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Diverse mitochondrial effects, antiplasmodial and anti-inflammatory potentials of *Costus afer* (Ker Gawl), *Nauclea latifolia* (Sm) and *Sphenocentrum jollyanum* (Pierre) in mice infected with *Plasmodium berghei*

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Abstract

Costus afer, Nauclea latifolia and *Sphenocentrum jollyanum* are common folkloric herbs for malaria treatment in Africa with limited scientific reports. Also, scientific evidence about their mitochondrial dysfunctional effects in the host during malaria treatment is lacking. This experimental report aims to investigate the antiplasmodial, anti-inflammatory, coupled with the mitochondrial dysfunctional effects of *C. afer, N. latifolia* and *S. jollyanum* extracts during malaria treatment in mouse model.

Air-dried *C. afer* rhizome, *N. latifolia* and *S. jollyanum* leaves were extracted with methanol. Antiplasmodial activity of these extracts were monitored separately using *Plasmodium berghei* strains that are susceptible and resistant to chloroquine. Total heme and hemozoin contents, interleukins (1-beta, 6 and 10), TNF-α and C-reactive protein were used to determine the anti-inflammatory potentials of these extracts. Host mitochondrial effects were assessed using its

membrane permeabilization (mPT) pore opening, peroxidation of mitochondrial lipid (mLPO) and F_0F_1 ATPase activity. Phytochemical constituents were determined using MADI/TOF/MS and detailed profiling was done using NMR spectroscopy. On Day 7, CA, NL and SJ at 200 mg/kg decreased percentage parasitemia to 0.25±0.07; 0.30±0.14 and 0.25±0.07% relative to control (8.60±0.15%) in the chloroquine-sensitive model. In chloroquine- resistant model, they decreased the parasite burden to 0.40±0.14; 0.30±0.14 and 0.45±0.07 as against 10.88±0.26% of the infected control with corresponding parasite suppression. In mice infected with chloroquine-resistant strain of *P. berghei*, NL (200 mg/kg) decreased mLPO (0.41±0.04) F_0F_1 ATPase (0.15±0.02mmol pi/mg protein /min). While CA enhanced mPT pore opening maximally at 100 mg/kg, SJ (50 mg/kg) reversed mPT pore opening caused by parasite infection (1.66 vs 9.4 folds). The NL (200 mg/kg) increased heme, decreased hemozoin, IL-6, CRP, TNF-α, while SJ (200 mg/kg) dose-dependently increased IL-10. MADI/TOF/MS analysis showed that coumaric acid, divaricatinic acid, diocin and aferiosides A and C were present in CA, 3-caffeoylquinic acid, 18, 19-dihydroangustine, jatrorrhizine, 17-epinaucleidinal, strictosamide and quinovic acid 3-O-rhamnoside were present in NL and quinic acid, jatrorrhizine and mabioside B in SJ. While the three medicinal plants have varying antimalarial effects, their decoction will be better for a synergistic purpose.

Keywords: Cytokines, Heme, Hemozoin, Malaria, Mitochondria, Natural Products

1. Introduction

Of all infectious diseases, malaria has become a difficult disease to treat and be prevented. This is because of the resistance of the parasites to various orthodox drugs that have been used for the treatment of the disease. Currently, resistance to artemisinin-combinative therapy has been on the increase. Further to this, it has been difficult to design effective vaccine for the disease because of the complex stages of the parasites' life cycle. Pregnant women and children are the worst hit and recovery from cerebral malaria is almost impossible because of the blood brain barrier. In most parts of Africa and other continents of the world, this disease is common and, the locals depend on the use of herbs from which decoctions are made and consumed without any specific dose regimen. The hope of malaria chemotherapy from medicinal plants is evident with the discovery of artemisinin from *Artemisia annua*. Since then, the hope of getting a permanent cure, via medicinal plant, from malaria scourge has risen tremendously. In ethno-medicine, *Costus afer's* rhizome, *Nauclea latifolia* and *Sphenocentrum jollyanum* leaves have been used in the treatment of malaria either as single plant preparation (Omokhua, 2011) or in combination with other plants. *C. afer* belongs to the Costaceae family formerly known as Zingiberaceae (Aweke, 2007). They use it to treat inflammation (Soladoye and Oyesika, 2008), hepatic problems and malaria. *N. latifolia (Smith)* classified as Rubiaceae is a spreading, evergreen plant with multiple stems; it is a ubiquitous plant (Gidado *et al*., 2005). *N. latifolia* herbal remedies are used to treat pain, malaria and hypertension (Elujoba, 1995). Folklorically, parts of *Sphenocentrum jollyanum* can be prepared to treat malaria (Odugbemi et al., 2006; Olorunnisola and Afolayan, 2011). Although it may not have been reported in some cases, it is likely that most plants with antimalarial properties equally have inflammatory potentials (Chukwujekwu et al., 2004). This is because such plants will

be useful in the prevention of associated complications such as oxidative stress with malaria. It is not new therefore, that oxidative stress can affect important organelle such as the mitochondrion in the host leading to its dysfunction via the irreversible permeabilization of mitochondrial membranes (Kent et al., 2021). Malaria infection causes oxidative damage and both host and parasites are affected. It is pertinent therefore, for the disease to be managed in such a way that both the immediate cause of the infection (parasite) and secondary side effects such as inflammation, bioenergetics stress and mitochondrial dysfunction are well managed. The folkloric treatment of uncomplicated malaria using *C. afer, N. latifolia* and *S. jollyanum* has been well documented. However, the protective mechanism of this medicinal plants on mitochondria has not been reported and current information on the bioenergetics status of the infected host treated with this medicinal plants is sketchy. Here, we report the antiplasmodial potential of *C. afer, N. latifolia* and *S. jollyanum* on chloroquine-sensitive and resistant strains of *Plasmodium berghei* using mice model, anti-inflammatory, lipid peroxidation and bioenergetics status of infected mice.

2. Materials and methods

2.1 Plant sources and their extraction

Aerial parts of *C. afer,* stem bark of *Nauclea latifolia,* and root bark of *Sphenocentrum jollyanum* were validated after collection by the Botany Department in the Faculty of Science, University of Ibadan. The plant samples were spread on wooden slabs in the laboratory until dry for 2 weeks and grounded to powder. The powdered form of *Costus afer Ker-Gawl, Nauclea latifolia, and Sphenocentrum jollyanum* were soaked in 100% methanol for 72 hours in a 5 L conical flask. The soaked plant was decanted and filtered using cotton wool plug in a glass funnel. Plant sediments were then re-soaked for 72 hours, decanted, and filtered. This cold extraction continued until exhaustion.

The filtrate was then concentrated in a rotary evaporator and the concentrated extracts were made solvent-free using a water bath.

2.2 Experimental Animals and Research Design

This study was divided into two phases. In Phase 1, mice were infected in the peritoneal cavitywith *P. berghei* (chloroquine-sensitive strain) while phase 2 containing the samemice number were infected with resistant (ANKA) *P. berghei*. In Phase 1, sixty mice (Swiss strain, all male of 18 ± 2 g body weight) were procured from the Institute (Advanced Medical Research and Training), College of Medicine University of Ibadan, Nigeria. They were kept in well-ventilated hutch in the Department of Biochemistry and were fed as often as necessary. Malaria parasites were transmitted into fifty-five of these using infected erythrocytes administered into the peritoneum of the animals. The infected mice were then grouped (n=5) and the remaining five not-infected mice were classified as normal control. Parasitemia was then confirmed after 72 hours via microscopy. In phase 2 also, the same number of mice were infected with resistant *Plasmodium* and similarly grouped. Infection was confirmed after 72 hours in each case via microscopy.

2.3 The design of the experiment

The treatment of the animals was designed as follows for both phases 1 and 2:

The animals in the positive control group in both cases received 10 mg/kg body weight of Artemether-Lumefantrine, constituted in five percent dimethyl sulfoxide solution as vehicle. The vehicle only was administered to the normal and infected control groups. The other test groups were treated with specific doses (50, 100 and 200 in mg/kg) of crude methanol of *Costus afer, Nauclea latifolia, and Sphenocentrum jollyanum*.

