COMPARATIVE ANTIPLASMODIAL ACTIVITY OF ALKALOID, FLAVONOID AND SAPONIN RICH-FRACTIONS OF METHANOL LEAF EXTRACT OF *CRYPTOLEPIS OBLONGIFOLIA* (*APOCYNACEAE*) (MEISN) SCHLTR IN MICE

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Abstract: Malaria is a parasitic disease caused by *plasmodium* parasite. It causes severe disease and death in children and pregnant women. This study aims to compare the antiplasmodial activity of alkaloid, flavonoid and saponin-rich fractions (ARF, FRF, and SRF) of methanol leaf extract of *Cryptolepis oblongifolia* (MLECO) in *Plasmodium berghei* infected mice. The LD_{50} of the fractions was estimated using limit test (OECD guidelines 425). The curative, suppressive and prophylactic models were used for antiplasmodial study. The effect of each fraction on the Packed Cell Volume (PCV) of mice in the curative groups was determined. The data were analysed using one way and repeated measure ANOVA followed by Dunnett's and Bonferroni *post hoc* tests. The oral LD_{50} of the fractions was estimated to be >5000 mg/kg. The ARF has the highest ($p < 0.001$) parasite clearance (17, 26.6 and 57.3%) followed by FRF (9.3, 36.3 and 36.3%) and SRF (5.1, 14.8 and 32.9%) in the curative test. The ARF protected the mice from death and produced the highest ($p < 0.001$) level of parasitaemia suppression. This is followed by SRF and FRF. All the fractions tested showed significant ($p < 0.001$) prophylactic parasite suppression. The ARF has no significant ($p > 0.05$) effect on the PCV levels of the treated mice.

Conclusion: The ARF has the highest antiplasmodial activity followed by FRF and SRF. All the fractions have no significant effect on the hematocrit of the treated mice.

Keywords: *Cryptolepis oblongifolia*, alkaloid-rich, flavonoid-rich, saponin-rich, antiplasmodial, *Plasmodium berghei*

Introduction

Malaria is a serious infection and important public health problem. It is caused by the *plasmodium* parasite. The parasite is transmitted to humans by the bites of female *anopheles* mosquitoes. More than 90% of malaria cases and deaths are found in sub-Saharan Africa (WHO, 2021). In 2021, an estimated 627,000 malaria deaths were recorded globally (WHO, 2021). The mainstay of malarial treatment is the artemisinin-based combination therapy (ACTs) which was proven to be efficacious and safe over the years. However, investigations in Southeast Asia, Rwanda and Northern Uganda have confirmed the presence of Artemisinin-resistant strain of *Plasmodium falciparum.* (Ashley, *et al.*, 2014; Uwimana, *et al*., 2021; Balikagala, *et al.*, 2021). These findings indicate potential devastating effects on the existing high malarial morbidity and mortality data and calls for urgent and aggressive search for new molecules/drugs from medicinal plants with traditional history of usage in malaria treatment. Medicinal plants/herbs have a long-standing history in the treatment of malaria, as the oldest antimalarial drug, Quinine (Gachelin, *et al.*, 2017), and the currently accepted ACTs (Tu, 2011) were discovered from medicinal plants. *Cryptolepis oblongifolia* has been used in traditional medicine in many African countries, including Nigeria, in the treatment of malaria, diarrhea, cough and stomach disorders (Bullock, 1963). *Cryptolepis oblongifolia* is a multi-stemmed shrub belonging to the family *Apocynaceae*. It is called red-stemmed milk rope; "*Kahon Bitsi*" in Hausa, "*Lali pupa*" in Yoruba and "*Nmanu Ngborogwu*" in Igbo. The plant is native to moist regions of southern Africa, where it occurs in rocky grassland, grassy woodland or riverine vegetation (Bingham, 2013).

Material and Methods:

Plant Collection and Identification

Fresh leaves of *Cryptolepis oblongifolia* were collected from Karau-Karau village of Giwa local government area, Kaduna State-Nigeria. The plant was identified and authenticated by a Botanist at the Department of Plant Biology, Faculty of Life Sciences, Ahmadu Bello University, Zaria. A voucher specimen number (ABU03359) was collected for feature reference.

Preparation of Plant Extracts

The leaves of the plant were shade dried and ground into powder with a pestle and mortar. One kilogram (1kg) of the powdered plant was cold macerated with 7.5 L of 70 % (v/v) methanol for five days. The extract was concentrated and evaporated to dryness using water bath maintained at 45^{o}C. The crude extract was subjected to successive solvents partitioning (Woo, *et al*., 1980), alkaloid, flavonoid and saponin-rich fractions were obtained and evaporated to dryness using water bath. The extracts were stored in an air-tight desiccator and kept in a refrigerator prior to the experiments (Figure 1).

