Romanian Biotechnological Letters*, 19 (2): 9128 – 9140.

Farahna, M., Bedri S., Khalid S., Idris M., Pillai, C.R. and Khalil E.A. 2010. Anti-plasmodial effects of *Azadirachta indica* in experimental cerebral malaria: Apoptosis of cerebellar Purkinje cells of mice as a

marker. *North American Journal of Medical Science*, 2**:** 518-525.

Hermenean, A., Popescu, C., Ardelean, A., Stan, M., Hadaruga, N., Mihali, C.V., Costache, M. and Dinischiotu, A. 2012. Hepatoprotective Effects of *Berberis vulgaris*n L. Extract /β cyclodextrin on Carbon Tetrachloride–Induced Acute Toxicity in Mice. *International Journal of Molecular Sciences,* 13: 9014-9034.

Hirlekar, R.S., Sonawane, S.N. and Kadam, V.J. 2009. Studies on the Effect of Water-Soluble Polymers on Drug–Cyclodextrin Complex Solubility. *AAPS Pharm Sci Tech*, 10(3): 858-863. DOI: 10.1208/s12249-009-9274-6.

Hu, C.M., Yu, S.C., Tsai, F.J. and Tsai, Y. 2013. Enhancement of rhubarb extract solubility and bioactivity by 2-hydroxypropyl-β-cyclodextrin. *Carbohydrates Polymers*, 98(2): 1422-1429.

Hu, C.M., Tsai, F.J. and Tsai, Y. 2014. Inhibitory effect of *Angelica sinensis* extract in the presence of 2-hydroxypropyl-β-cyclodextrin. *Carbohydrates Polymers*, 114: 115-22.

Isah, A.B., Ibrahim, Y.K. and Iwalewa, E.O. 2003. Evaluation of the antimalarial properties and standardization of tablets of *Azadirachta indica* (Meliaceae) in mice. *Phytotherapy Research,* 17(7): 807-810.

Iwu, M.M. 2014. Handbook of African Medicinal Plants, CRC Press, Inc., Florida, 150 - 153.

Kalogeropoulos, N., Konteles, S., Mourtzinos, I., Troullidou, E., Chiou, A. and Karathanos, V. T. 2009. Encapsulation of complex extracts in β-cyclodextrin: An application to propolis ethanol extract. *Journal of Microencapsulation*, 26(7): 603–613.

Kaur, K., Jain, M., Kaur, T. and Jain, R. 2009. Antimalarials from nature. *Bioorganism and Medicinal Chemistry,* 17(9): 3229–3256.

Khalid, S.A., Duddeck, H. and Gonzalez-Sierra, M. 1989. Isolation and characterization of an antimalarial agent of the neem tree *Azadirachta indica*. *Journal of Natural Products*, 52(5): 922-926.

Khalid, S.A., Farouk, A., Geary, J.G. and Jenson, J.B. 1986. Potential antimalarial candidates from African plants: an *in-vitro* approach using *Plasmodium falciparum*. *Journal of Ethnopharmacology*, 15(2): 201-209.

Lim, T.K. 2014. *Azadirachta indica*. In: Edible Medicinal and Non-Medicinal Plants. Lim T.K. (ed.), Springer, Dordrecht. pp. 409-455.

Loftson, T. and Brewster, M.E. 1996. Pharmaceutical applications of cyclodextrins.1.Drug solubilization and stabilization. *Journal of Pharmaceutical Sciences*, 85**(**10): 1017-1025.

López, M.D. and Pascual-Villalobos, M. J. 2010. Analysis of monoterpenoids in inclusion complexes with *β*-cyclodextrin and study on ratio effect in these microcapsules. *Julius-Kühn-Archives*, 425: 1-5.

Mackinnon, S., Durst, T., Arnason, J. T., Angerhofer, C., Pezzuto, J. M., Sanchez-Vindas, P. E., Povedan, L. J. and Gbeassor, F. M. 1997. Antimalarial activity of Tropical Meliaceae extracts and Gedunin Derivatives. *Journal of Natural Products,* 60: 336-341.3

Mourtzinos, I., Salta, F., Yannakopoulou, K., Chiou, A. and Karathanos, V.T. 2007. Encapsulation of Olive Leaf Extract in β-cyclodextrin. Journal of Agricultural Food Chemistry, 55: 8088–8094.