2.4 Antimalarial effects of *Costus afer Ker-Gawl, Nauclea latifolia, and Sphenocentrum jollyanum* parts extracted with methanol

The antimalarial potentials of *C. afer, N. latifolia* and *S. jollyanum* for *P. berghei* (chloroquine sensitive and resistant strains) were carried out using the established infection method as described by Ryle and Peters (1970). Briefly, after the confirmation of parasitemia, the animals were treated via oral gavage for seven days (susceptible model) and five consecutive days (resistant model). At every other day (forty-eight hours interval), thin film slides were prepared via tail snip and were allowed to dry after which they were stabilized in (absolute) methanol for few minutes, dried, stained (in ten percent stock of Giemsa stain) for some minutes. To enhance the stability of the red cells, slides were then rinsed in buffered water, mopped to remove dye particles and air-dried. Air-dried stained slides were initially focused using x10 objective of a binocular microscope and then erythrocytes, both infected and un-infected were counted in each field using x100 objective. Parasitemia and chemosuppression, expressed as percentages were then calculated.

2.5 Heme and hemozoin determination

The concentration of heme in the erythrocytes was determined by using an established method of Asakura et al., 1977. Blood sample (10 µL) was lysed in Sodium duodecyl sulfate (SDS) and dissolved in 1 M NaOH was later added and this was followed by sonication for 10 minutes. At 37°C, the samples were then incubated for 2 hours in a water bath. Total heme was determined by reading the absorbance of the samples at 404 nm and the total heme calculated using 9.08×10^{4}/M/cm, its molar absorption coefficient. The hemozoin content was estimated from 10 µL of blood withdrawn from the mice as described by Orjih and Fitch, 1993. Ten (10) microliter of blood sample was lysed using a 0.08% and centrifuged at 18,000 rpm. As the hemozoin pellet

sedimented, the clear liquid on it was poured out and the pellet was washed repeatedly (three times) using 250 μL of 25% SDS buffered with 25 mM Tris-HCl buffer (pH 7.4). Incubation at 37°C for twenty-four hours followed the washing of the hemozoin pellets at 18,000 rpm. The washed pellets were then dissolved in sodium hydroxide (1 M) and the absorbance was read also at 404 nm.

2.6 Preparation of serum

After the due date of the experiment, the experimental mice were terminated by using cervical dislocation and opened up. The heart was located and using hypodermic needle (1 mL), cardiac blood was drawn and gently aspired into the sample bottles after the removal of the needle, gently placed on the table to form a clot. The blood, after clotting was centrifuged at 3.500 rpm for five minutes to obtain the serum. The Pasteur pipette was used to gently aspirate the serum into another clean sample bottle and preserved in the refrigerator until used.

2.7 Mitochondria isolation

Johnson and Lardy method (1967) was used to isolate mitochondria from the liver. Quickly after the blood was drawn from the heart, liver was removed and it was first rinsed in isolation buffer that contained sucrose (70 mM), Hepes (5 mM) basified with potassium hydroxide to pH 7.4, mannitol (210 mM) and EGTA (1 mM) and weighed. Thereafter, the liver was cut into pieces with the aid of scissors. A 10% preparation of chopped liver suspended in isolation buffer was evenly homogenized. The homogenized liver tissue was then poured into the centrifuge tubes and then spun in refrigerated centrifuge (Sigma 3-30KS, Berlin, Germany) twice at 2,500 rpm for five minutes each time. The supernatant was decanted into another centrifuge tube while the sediment was poured away each time. Mitochondria was pelleted at 13,000 rpm for ten minutes.

Mitochondrial pellets were suspended in washing buffer that contained Hepes (5 mM) basified to physiological pH (7.4) by using potassium hydroxide, sucrose (70 mM), mannitol (210 mM) and BSA (0.5%) and washed twice at 12000 rpm. The mitochondrial pellets were then dispensed in small volumes using the suspension buffer that contained Mannitol (210 mM), Sucrose (70 mM) and Hepes that was basified to physiological pH (pH 7.4) using potassium hydroxide. Washed mitochondria pellets were then reconstituted with the suspension buffer and kept in Eppendorf vials kept on ice.

2.8 Determination of total protein in mitochondria

The protein content of mitochondrial isolate was determined using the method of Lowry and his co-workers (1951). Mitochondria sample (10μl) were made up to 1mL and thereafter, 3 mL of a mixture of Na_2CO_3 (2 g) and$CuSO_4.5H_2O$ (1%) in sodium hydroxide prepared in ratio 100:1:1, respectively was added to each test tube and the mixture was vortexed and later, 0.3 mL of diluted Folin reagent was added and incubated again at room temperature for another 30 minutes. The absorbance was read at 750 nm and mitochondria protein was quantified by using protein standard curve.

2.9 Determination of mitochondrial membrane permeabilisation by *P. berghei* infection and extract intervention

The quality of isolated mitochondria for permeabilization assay was first confirmed using the Lapidus and Sokolove (1993) assay and mPT was subsequently conducted using the same assay method as stated below: mitochondria quantity from the normal control group equivalent to 0.4 mg/mL mitochondrial protein was incubated in the buffer used for reconstitution with rotenone (8 μM) three and half minutes. After this incubation, succinate (5 mM) (in the form of sodium

succinate) was added to the system and the absorbance read at 540 nm for 12 minutes at 30 seconds interval to ascertain that the isolated mitochondria were intact. To ascertain the responsiveness of the mitochondria to calcium, the same quantity of mitochondria was added to the suspension buffer and rotenone and the mixture was incubated for 3 minutes and then calcium (3 μM) in the form of $CaCl_2$ was added and the system was energized 30 seconds after and the absorbance was read at the same wavelength. The above experiments were conducted to determine how good the mitochondria were for mPT assay. Mitochondria with little or no decrease in absorbance when calcium has not been added but with large amplitude swelling in the presence of calcium are considered intact and good enough for mitochondria permeabilization assays. Mitochondrial volume from the treated groups with the same protein content were subjected to the same permeability transition under the same condition. The mPT opening effects of calcium, *Plasmodium* infection and the modulatory effect of *C. afer*, *N. latifolia* and *S. jollyanum* were then compared.

2.10 Assay of Mitochondrial F_0F_1 ATPase activity

The activity of mitochondrial F_0F_1 ATPase enzyme was assessed using modified method of Lardy and Wellman (1953). In test tubes arranged in triplicates, sucrose, potassium chloride (25 and 0.5 mM, respectively), and Tris (65 mM) whose pH was adjusted to 7.4 using HCl were pipetted into the test tubes and made uo to 1 mL using distilled water. Mitochondrial protein (0.5 mg/mL) from mice each group was added to their respective test tubes. To a set of three test tubes, ATP (1 mM) only was added (to determine if the ATP was hydrolyzed already). To another set of three test tubes 'mitochondria only' (0.5 mg/mL mitochondria protein from the normal control group, to monitor the integrity of isolated mitochondria). Mitochondria protein and ATP were added to another set of three test tubes but 10% SDS was immediately added to show that inorganic

phosphate release from ATP by the mitochondrial enzyme is time-dependent. The uncoupler test tubes contained mitochondria and ATP with 25 μM of 2, 4-dinitrophenol as uncoupler. The test tubes for the test groups contained ATP, mitochondria and reagents and the whole set up was incubated at 25°C in a shaker water bath for thirty minutes. After the incubation, the reactrion was stopped by adding SDS to all the test tubes except zero time test tubes whose reaction has been stopped already. One mLfrom each test tube was taken and diluted with 4 mL of distilled water. Ammonium molybdate (1 mL) and ascorbic acid (1 mL) were sequentially added and the absorbance was read at 660 nm. Inorganic phosphate concentration was estimated from phosphate standard curve. **2.11 Assay of mitochondrial membrane lipids peroxidation**

Varshney and Kale (1990) and Adam-Vizi and Seregi (1982) methods were used for this study. Specific volume of isolated mitochondria (0.4 mL) was pipetted into 1.6 mL of Tris-KCl (0.15 M) preparation. Later, 0.5 mL of trichloroacetic acid (30%) was added, together with 0.5 mL of thiobarbituric acid (0.75%). The mixture was heated in a water bath for 45 minutes at 80°C. The mixture was later cooled, spun at 3000 rpm for 10 minutes and the absorbance of 2 mL of the supernatant was read at 532 nm. Thiobarbituric acid reactive substances formed was quantified by using its extinction coefficient (0.156 $\mu M^{-1}cm^{-1}$.)

2.12 Measurement of inflammatory markers interferon gamma, C-reactive protein and interleukins

Several serum interleukins such as IFN-γ, CRP, TNF-α and IL-1β,-6 and10 levels were determined using their corresponding ELISA assay kits manufactured by Elabscience, United States of America.