Partitioning of Crude Methanol Leaf Extract of *Cryptolepis oblongifolia* (CMLECO)

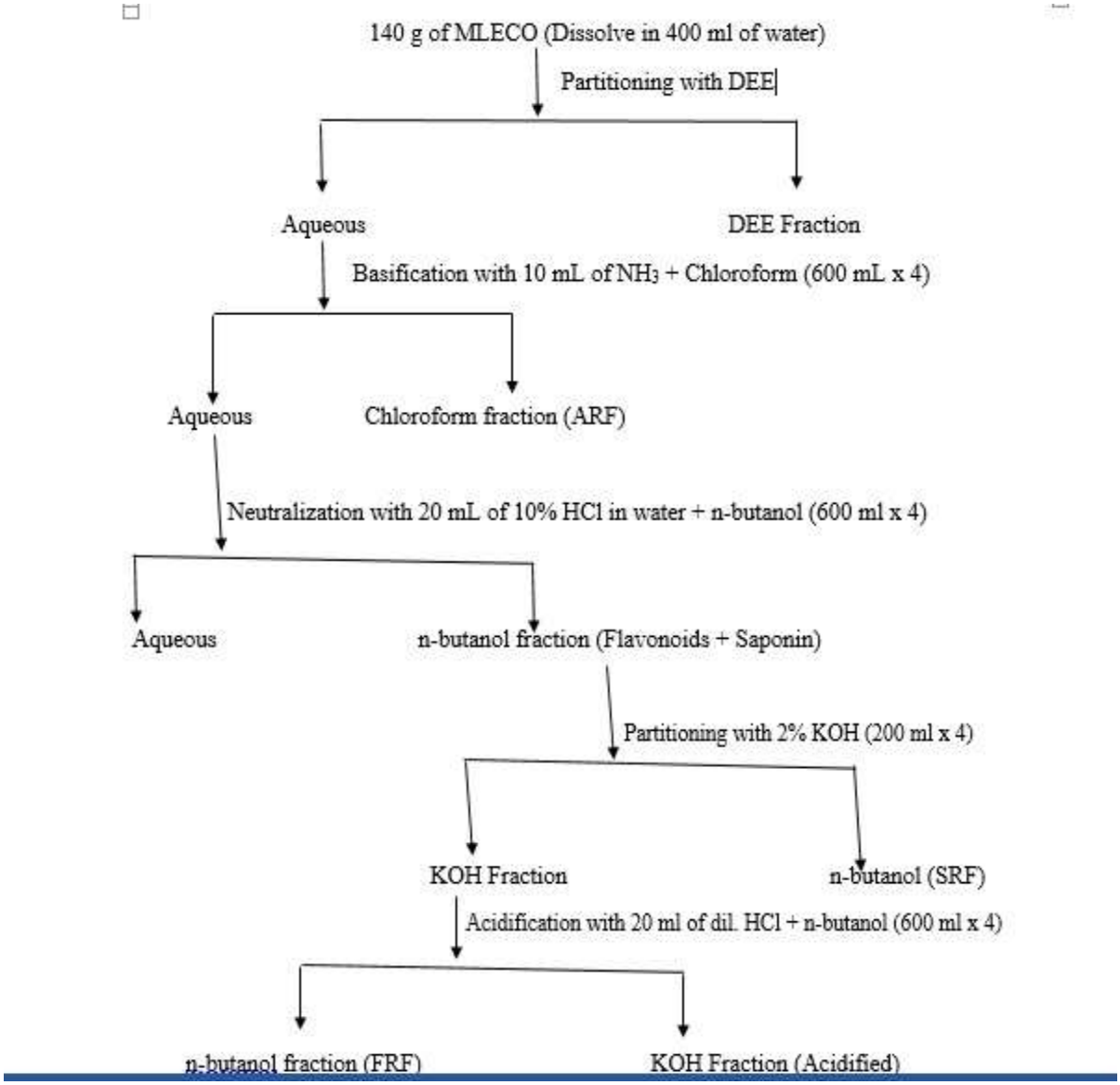

Figure 1: Solvent fractionation of Alkaloid, Saponin and Flavonoid-rich fractions from **MLECO. MLECO =** Methanol Leaf Extract of *Cryptolepis oblongifolia*, **DEE** = Diethyl ether, **NH_3** = Ammonia, **ARF** = Alkaloid-rich fraction, **KOH** = Potassium Hydroxide, **SRF** = Saponin-rich fraction, **FRF** = Flavonoid-rich fraction, HCl = Hydrochloric acid (Woo *et al.*, 1980)

Experimental Animals

Adult *Swiss* albino mice of either sex, weighing 20-28 g, were obtained from the Animal House Facility of Pharmacology and Therapeutics Department, Ahmadu Bello University, Zaria. They were maintained in a transparent and well-ventilated cages, fed with vital feeds and allow free access to water *ad libitum*. Ethical approval was obtained from Ahmadu Bello University, Zaria Committee on Animal Use and Care (ABUCAUC/2022/010).