Muankaew, C. and Loftsson, T. 2018. Cyclodextrin-Based Formulations: A Non-Invasive Platform for Targeted Drug Delivery. *Basic and Clinical Pharmacology and Toxicology*, 122: 46–55.

Ogbuewu, I.P., Odoemenam, V.U., Obikaonu, H.O., Opara, M.N., Emenalom, O.O., Uchegbu, M.C., Okoli, I.C., Esonu, B.O. and Iloeje, M.U. 2011. The Growing Importance of Neem (*Azadirachta indica* A. Juss) in Agriculture, Industry, Medicine and Environment: A Review. *Research Journal of Medicinal Plant,* 5(3): 230 – 245.

Omar, S., Zhang, J., MacKinnon, S., Leaman, D., Durst, T., Philogene, B.J.R., Arnason, J.T., Sanchez-Vindas, P.E., Poveda, L., Tamez, P.A. and Pezzuto, J.M. 2003. Traditionally-Used Antimalarials from the Meliaceae. *Current Topics in Medicinal Chemistry*, 3:133-139.

Oshikoya, K. A., Senbanjo, I. O., Njokanma, O. F. and Soipe, A. 2008. Use of complementary and alternative medicines

for children with chronic health conditions in Lagos, Nigeria. *BMC Complementary and Alternative Medicine*, 8: 66.

Pacheco, P.A., Rodrigues, L.N.C., Ferreira, J.F.S., Gomes, A.C.P., Verissimo, C.J., Louvandini, H., Costa, R.L.D., and Katiki, L.M. 2018. Inclusion complex and nanoclusters of cyclo dextrin to increase the solubility and efficacy of albendazole. *Parasitology Research*, 117: 705 - 712.

Peters, W. 1980 Chemotherapy of malaria, In: Malarial Epidemiology, Chemotherapy, Morphology and Metabolism. J. P. Kreier (ed), pp 145 – 176.

Phillipson, J. D. and Wright, C. W. 1999. Can Ethnopharmacology contribute to the Development of Antimalarial Agents? *Journal of Ethnopharmacology*, 32: 155-156.

Rukunga, G. and Simons, A. J. 2006. The potentials of plants as a source of Antimalarial agent-A review. Africa Herbal Antimalarial meeting. PlantaPhile Publications, Berlin. pp 72

Ryley, J.F. and Peters, W. 1970. The antimalarial activity of some quinolone esters. *Annals of Tropical Medicine and Parasitology*, 84: 209-222.

Sá Couto, A., Salústio, P. and Cabral-Marques, H. 2014. Cyclodextrins. In: Ramawat, K. and Mérillon, J.M. (eds) Polysaccharides. Springer Cham, Switzerland. https://doi.org/10.1007/ 978-3-319-03751-6_22-1.

Sharma, N. and Baldi, A. 2016. Exploring versatile applications of cyclodextrins: an overview. *Drug Delivery*, 23(3): 729-747.

Udeinya, I. J. 1993. Anti-malarial activity of Nigerian neem leaves. *Trans Royal Society Tropical Medicine and Hygiene*, 87(4): 471.

Udeinya, I.J., Brown, N., Shien, O., Udeinya, F.I. and Quakeyie, I. 2003. Fractions of an antimalarial neem-leaf extract have activities superior to chloroquine, and are gametocytocidal. *Annals of Tropical Medicine and Parasitology*, 100(1): 17-22.

Udeinya, J.I., Shu, E.N., Quakyi, I., and Ajayi, F.O. 2008. An antimalarial neem leaf extract has both schizonticidal and gametocytocidal activities. *American Journal of Therapeutics*, 15: 108-110.

Wankar, J., Kotla, N.G, Rasala, S.A., Pandit, A. and Rochev, Y.A. 2020. Recent Advances in Host Guest Self-Assembled Cyclodextrin Carriers: Implications for Responsive Drug Delivery and Biomedical Engineering. *Advanced Functional Materials*, 30: 1909049.