2.13 Chemical analysis of plant phytochemicals by Ultra Performance Liquid Chromatography coupled with-Quadrupole Time of Flight-Mass Spectrometry (UPLC-QTOF-MS)

Separation of the compounds and their detection from the three extracts was carried out on a Waters UPLC hyphenated with a Waters Synapt G2 QTOF instrument. An Acquity UPLC BEH C18 1.7dμm (2.1×100 mm column), running at 0.30 mL/min flow rate. Extracts were dissolved in 50% LC grade methanol, sonicated for 15 min before centrifuging at 15000 rpm and resulting supernatants injected for analysis. The mobile phase consisted 0.1% formic acid in LC grade water (A) and methanol + 0.1% formic acid (B). The MS source was Electrospray Ionization (ESI). It operated in both negative and positive ion modes with capillary and endplate voltages set at 2600V and 2000V, respectively. Nitrogen served as nebulizer gas, at 10 L/h, while m/z range was set from 50 to 1200 amu. Gradient elution was initiated with 97% A and 3% B which remain linear until 14 min. From 14 to 16 min, elution was kept constant with 0% A and 100% B. A linear gradient of 97% A and 3% B was then used to reach completion until 20 min. The MS data were processed through MassLynx version 4.1 (Waters Corporation, Milford, MA, USA) software, providing elemental formulae of possible compounds. Structures of compounds were further determined through library search and matching of high-resolution masses alongside MS/MS fragment ions with relevant databases.

2.14 Chemical analysis by Nuclear Magnetic Resonance (NMR) spectroscopy

To validate the presence of compounds identified, the extracts were subjected to NMR analysis. Spectra of ^{1}H, ^{13}C APT and selected 2D NMR spectroscopy were recorded using deuterated methanol (CD_3OD) at room temperature on a Bruker Avance III, 400 MHz spectrometer (Bruker,

Rheinstetten, Germany). Raw NMR data were visualized and processed using Bruker Top Spin (version 3.6.4) software.

2.15 Statistical analysis of data

Kinetic representation of changes in the absorbance of mitochondria observed over a period of 12 minutes was used to represent the mitochondrial permeabilization assays. Other assays where end points were determined was done three times per sample and the mean, as well as, the deviation from the mean were calculated. Data were analyzed by using descriptive statistics and multiple comparison between the means of the groups was carried out by using Tukey's post-hoc multiple comparison test in a GraphPad prism (9.0 version).

3. Results

3.1 Methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* decrease parasite burden in *P. berghei*-infected mice (chloroquine-sensitive model)

The percentage parasitemia and parasite clearance potency of *C. afer, N. latifolia* and *S. jollyanum* in *P. berghei-infected* mice (chloroquine sensitive strain) are presented in Figure 1. In this Figure, relative to the infected control (0.84±0.09), the 200 mg/kg *C. afer* reduced the parasite burden (percentage parasitemia) to 0.42±0.16, 200 mg/kg *N. latifolia* (0.35±0.05) and 200 mg/kg *S. jollyanum* 0.38±0.04. Although, *N. latifolia* gave the least percentage parasitemia when compared with other extracts, there was no significant change in this value among other extracts at the same

dose (200 mg/kg) (Figure 1a). The 200 mg/kg methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* significantly decreased the parasite burden in mice infected with *P. berghe* (chloroquine-sensitive strain) compared with the infected control. On the last day of the experiment, 200 mg/kg of all these extracts gave corresponding parasite clearance that are not significantly different from the effect of the standard drug (Figure 1b).

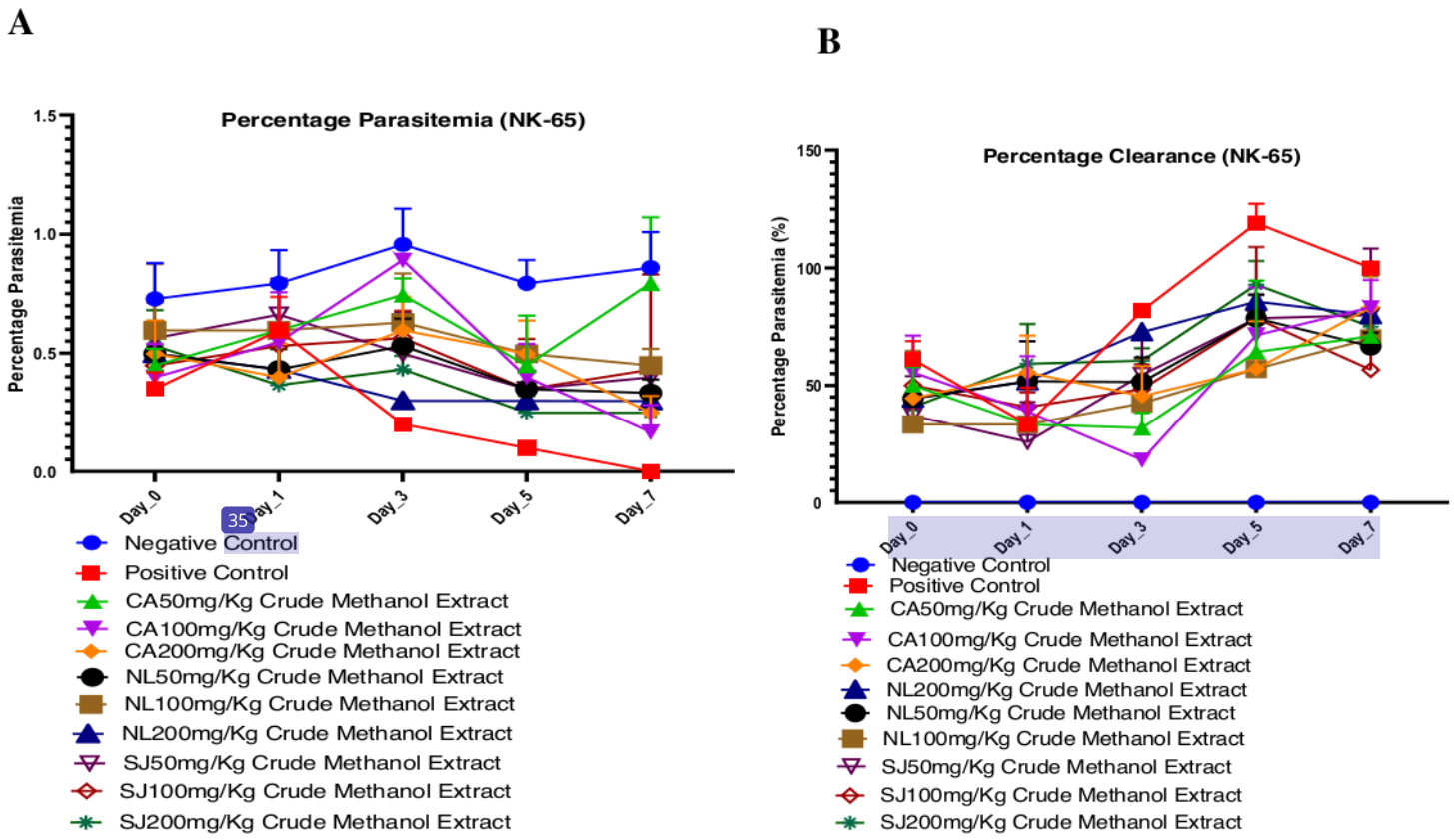


Figure 1: Antiplasmodial potentials of *C. afer, N. latifolia* and *S. jollyanum* methanol extracts in mice infected with chloroquine-sensitive *P. berghei* **Figure 1(A) shows the extent of decrease in parasite burden inparasitised mice treated with the methanol extracts of these medicinal plant and Figure 1(B) shows the percentage suppression in terms of parasite clearance in mice burdened with *Plasmodium* infection that were treated with the methanol extracts of the medicinal plants**

3.2 Therapeutic potentials of *C. afer, N. latifolia* and *S. jollyanum* against resistant (ANKA strain) *P. berghei* infection in mice

Since resistant malaria is the major challenge in malaria treatment as a result of ineffectiveness of available drugs in chemotherapy because of how infection leads to cerebral complications, we assessed the potency of these extracts against resistant (ANKA) *P. berghei*. At the percentage parasitemia observed in the infected control (1.01±0.13), 200 mg/kg *N. latifolia* decreased the parasite burden (0.48±0.13), followed by the *S. jollyanum* (0.52±0.04) and lastly the 200 mg/kg *C. afer* (0.57±0.06). However, there was no significant difference between the potency of the methanol extracts of the three medicinal plants (Figure 2a). The percentage clearance was corresponding to the decrease in the parasitemia as observed in the susceptible model (Figure 2b).