Acute Toxicity Study

The oral median lethal dose (LD_{50}) of alkaloid-rich fraction (ARF), flavonoid-rich fraction (FRF) and Saponin-rich fraction (SRF) was determined using OECD guidelines number 425 (OECD, 2008). A limit test was done for each extract and five rats per extract were treated orally with 5000 mg/kg of the extract sequentially. Each rat was monitored closely within the first 30 minutes, then once per hour for the first four hours and later daily, for signs of toxicity and/or

death. All the rats were observed for two weeks for signs of late onset of toxicity and/or mortality.

Plasmodium Parasite Inoculation

Blood sample was collected, retro-orbitally into ethylenediaminetetraacetic acid (EDTA) containing bottle, from a donor mouse with parasitaemia level of 32%. The collected blood was diluted with normal saline. Each mouse was inoculated intraperitoneally with 0.2 ml of the collected blood, containing approximately 1 x 10^7 infected RBCs (Peters, 1965).

Antiplasmodial Activity of ARF, FRF and SRF in Mice with Established Infection (Curative Test)

Evaluation of antiplasmodial potential of alkaloid, flavonoid and saponin-rich fractions of methanol leaf extract of *Cryptolepis oblongifolia* against established infection in mice was carried out using the method of Ryley and Peters (1970). A total of ninety (90) mice were used for this test, thirty mice (30) per fraction. On the first day (D_0), blood sample was collected retro orbitally to determine pre-infection hematocrit levels of the mice followed by parasite inoculation. The mice were left untreated for 72 hours (D_0-D_3) to establish the infection. On day three post parasite inoculation, each mouse was tail-bled, blood smear was prepared, fixed with absolute methanol and stained with Giemsa. Pre-treatment parasitaemia levels were determined by counting the number of parasitized erythrocytes in ten random fields. Post-infection blood sample was taken from each mouse to determine the post-infection hematocrit levels. The blood sampling was done once (retro orbitally) using capillary tube for both parasitaemia estimation and hematocrit determination. After the baseline blood samplings, all inoculated mice were randomly divided into 5 groups of 6 mice each and treated orally with distilled water (10 ml/kg, group I [negative control]), graded doses of ARF, FRF and SRF (375, 750 and 1500 mg/kg body weight, groups II-IV) and standard drug, chloroquine (5 mg/kg body weight, group 5 [positive control]) respectively for 5 days (D_3-D_7). Post-treatment parasitaemia levels were determined on day seven (D_7) of the experiment, as described above, using light microscope (Celestron CB1000CF 40-1000) at x100 magnification. Post-treatment hematocrit levels were also determined. All the mice were monitored and the mean survival time for each group was determined arithmetically by finding the average survival time (post-inoculation) in each group over a period of 28 days (Ryley and Peters, 1970).

Mean Survival Time

The Mean Survival Time (MST) of mice treated with ARF, FRF and SRF, in the curative groups, was determined by calculating the average survival time of mice after infection with *Plasmodium berghei* over a period of 28 days, as express below;

MST=

Sum of survival time of all mice in a group (Days)/Total nu mber of mice in that group

Effect of ARF, FRF and SRF on Hematocrit of the Mice in the Curative Groups

The effect of the extract on the hematocrit level of the mice in the curative group was investigated by determining the hematocrit level before parasite inoculation, three days after parasite inoculation and five days after daily extracts treatment. In each case, blood sample was collected retro-orbitally by using heparinized capillary tube, the tube was filled to 75% capacity. One end of the tube was sealed using bunsen flame and transferred to hematocrit centrifuge. The blood samples in the capillary tube were centrifuged at 15,000 revolutions per minute for 3 minutes. The hematocrit was determined by using the hematocrit reader and the result was recorded in percentage.

Antiplasmodial Activity of ARF, FRF and SRF Against 4-Days Peter's Suppressive Test (Early Infection)

The method described by Peters (1980) was used in this test. A total of ninety (90) mice were used for this test, thirty (30) mice per fraction. On the first day (D_0), thirty (30) adult mice per fraction were inoculated with *Plasmodium berghei* and thereafter the mice were randomly divided into 5 groups of 6 mice each. On the same day (D_0), treatment with the graded doses of the extract (375, 750 and 1500 mg/kg) was administered orally four hours after inoculation with 0.2 ml of infected erythrocyte and continued daily for three days. The negative and positive control groups were treated with 10 ml/kg of distilled water and 5 mg/kg of Chloroquine respectively. Twenty-four hours (24 hours) after administration of the last dose (D_4), blood sample was taken from the tail of each mouse and a smear was prepared, fixed in absolute methanol, stained with Giemsa solution and examined under microscope at X 100 magnification for determination of parasitaemia.