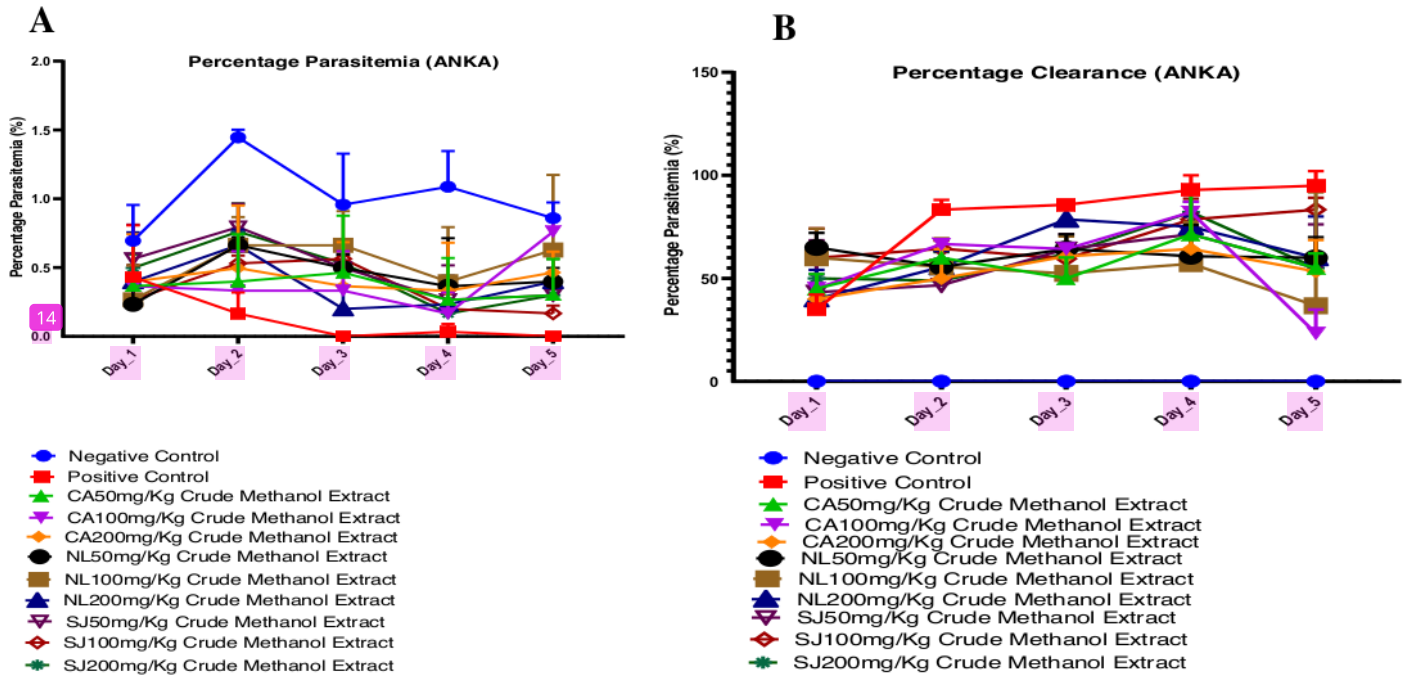
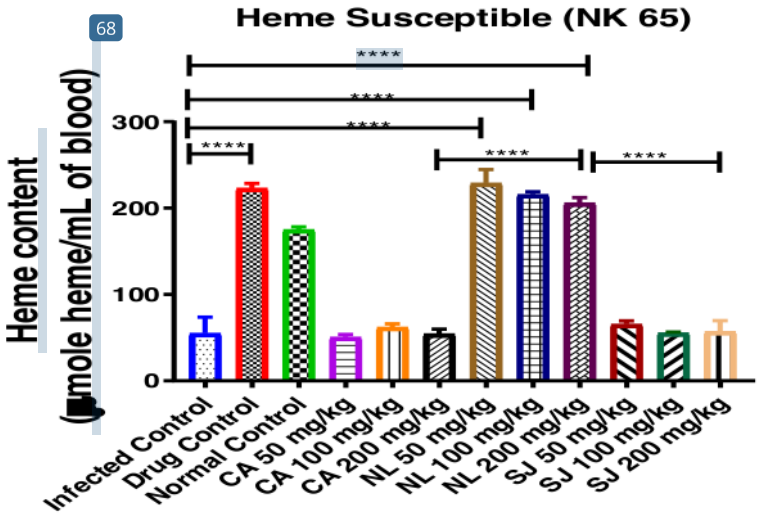
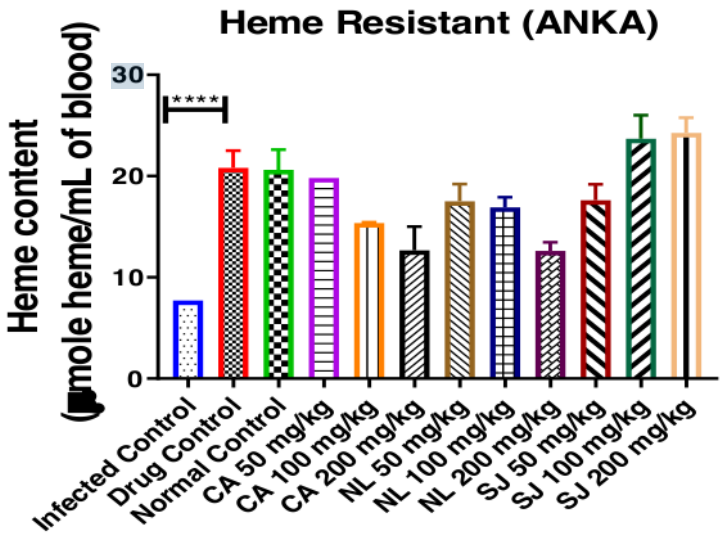


Figure 2: Assessment of the potency of methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* against chloroquine- resistant strain of *P. berghei* in mice. The parasite load decreased as the day increased (Figure 2A) while increase in parasite suppression was also observed as the days go by (Figure 2B)

3.3 Inverse relationship between Heme and hemozoin contents in infected mice treated with methanol extracts of *C. afer, N. latifolia* and *S. jollyanum*

To understand the level of hemolysis and assess the aerobic potentials of the erythrocytes and the damaging extent of parasite infection on the red blood cells, both in chloroquine-sensitive and resistant models, parasite infection decreased ($P<0.0001$) heme content when compared with the drug or normal controls (Figures 3a and 3b). In Figure 3a, although there was increase in the concentration of the bound heme in all the test groups ($P<0.0001$) compared with the infected control, it was only the concentration of the bound heme of the test groups treated with *N. latifolia* that compared insignificantly with that of the normal and drug control groups. Both *C. afer* and *S. jollyanum* at 200 mg/kg decreased heme content when compared with the infected control. The heme content of mice treated with *N. latifolia* (200 mg/kg dose) in chloroquine-sensitive model was higher ($P<0.0001$) when compared with methanol extracts of *C. afer* and *S. jollyanum*. In the chloroquine-resistant groups, heme content of all the treated groups increased than the infected control, although there was variation in the response to treatment that necessitated a corresponding variation in the heme content (Figure 3b). While heme content decreased in the infected control, hemozoin content of this set of mice increased significantly compared with that of the treated groups. It was further observed that the methanol extracts of *C. afer, N. latifolia* and *S. jollyanum*, all at 200 mg/kg, significantly decreased hemozoin contents of mice treated with these extracts. We did not observe any differential significant effect among these extracts (Figure 3c).





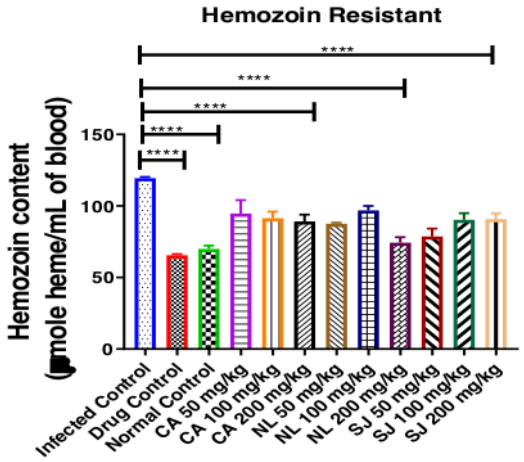


Figure 3:The impact of*Plasmodium berghei* infection and treatment with methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* on bound heme in chloroquine-sensitive *P. berghei*-infected mice (Figure 3A), chloroquine-resistant, P. berghei-infected mice.(Figure 3B) as well as the extent of the formation of malaria pigment (hemozoin) in mice infected with resistant *P.berghei*. ****=P<0.001.

3.4 Extracts of *C. afer, N. latifolia* and *S. jollyanum* reverse parasite-mediated mitochondrial pore opening, F_oF_1 ATPase and peroxidation of mitochondrial lipids

Plasmodium berghei infection caused large amplitude swelling of host mitochondria as evidently observed in Figure 4a. The parasite infection opens the mitochondrial pore in 6 folds relative to when compared with calcium. Here, there was a continuous increase in the host mitochondrial pore opening with consequent decrease in the absorbance observed for twelve minutes at 30 seconds interval. This occurs both in the chloroquine-sensitive (6 folds) and chloroquine-resistant models (9 folds) of experiments (Figure 4b). Similarly, the drug control (Artemether Lumefantrine) equally mediated host mitochondrial pore opening as a consequence of parasite clearance. However, in the susceptible model, artemether lumefanthrine (AL) increased the amplitude swelling to 8 folds as against the 6 folds caused by the infection while in the resistant model, AL decreased the pore opening from 9 folds to 3 folds. In the susceptible model, *C. afer,* mediated a dose dependent reversal of pore opening from 5 folds (50 mg/kg) through 4.7 folds (100 mg/kg) to 2.6 folds (200 mg/kg). Similarly, *N. latifolia* decreased amplitude swelling from 7 folds (50 mg/kg) through 3 folds (100 mg/kg) to 3.8 folds (200 mg/kg). Only the lower doses of *S. jollyanum* decreased the pore opening maximally at 100 mg/kg to 4.6 folds. In the resistant model also, *C. afer* decreased the mitochondrial pore opening maximally at 200 mg/kg to 3 folds while similar effect was noticed by the 50 mg/kg of *N. latifolia*. *Sphenocentrum jollyanum* reversed the pore opening caused by *Plasmodium* infection to near normal at 50 mg/kg (approximately 2 folds). Mitochondrial ATPase activity of the bifunctional ATP synthase was determined to assess the bioenergetics stress status both in mice infected and those treated with methanol extracts of *C. afer, N. latifolia* and *S. jollyanum*. In the susceptible model, ATP hydrolyzing effect of the enzyme increased ($P<0.01$) relativeto the drug control. While ATP hydrolyzing effect of the enzyme in

the infected control and those treated with 200 mg/kg of *C. afer* did not vary significantly, *N. latifolia* and *S. jollyanum* (200 mg/kg) reversed F_0F_1 ATPase activity significantly at P<0.0001. *N. latifolia* and *S. jollyanum* (200 mg/kg) significantly (P<0.0001) decreased ATP hydrolysis compared to same dose of *C. afer*. Although, AL decreased ATP hydrolysis, *N. latifolia* (200 mg/kg) further decreased ATP hydrolysis (P<0.05) better than the drug control (Figure 4c). In Figure 4d, methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* also decreased the ATP hydrolysis albeit insignificantly. The susceptibility of mitochondrial membrane lipids to oxidative damage was assessed as a measure of lipid peroxidation. Infection by *P. berghei* caused oxidative damage to mitochondrial membrane both in the susceptible and resistant models. In Figure 4e, infection caused lipid peroxidation (P<0.01) than the drug control while it was not significant in the resistant model. *C. afer* (200 mg/kg) decreased (P<0.0001) peroxidation compared with the drug control. *S. jollyanum* (200 mg/kg) further decreased lipid peroxidation (P<0.0001) more than *N. latifolia* (200 mg/kg) and is also preferred to *C. afer* at the same dose because it inhibited lipid peroxidation (P<0.01) as observed in Figure 4e. In the resistant model, all doses of *C. afer, N. latifolia* and *S. jollyanum* decreased oxidative damage of mitochondrial membrane lipids relative to the infected control; specifically, the highest dose (200 mg/kg) decreased (P<0.0001) lipid peroxidation. It was observed that although, effects of *C. afer* and *N. latifolia* (200 mg/kg) are not statistically significant, *N. latifolia* significantly (P<0.0001) decreased lipid peroxidation when compared with the *S. jollyanum* (Figure 4f).

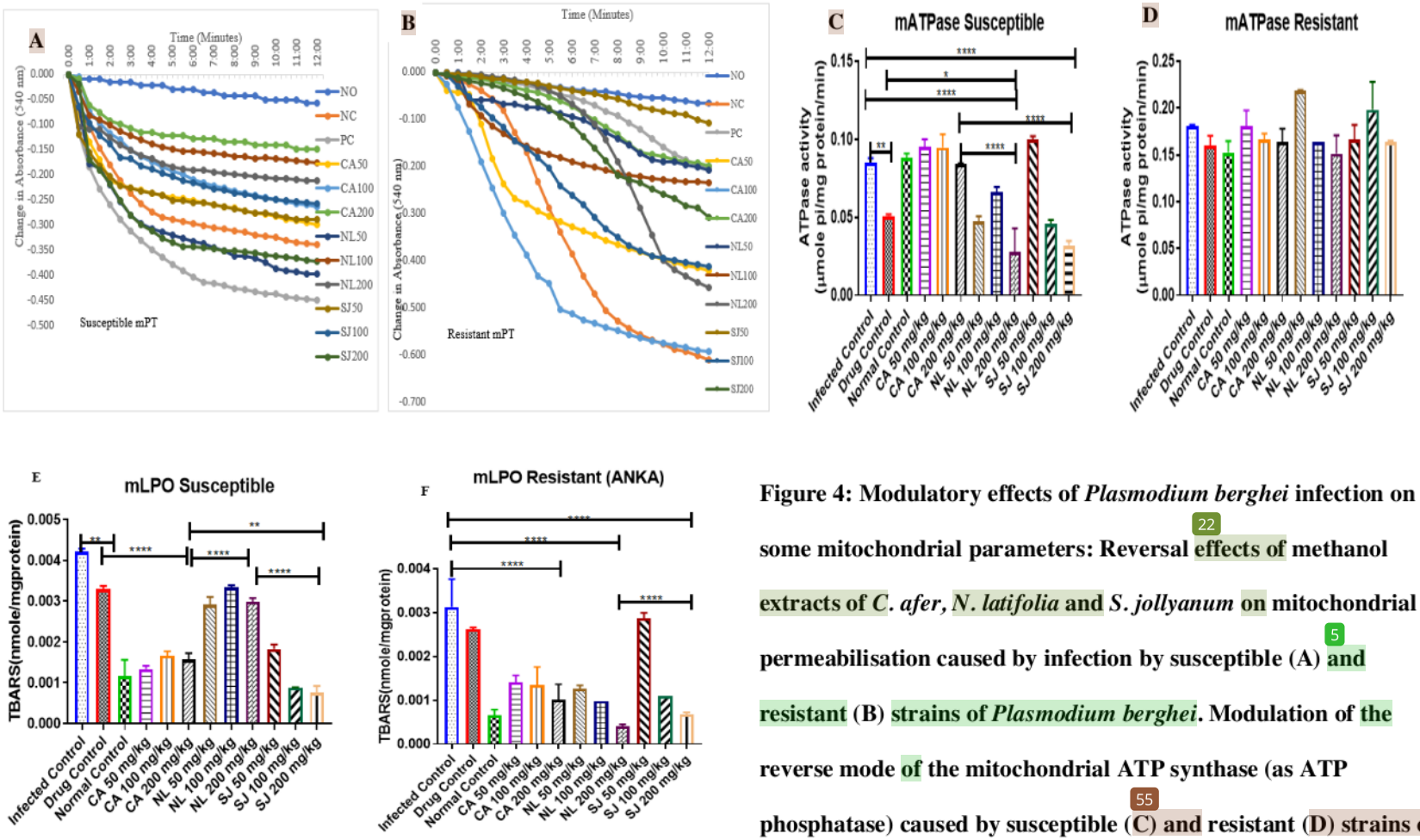


Figure 4: Modulatory effects of *Plasmodium berghei* infection on some mitochondrial parameters: Reversal effects of methanol extracts of *C. afer*, *N. latifolia* and *S. jollyanum* on mitochondrial permeabilisation caused by infection by susceptible (A) and resistant (B) strains of *Plasmodium berghei*. Modulation of the reverse mode of the mitochondrial ATP synthase (as ATP phosphatase) caused by susceptible (C) and resistant (D) strains of *Plasmodium berghei*; inhibition of peroxidation of mitochondrial membrane phospholipids caused by mice infection with susceptible (E) and and resistant (F) strains of *Plasmodium berghei*. *=P<0.05; **=P<0.01; ****=P<0.0001.