Prophylactic Antiplasmodial Activity of ARF, FRF and SRF in Mice Infected with Plasmodium berghei

The method of Peters (1965) was used to determine the prophylactic activity of ARF, FRF and SRF. A total of ninety (90) mice were used for this test. Thirty (30) adult mice per each extract were randomly grouped into 5 groups of 6 mice each and treated with the graded doses of the extract (375, 750 and 1500 mg/kg orally, group II-IV) and standard drug (Pyrimethamine 1.2 mg/kg, group V) orally for five 5 days (D_0-D_4). The negative control group was treated with distilled water (10 ml/kg). On the sixth day (D_5), the mice were inoculated with 0.2 ml of *Plasmodium berghei* infected erythrocyte intraperitoneally. Blood sample was taken from the tail of each mouse 72 hours post inoculation for parasitaemia level determination as described above.

Data Analysis

Results were expressed as mean plus or minus standard error of mean (Mean ± SEM) and analysed using One-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test for antiplasmodial tests. Results of repeated hematocrit measurements were analysed using repeated measures ANOVA and Bonferroni *post hoc* test. Results were considered statistically significant at $p \leq 0.05$. Data analysis was done using SPSS software version 20.0

Results

Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the three fractions was found to be > 5000 mg/kg. The rats in all the three groups did not show any noticeable sign of toxicity within the first and fourth hour following extract administration. All the rats in the three treatment groups were monitored for 14 days with no evidence of late onset toxicity and/or death.

Curative Antiplasmodial Activity of ARF, FRF and SRF in Mice with Established Infection (Curative Test)

The ARF of *Cryptolepis oblongifolia*, at the tested doses of 375, 750 and 1500 mg/kg, has produced significant (p < 0.001) dose-dependent curative antiplasmodial activity (parasite clearance: 17%, 26.6% and 57.3%), followed by FRF (parasite clearance: 9.3%, 36.3% and 36.3%) and SRF (parasite clearance: 5.1%, 14.8% and 32.9%) compared to the distilled water treated group. The standard drug chloroquine, at 5 mg/kg body weight, produced significant (p < 0.001) parasite clearance of 81.2, 91.6 and 58.5% (Figure 2).

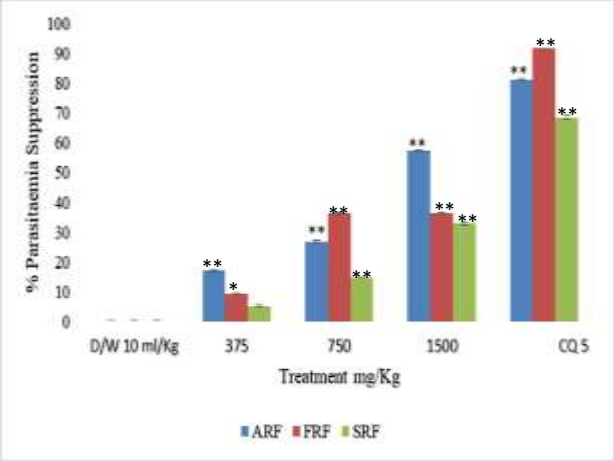

Figure 2: Curative Effect of Alkaloid, Flavonoid and Saponin-Rich Fractions of Methanol Leaf Extract of *Cryptolepis oblongifolia* in Mice Infected with *Plasmodium berghei.*

Mean Survival Time

The group treated with ARF at 1500 mg/kg survived the 28 days observation period, followed by SRF (26.8 days) and FRF (24.7 days) compared to the mice in distilled water treated groups. All the mice treated with Chloroquine survived the 28 days observation period. (Table 1).