3.5 *C. afer, N. latifolia* and *S. jollyanum* modulate inflammatory cytokines in resistant (ANKA) *P. berghei*-infected mice

Serum inflammatory cytokines were assayed because inflammation is a residual effect of *Plasmodium* infection and treatment. This study is necessary to determine the resolution of one of the pathological events of malaria. Again, the resistant model was selected as a representative for the two models. It was observed in this study, that *Plasmodium* infection caused a significant increase in serum interleukin-1beta (IL-1β) and this was reduced by the AL meaning that decrease in IL-1β accompanies parasite clearance. This was observed when *P. berghei* infection was treated with graded doses ofe methanol extracts of *C. afer, N. latifolia* and *S. jollyanum*. Further to this, although, all the doses of the methanol extracts of the three medicinal plants exhibited decrease in the levels of IL-1β, 200 mg/kg of *S. jollyanum* had the highest inhibitory effects as shown in Figure 5a. Figure 5b depicts the serum interleukin-6 concentration in mice infected with resistant *P. berghei*. We discovered that the serum level of this cytokine was significantly (P<0.0001) higher in infected mice and it was reversed upon AL administration. Similarly, *C. afer* and *N. latifolia* at200 mg/kg significantly (P<0.0001) decreased serum level of this cytokine in infected mice. Although, the serum level of this cytokine remains significantly (P<0.0001) high in mice treated with 200 mg/kg of *S. jollyanum*. It is interesting to note that of all the test drugs used, *C. afer* (200 mg/kg) decreased (P<0.0001) the serum IL-6 level relative to *N. latifolia* (200 mg/kg). Interleukin-10 is an anti-inflammatory cytokine and its serum level decreased in infected mice but still higher (P<0.0001) than in infected mice treated with the drug control. The level of this cytokine was the highest in the serum of mice administered with 200 mg/kg of *S. jollyanum*, followed by *C. afer* (200 mg/kg0 and *N. latifolia* (Figure 5c). While there was no significant difference between serum C-reactive proteins (CRP) level of infected and drug controls, infected control, treatment of *P. berghei* infection with 200 mg/kg of the extracts significantly (P<0.05)

decreased serum CRP level (Figure 5d). Serum level of TNF-α was determined and it was discovered that *N. latifolia* (200 mg/kg) was the most potent extract that decreased ($P<0.0001$) serum TNF-α level relative to the infected control. In order of decreasing potency, next to this was *S. jolyanum* (200 mg/kg), and lastly *C. afer* (200 mg/kg). Although, the effect of *N. latifolia* was dose-dependent, the effects of *C. afer* and *S. jollyanum* assumed a hormetic response; 200 mg/kg of *C. afer* and 50 mg/kg of *S. jollyanum* being the doses that maximally decreased serum TNF-α levels (Figure 5e),

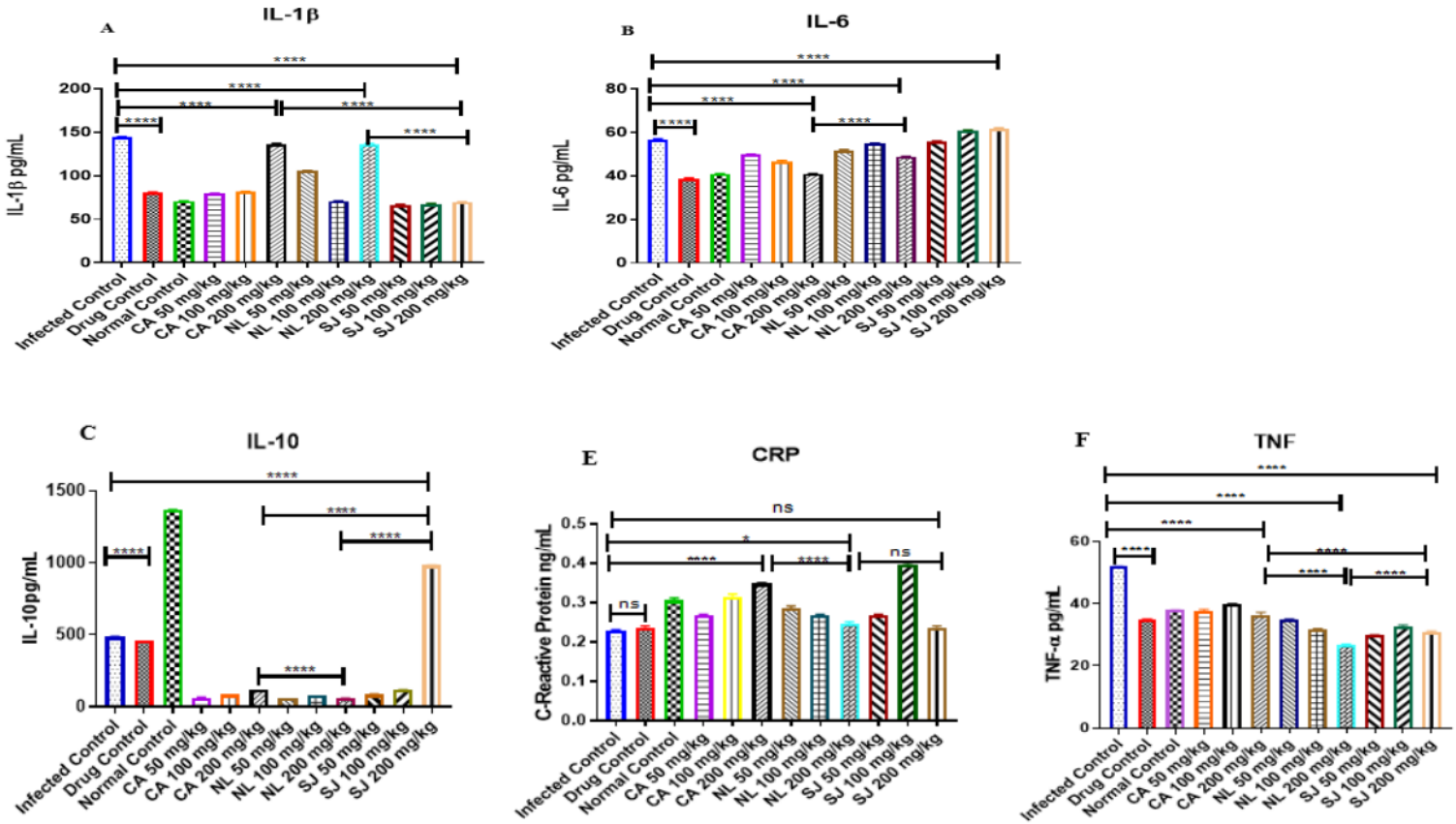


Figure 5: Anti-inflammatory potentials of the methanol extracts of *C. afer*, *N. latifolia* and *S. jollyanum* via the determination of serum interleukins 1β, (A), interleukin 6 (B) interleukin 10 (C), C-reactive protein € and Tumour Necrosis Factor alpha (F) in mice infected with resistant (ANKA strain) Plasmodium berghei. Ns= not significant, *=P<0.05; ****=P<0.0001

3.6 Chemical profile of the extracts of *C. afer, N. latifolia* and *S.jollyanum*

3.6.1 Compounds profile by UPLC-QTOF-MS analysis

Identification of compounds (Figure 6) present in the methanol extracts of *C. afer*, *N. latifolia* and *S. jollyanum* was achieved using a Waters Synapt G2 QTOF in both positive and negative ionization modes. The mass spectrometry data features such as quasimolecular ion, chemical formula MS/MS fragment ions were generated from MassLynx and used for tentative compounds identification. *C. afer* was found to contain as part of its constituents; two *p*-coumaroyl compounds, coumaric acid and coniferyl aldehyde, two phenolic acids, divaricatinic acid and 3-hydroxy-4-(2-hydroxy-6-methylheptan-2-yl)benzoic acid, two hydroxylated unsaturated fatty acids, (*Z*)-9,12,13-trihydroxyoctadec-15-enoic acid, and 9-hydroxy-10,12-octadecadienoic acid, unsaturated fatty alcohol, icos-19-ene-1,2,4-triol and three steroidal saponins, dioscin, aferoside A and C. Methanol extract of *N. latifolia* reveals the presence of the quinic acid derivative, 3-*O*-caffeoylquinic acid, isoquinoline alkaloids, tetrahydropalmatine, palmatine, jatrorrhizine, indole alkaloids, 18,19-dihydroangustine, 17-epinaucleidinal and strictosamide with a saponin, quinovic acid 3-*O*-rhamnoside. Lastly, *S. jollyanum* methanol extract revelaed the presence of a phenolic acid, protocatechuic acid, quinic acid and its derivatives, 3-*O*-caffeoylquinic acid and 3,4-dicaffeoylquinic acid, a saponin, mabioside B while the only identified alkaloid was jatrorrhizine which was also detected in *N. latifolia*.

Table 1. Phytochemical profiles of *C. afer*, *N. latifolia* and *S. jollyanum*

RT (min)	Compound name	Molecular formula	Theoretical mass (m/z)	Found mass (m/z)	Adduct	DBE count	Fragment ions
Costus afer							
3.68	Coumaric acid (**1**)	$C_9H_8O_3$	164.04734	163.0374	$[M-H]^-$	6	119.1
6.12	Coniferyl aldehyde (**2**)	$C_{10}H_{10}O_3$	178.06299	177.0565	$[M-H]^-$	6	177.1; 145.1
6.76	Divaricatinic acid (**3**)	$C_{11}H_{14}O_4$	210.08921	209.0842	$[M-H]^-$	5	209.1; 97.0
7.40	(Z)-9,12,13-trihydroxyoctadec-15-enoic acid (**4**)	$C_{18}H_{34}O_5$	330.24062	329.2308	$[M-H]^-$	2	329.2; 229.1; 211.1; 171.1
10.42	3-hydroxy-4-(2-hydroxy-6-methylheptan-2-yl)benzoic acid (**5**)	$C_{15}H_{22}O_4$	266.15181	265.1440	$[M-H]^-$	5	265.1; 183.0
10.75	Dioscin (**6**)	$C_{45}H_{72}O_{16}$	868.48204	867.4802	$[M-H]^-$	10	N/D
10.90	Aferoside A (**7**)	$C_{44}H_{70}O_{16}$	854.46639	853.4674	$[M-H]^-$	10	N/D
11.50	9-hydroxy-10,12-octadecadienoic acid (**8**)	$C_{18}H_{32}O_3$	296.23514	295.2271	$[M-H]^-$	3	295.2; 277.2; 171.1
11.80	Aferoside C (**9**)	$C_{38}H_{60}O_{12}$	708.40848	707.3923	$[M-H]^-$	9	N/D
16.03	Icos-19-ene-1,2,4-triol (**10**)	$C_{20}H_{40}O_3$	328.29775	327.2925	$[M-H]^-$	1	327.3; 281.3
Nauclea latifolia							
7.12	3-Caffeoylquinic acid (**11**)	$C_{16}H_{18}O_9$	354.09508	354.1356	$[M]^+$		
7.54	Tetrahydropalmatine (**12**)	$C_{21}H_{25}NO_4$	355.17836	356.1819	$[M+H]^+$	10	340.1; 309.1; 294.1; 192.1
8.54	18,19-Dihydroangustine (**13**)	$C_{20}H_{17}N_3O$	315.13716	338.1354	$[M+Na]^+$	14	N/D

8.64	Jatrorrhizine (**14**)	$C_{20}H_{20}NO_4$	338.13923	338.1375	$[M]^+$	12	322.1; 307.1; 294.1; 279.1
9.37	Palmatine (**15**)	$C_{21}H_{22}NO_4$	352.15488	352.1554	$[M]^+$	12	336.1; 320.1; 308.1; 294.1
10.14	17-*epi*-Naucleidinal (**16**)	$C_{20}H_{20}N_2O_3$	336.14739	337.1557	$[M+H]^+$	12	N/D
10.15	Strictosamide (**17**)	$C_{26}H_{30}N_2O_8$	498.20022	497.1906	$[M-H]^-$	13	N/D
12.14	Quinovic acid 3-*O*-rhamnoside (**18**)	$C_{36}H_{56}O_9$	632.39243	631.3870	$[M-H]^-$	9	587.4
Sphenocentrum jollyanum							
0.86	Quinic acid (**19**)	$C_7H_{12}O_6$	192.06339	191.0532	$[M-H]^-$	2	N/D
1.72	Protocatechuic acid (**20**)	$C_7H_6O_4$	154.02661	153.0189	$[M-H]^-$	5	109.0
2.10	3-Caffeoylquinic acid (**11**)	$C_{16}H_{18}O_9$	354.09508	353.0911	$[M-H]^-$	8	191.1 179.0 173.0
4.49	3,4-Dicaffeoylquinic acid (**21**)	$C_{25}H_{24}O_{12}$	516.12678	515.1179	$[M-H]^-$	14	353.1 191.1 179.0 173.0
4.76	Jatrorrhizine (**14**)	$C_{20}H_{21}NO_4^+$	338.13923	338.1346	$[M]^+$	12	322.1 307.1 294.1 279.1
5.09	Mabioside B (**22**)	$C_{42}H_{66}O_{15}$	810.44017	809.4332	$[M-H]^-$	10	765.4 645.4 603.4
8.44	16-(2-Prop-1-en- yl)-2-O-*β*-D-glucopyranosyl cucurbitacin I (**23**)	$C_{39}H_{56}O_{11}$	700.38226	699.3698	$[M-H]^-$	12	587.4

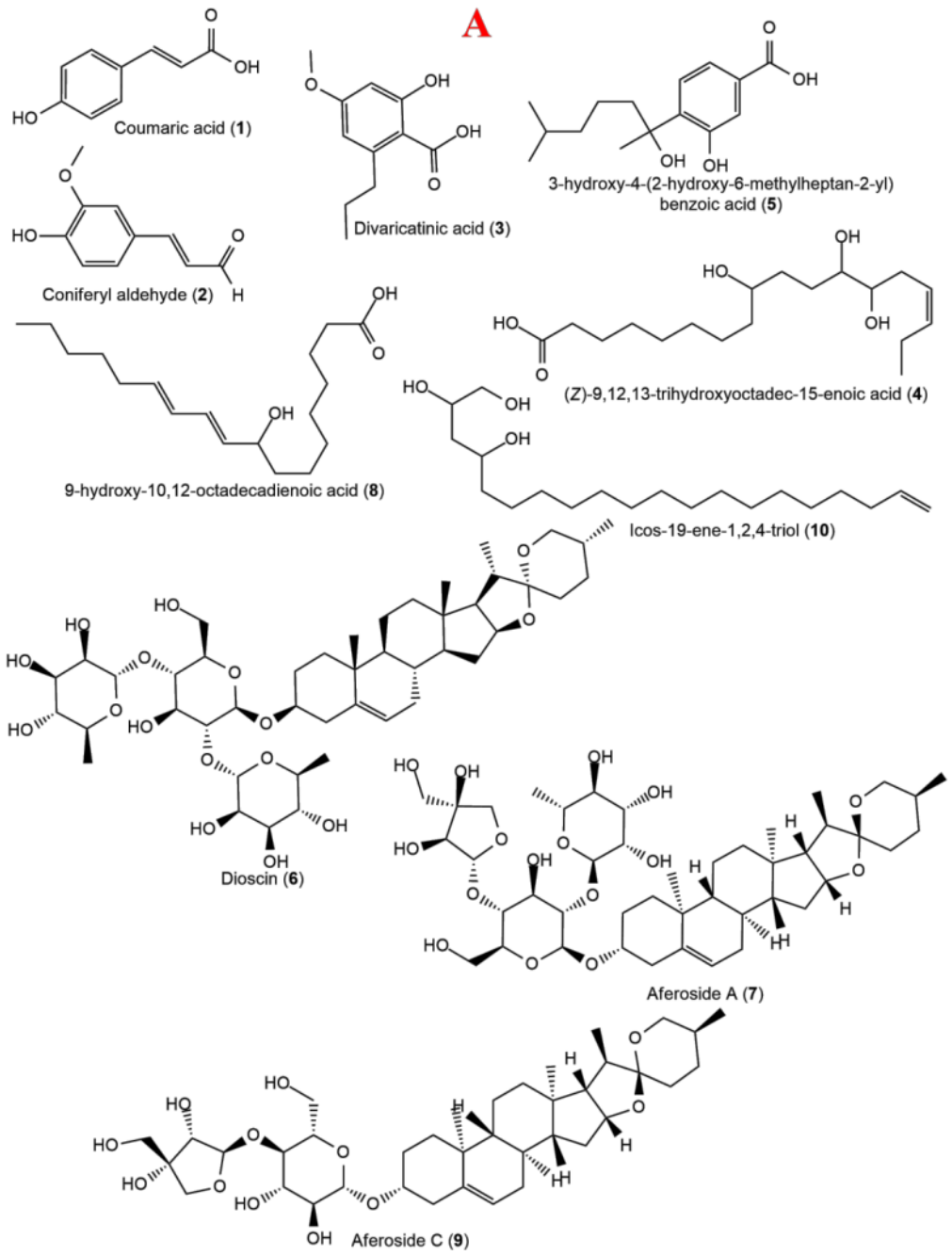


Figure 6. Chemical structures of compounds identified in *Costus afer* (A), *Nauclea latifolia* (B) and *Sphenocentrum jollyanum* (C)