Table 1: Survival Time of *Plasmodium berghei* infected Mice Treated with Alkaloid, Flavonoid and Saponin-Rich Fractions of *Cryptolepis oblongifolia* Following Curative Test

Fractions	Mean Survival Time (Days)		
	ARF	FRF	SRF
DW 10ml/kg	15.0 ± 4.12	12.3 ± 3.60	14.6 ± 2.28
375	25.2 ± 0.87	16.0 ± 1.18	24.3 ± 0.61
750	23.6 ± 3.74	23.8 ± 2.53	25.7 ± 0.98
1500	28.0 ± 0.00	24.7 ± 0.95	26.8 ± 0.65
CQ 5	28.0 ± 0.00	28.0 ± 0.00	28.0 ± 0.00

Values are presented as Mean ± SEM, n = 6, ARF = Alkaloid-Rich Fraction, FRF = Flavonoid-Rich Fraction, SRF = Saponin-Rich Fraction, DW = Distilled Water, CQ = Chloroquine

Effects of Alkaloid, Flavonoid and Saponin-Rich Fractions of Cryptolepis oblongifolia on Hematocrit of Mice Infected with Plasmodium berghei Following Curative Test

The ARF produced non-statistically significant (p > 0.05) increase in the hematocrit levels of the treated mice at 375 and 750 mg/kg. The standard drug, Chloroquine, at 5 mg/kg, has produced significant (p < 0.004) increase in the hematocrit of the treated mice after 5 days of treatment. The mice in the group treated with ARF at 1500 mg/kg has non-significant declined in hematocrit compared to the pre infection levels. There was continues non-significant reduction in the hematocrit of the mice treated with distilled water (negative control) throughout the experiment (Table 2).

The FRF has not produced any significant effect on the hematocrit of the treated mice at all the doses tested. There was reduction in the post-treatment hematocrit levels of the mice treated with the extract at 375, 750 and 1500 mg/kg. The mice treated with standard drug, Chloroquine at 5 mg/kg, have shown non-significant (p > 0.05) increase in the post treatment hematocrit level compared to their post infection level. (Table 3).

The SRF, at all the doses tested, has not protected the treated mice from falling hematocrit levels when post infection and post treatment hematocrit levels are compared. The mice treated with the standard drug, Chloroquine 5 mg/kg, had significant (p < 0.001) increase in their post treatment hematocrit level compared to the post infection level. (Table 4).

Table 2: Effect of Alkaloid-Rich Fraction of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Haematocrit Level of Mice infected with *Plasmodium berghei* Following Curative Test

Treatment (mg/kg)	PRE-INFECTION PCV (%)	POST-INFECTION PCV (%)	POST-TREATMENT PCV (%)
DW	47.8 ± 2.05	42.8 ± 2.03	40.3 ± 1.08
ARFCO 375	48.0 ± 2.05	43.5 ± 2.03	44.5 ± 2.03
ARFCO 750	46.3 ± 2.05	43.3 ± 2.03	43.8 ± 1.08
ARFCO 1500	42.0 ± 2.05	42.2 ± 2.03	42.0 ± 1.08
CQ 5	46.5 ± 2.05	43.2 ± 2.03	47.5 ± 1.08*

Values presented as Mean ± SEM, n = 6, * significantly different at p < 0.04, using Repeated measures ANOVA and Bonferroni *post hoc* test. Distilled water, ARFCO = Alkaloid-Rich Fraction of *Cryptolepis oblongifolia*, CQ= Chloroquine, PCV = Packed Cell Volume

Table 3: Effect of Flavonoid-Rich Fraction of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Haematocrit of Mice infected with *Plasmodium berghei* Following Curative Test

Treatment (mg/kg)	PRE-INFECTION PCV (%)	POST-INFECTION PCV (%)	POST-TREATMENT PCV (%)
DW	43.5 ± 1.31	41.7 ± 1.46	42.0 ± 1.01
FRFCO 375	47.7 ± 1.31	45.3 ± 1.46	43.2 ± 1.01
FRFCO 750	45.0 ± 1.31	42.0 ± 1.46	40.3 ± 1.01
FRFCO 1500	45.0 ± 1.31	42.0 ± 1.46	40.3 ± 1.01
CQ 5	45.5 ± 1.31	42.7 ± 1.46	44.5 ± 1.01

Values presented as Mean ± SEM, n = 6, using Repeated measures ANOVA and Bonferroni *post hoc* test. Distilled water, FRFCO = Flavonoid-Rich Fraction of *Cryptolepis oblongifolia*, CQ= Chloroquine, PCV = Packed Cell Volume

Table 4: Effect of Saponin-Rich Fraction of Methanol Leaf Extract of *Cryptolepis oblongifolia* on Haematocrit Level of Mice infected with *Plasmodium berghei* Following Curative Test

Treatment (mg/kg)	PRE-INFECTION PCV (%)	POST-INFECTION PCV (%)	POST-TREATMENT PCV (%)
DW	49.2 ± 1.17	46.5 ± 1.22	42.5 ± 1.21
SRFCO 375	47.5 ± 1.17	44.3 ± 1.22	42.2 ± 1.21
SRFCO 750	48.0 ± 1.17	45.2 ± 1.22	42.3 ± 1.21
SRFCO 1500	44.2 ± 1.22	41.3 ± 1.21	41.3 ± 1.21
CQ 5	46.0 ± 1.17*	43.3 ± 1.217	47.5 ± 1.21*

Values presented as Mean ± SEM, n = 6, *, significantly different at $p < 0.001$, using Repeated measures ANOVA and Bonferroni *post hoc* test. Distilled water, SRFCO = Saponin-Rich Fraction of *Cryptolepis oblongifolia*, CQ= Chloroquine, PCV = Packed Cell Volume

Suppressive Antiplasmodial Activity of ARF, FRF and SRF in Mice with Early Infection (4 Days Peter's Suppressive Test)

The ARF of *Cryptolepis oblongifolia* produced significant ($p < 0.01$ and 0.001) dose-dependent chemosuppressive antiplasmodial activity (percentage chemosuppression: 18.2%, 27.3% and 52.0%) followed by FRF (percentage chemosuppression: 2.8%, 21.6% and 28.2%) and SRF (percentage chemosuppression: 2.5%, 7.9% and 48.8%) compared to the distilled water treated group at tested doses of 375, 750 and 1500 mg/kg. The standard drug Chloroquine, at a dose of 5 mg/kg produced significant ($p < 0.001$) chemosuppression of 79.3, 70.4 and 69%. (Figure 3).

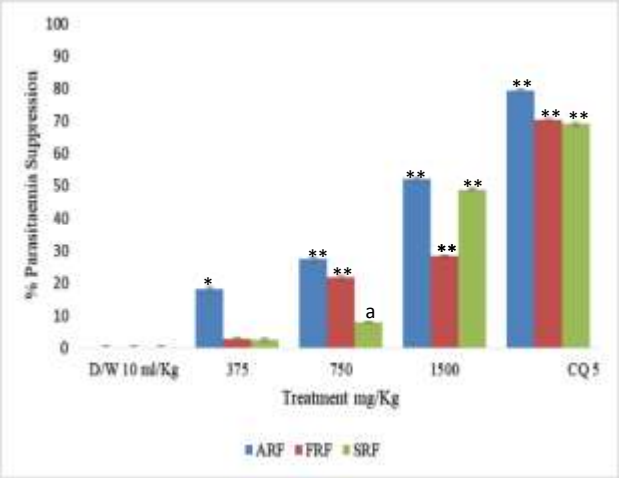

Figure 3: Suppressive Activity of Alkaloid, Flavonoid and Saponin-Rich Fractions of Methanol Leaf Extract of *Cryptolepis oblongifolia* in Mice Infected with *Plasmodium berghei.*

Values presented as Mean ± SEM, n = 6, a,* and ** significantly different form DW group at $p < 0.04$, 0.01 and 0.001 using one way ANOVA and Dunnett's *post hoc* test. DW = Distilled water, ARF = Alkaloid-Rich Fraction, FRF = Flavonoid-Rich Fraction, SRF = Saponin-Rich Fraction, CQ = Chloroquine

Prophylactic Antiplasmodial Activity of ARF, FRF and SRF in Mice (Repository Test)

The SRF produced the highest ($p < 0.001$) and dose-dependent prophylactic antiplasmodial activity (percentage chemoprophylaxis: 10.8%, 37.8% and 65.8%) followed by ARF, in a non-dose dependent manner, (percentage chemoprophylaxis: 12.8%, 58.8% and 30.3%) and FRF (percentage chemoprophylaxis: 15.6%, 37.5% and 21.4%) compared to the distilled water treated groups at tested doses of 375, 750 and 1500 mg/kg. The standard drug Pyrimethamine, at a dose of 1.2 mg/kg produced significant ($p < 0.001$) chemoprophylaxis of 84.5, 82.6 and 78.2, %. (Figure 4).

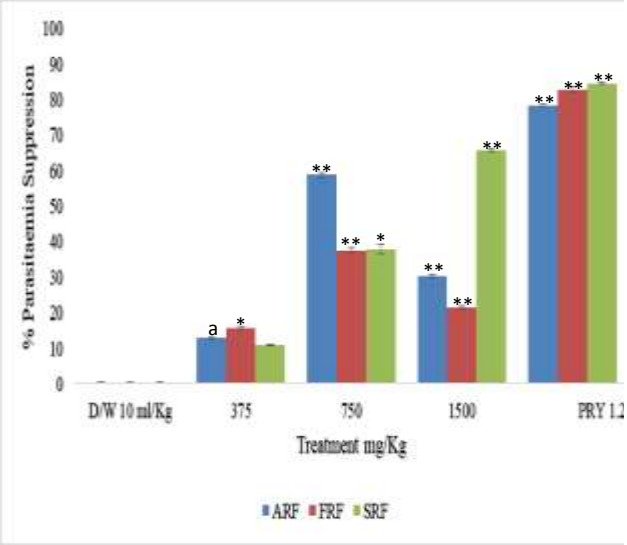

Figure 4: Prophylactic antiplasmodial Activity of Alkaloid, Flavonoid and Saponin-Rich Fractions of Methanol Leaf Extract of *Cryptolepis oblongifolia* in Mice Infected with *Plasmodium berghei.*

Values presented as Mean ± SEM, n = 6, a,** and ** significantly different form DW group at $p < 0.04$, 0.01 and 0.001 using one way ANOVA and Dunnett's *post hoc* test. DW = Distilled water, ARF = Alkaloid-Rich Fraction, FRF = Flavonoid-Rich Fraction, SRF = Saponin-Rich Fraction, PRY = Pyrimethamine

Discussion

The plant, *Cryptolepis oblongifolia*, has been used in traditional medicine of Africa including Nigeria to treat fever, malaria, cough and diarrhea (Alaribe, *et al*, 2021). This work aims at scientific investigation of the antimalarial claim of the plant. Three validated rodent malaria models: curative, suppressive and prophylactic models, were used to screen the ARF, FRF and SRF of the methanol leaf extract of the plant. Curative model tests the fraction on its potential usefulness to produce clinical cure of malaria, suppressive model tests for extract ability to suppress early infection by *Plasmodium berghei*, while prophylactic models tests for extracts ability to protect the treated mice from getting infected by the parasite. The *Cryptolepis oblongifolia* methanol leaf extract was reported to contained alkaloid, flavonoids, saponin, tannins, cardiac glycosides, terpenoids, phenols and steroids (Abdussalam *et al*., 2022). These are phytochemicals responsible for the observed pharmacological activities of the plant.

The ARF produced the highest antiplasmodial effect on the curative and suppressive models. This is evident by the significant suppression of the parasitaemia in the treated mice and protection of the mice from death throughout the observation period. *Cryptolepis oblongifolia* contains alkaloid and the oldest antimalarial drug quinine was also an alkaloid isolated from the bark of Cinchona tree (Sexana *et al.,* 2003). It was reported that quinine produced parasite clearance by disrupting the parasite DNA, parasite replication and transcription processes as well as inhibition of schizont maturation into infective merozoites (Inbaneson, *et al*., 2012). The ARF of *Cryptolepis oblongifolia* might have acted by one or more of these mechanisms to account for the significant parasitaemia suppression seen in this study. This finding is similar to that of Abdussalam, *et al.*, (2018), who reported significant curative antiplasmodial activity of alkaloid-containing *Marrubium vulgare* leaf extract in mice.

The FRF also produced significant antiplasmodial effect and prolonged the mean survival time of the treated mice compared to the negative control. It was reported that flavonoids inhibit *plasmodium* parasite protein synthesis thereby leading to the parasite death (Ferreira, *et al.*, 2010). This may account for the observed significant parasite clearance in the treated mice and protection from malarial death by the FRF of *Cryptolepis oblongifolia*. The SRF produced the least antiplasmodial effect compared to the ARF and FRF. However, SRF has produced significant parasite suppression in the prophylactic model compared to the negative control group. These findings were similar to those of Abdullahi, *et al*., (2020) and Abdulkadir, *et al.*, (2022) who reported significant antiplasmodial activity of *Detarium microcarpum* and *Piliostigma reticulatum* in mice infected with *Plasmodium berghei* parasite, respectively.

The merozoites of *plasmodium* parasite invade red blood cells of all ages and this results in clinical symptoms and pathogenicity of malaria including anaemia (Gilson and Crabb, 2009). The potential of the three fractions on the hematocrit of the treated mice in the curative group was measured by comparing the post infection hematocrit levels with post treatment levels after five days of treatment with the three fractions. The ARF protected the treated mice from reduction in hematocrit compared to their pre-treatment hematocrit levels. The FRF and SRF have not shown any protection on the continuous reduction of the hematocrit of the treated mice compared to pre-treatment levels.

Conclusion: The alkaloid-rich fraction of *Cryptolepis oblongifolia* has produced the highest curative and suppressive antiplasmodial activity in mice infected with *Plasmodium berghei*. All the three fractions have shown significant prophylactic antiplasmodial activity. These findings indicate that *Cryptolepis oblongifolia* may be useful in developing new antimalarial drugs.

Conflict of interest

The authors declared no conflict of interest

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References

Abdulkadir, S. S., Jatau, A. I., Abdussalam, U. S., Bichi, L. A., Abubakar, B., and Malami, S. (2022). *In vivo* antiplasmodial activity of the methanol leaf extract of *Piliostigma reticulatum* (Dc.) Hochst (*Fabaceae*). *Bulletin of the National Research Centre*, *46*(1), 1- 8.

Abdullahi, A. R., Malami, S. A. N. I., Abdussalam, U. S., and Bichi, L. A. (2020). Antiplasmodial activity and safety assessment of methanol leaf extract of *Detarium microcarpum* Guill. & Perr.(*Fabaceae*). *African Journal of Pharmaceutical Research*, *12*(3), 288-298.

Abdussalam U., S, Aliyu M, Maje I., M. (2018). *In vivo* Antiplasmodial Activity of Ethanol Leaf Extract of *Marrubium Vulgare* L. (Lamiaceae) in *Plasmodium Berghei-Berghei* Infected Mice. *Tropical Journal of Natural Product Research.* 2(3):132-135.

Abdussalam, U., S., Maje, I., M., Anafi, B., S. and Jatau, I., D. (2022). Antiplasmodial activity of methanol leaf extract of *Cryptolepis oblongifolia* (*Apocynaceae*) (Meisn) Schltr in mice infected with *Plasmodium berghei*, *Bayero Journal of Pure and Applied Sciences,* 13(1): 255-259

Alaribe, S. C., Oladipo, A., R., Uche, G., C., Onumba, M., U. (2021). Suppressive, curative, and prophylactic potentials of an antimalarial polyherbal mixture and its individual components in *Plasmodium berghei*-infected mice. *Journal of Ethnopharmacology*. 277, 105-114.

Ashley, E. A., Dhorda, M., and Amaratunga, C. (2014). Spread of artemisinin resistance in *Plasmodium falciparum* malaria. *New England Journal of Medicine*, (371) 411–423

Balikagala, B., Fukuda, N., Ikeda, M., and Katuro, O., T., *et al*. (2021). Evidence of artemisinin-resistant malaria in Africa. *New England Journal of Medicine*. 385, 1163– 1171.

Bingham, M. (2013). "*Cryptolepis oblongifolia* Schltr" *Flora of Zambia Bioorganic and Medicinal Chemistry*, 23: 2829–2843.

Bullock, A., A. (1963). *Periplociideae* In: Hepper, F. N., Flora of West Tropical Africa. Volume 2, 2nd Edition. Crown Agents for Oversea Governments and Administrations, London, United Kingdom. pp. 80-85.

Ferreira, J. F., Luthria, D. L., Sasaki, T. and Heyerick, A. (2010). Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules* **15**, 3135–3170

Gachelin, G., Garner, P., Ferroni, E., Trohler, U. (2017). Evaluating Cinchona bark and quinine for treating and preventing malaria. *Journal of the Royal Society of Medicine.* 110, 31–40.

Gilson, P. R, and Crabb, B. S. (2009). Morphology and kinetics of the three distinct phases of red blood cell invasion by *Plasmodium falciparum merozoites*. *International Journal of Parasitology*. 39:91–96.

Inbaneson, S., J., Sundaram R., and Suganthi, P. (2012). *In vitro* antiplasmodial effect of ethanolic extracts of traditional medicinal plant *Ocimum* species against *Plasmodium falciparum*. *Asian Pacific Journal of Tropical Medicine*, 5(2):103-06.

OECD, (2008). Organization for Economic Cooperation and Development. Guidelines for the Testing of Chemicals. Acute Oral Toxicity. Test No. 425

Peter, W. (1980). Antimalarial drug resistance. British Medical Bulletin, (32):187–192.

Peters, W. (1965). Drug resistance in Plasmodium berghei. Experimental Parasitology, (17): 80- 89.

Ryley, J. F., Peters, W. (1970). The antimalarial activity of some quinolone esters. *American Tropical Medicine and Parasitology*, (84): 209-22.

Saxena, S., Pant, N., Jain, D., C. and Bakuni, R. S., (2003). Antimalarial agents from plant sources. *Current science*, 85, 1314-1329.

Tu, Y. (2011). The discovery of *artemisinin* (qinghaosu) and gifts from Chinese medicine. *Nature Medicine.* 17, 1217–1220.

Uwimana, A., Umulisa, N., Venkatesan, M., and Svigel, S.S. *et al*. (2021). Association of *Plasmodium falciparum* kelch13 R561H genotypes with delayed parasite clearance in Rwanda: an open-label, single-arm, multicenter, therapeutic efficacy study. *Lancet Infectious Disease,* (21) 1120–1128.

W.H.O (2021). World malaria report. Available at https//www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2021 [Accessed on 15th January, 2022].

Woo, W., S., Shin, KH, and Kang, S., S. (1980). Chemistry and Pharmacology of Flavone -C- Glycoside from *ziziphus* seeds. *Journal of Pharmacology* 11:141-148.