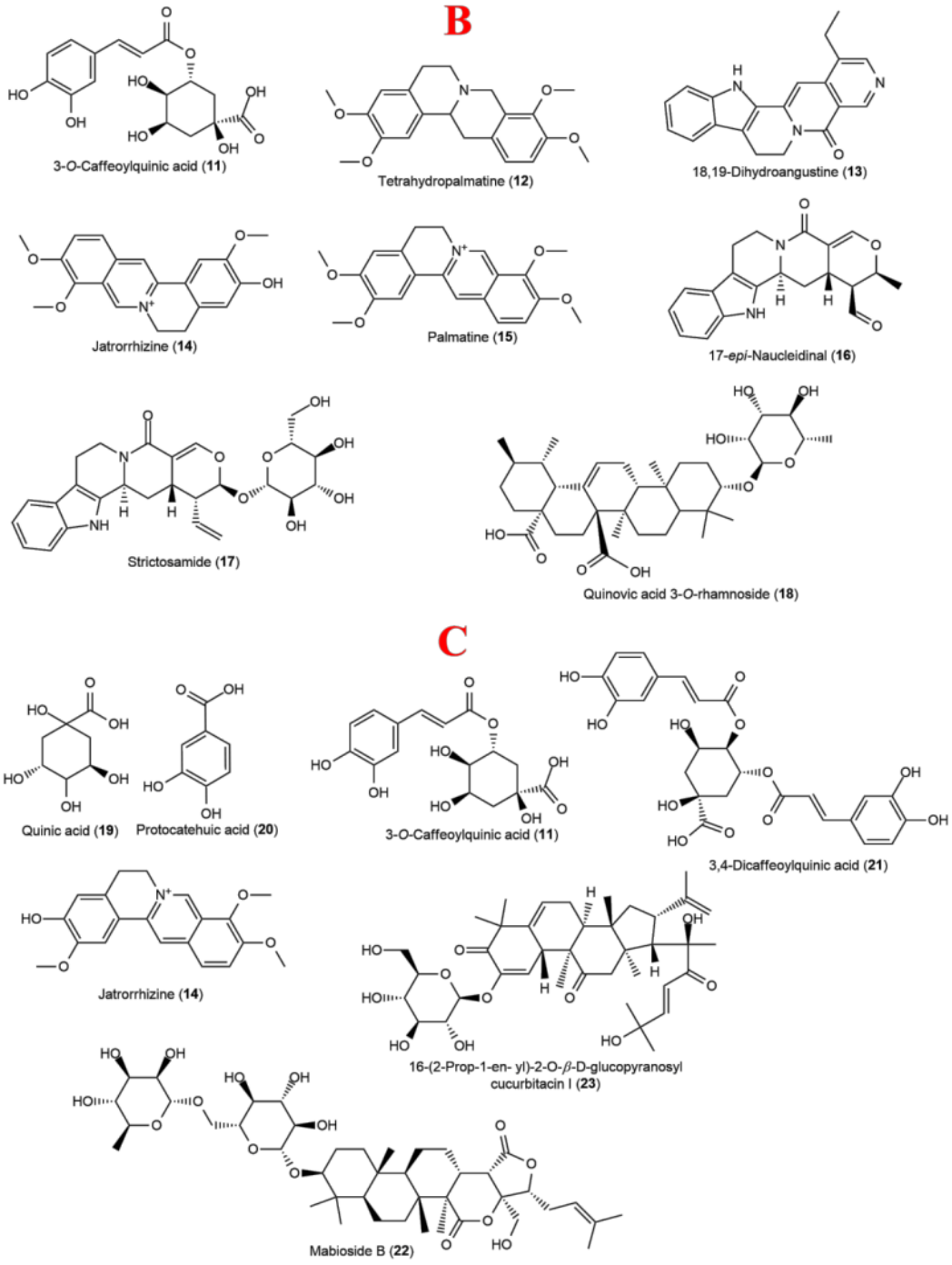


Figure 6 contd. Chemical structures of compounds identified in *Costus afer* (**A**), *Nauclea latifolia* (**B**) and *Sphenocentrum jollyanum* (**C**)

Compounds profile by NMR analysis

The ^{1}H NMR fingerprints of the three extracts (Figure 2) revealed common chemical shifts in most regions of the spectra. Thus, presenting possible common pharmacophores occurring in structurally diverse metabolites of the extracts.

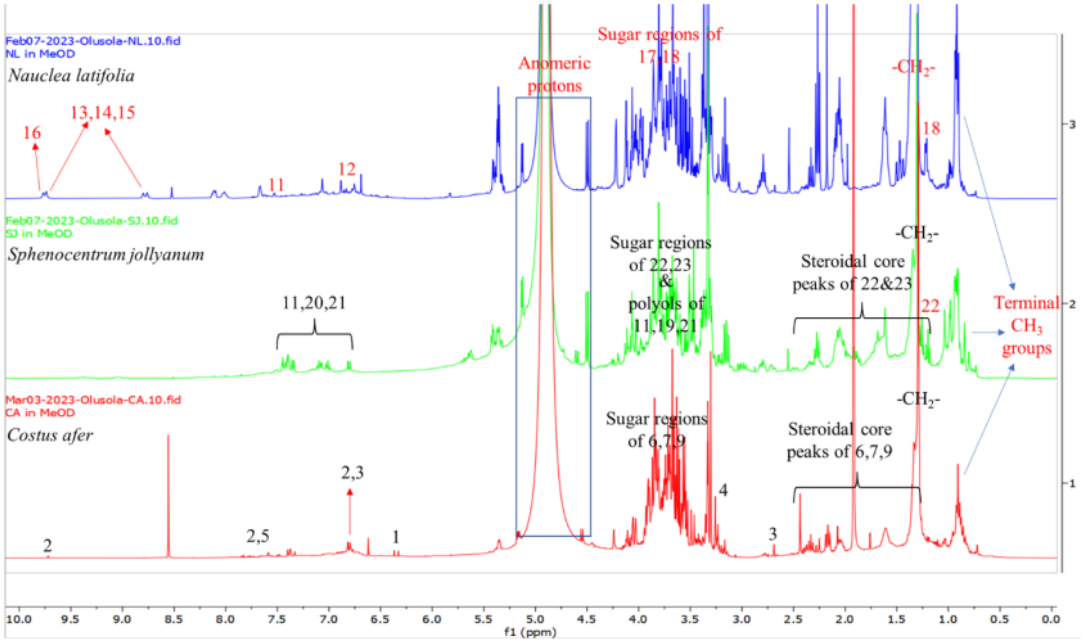


Figure 7: ^{1}H NMR spectra of the methanolic extracts of *Costus afer* (red fingerprint), *Nauclea latifolia* (blue fingerprint) and *Sphenocentrum jollyanum* (green fingerprint)

The ^{1}H NMR spectrum of *C. afer* had a unique doublet at δ_H 6.36 with a coupling constant (*J* value) of 15.8 Hz confirms the presence of coumaric acid (**1**). This peak was supported with the immediate neighbouring proton at δ_H 7.37 observed with overlaps of other compounds' signals. The δ_H 6.36 peak was unambiguously absent or insignificant in the other two extracts indicating that coumaric acid may be a unique signature molecule for *C. afer* in comparison to other species in this study. A similar pattern was observed for coniferyl aldehyde (**2**) although with more

deshielded protons around δ_H 6.76 and 7.78, which were further supported by the aldehydic singlet proton at δ_H 9.72.

The ^{1}H spectrum of *N. latifolia* showed some similarities with that of *C. afer* in regions belonging to caffeoylquinic acid which has a common *p*-coumaric acid moiety as **1** and **2** in addition to the quinic acid's polyol and saponins' sugar chemical shifts between δ_H 3.15 and 4.50. However, additional peaks of higher deshielded protons at δ_H 7.64 and 9.76 which are characteristic of unique aromatic skeletons of indole and isoquinoline alkaloids with 17-*epi*-naucleidinal claiming one of the aldehydic singlets between δ_H 9.73 and 9.76. The peaks at δ_H 8.52 and 8.79 could be assigned to the two singlets of the pyridinyl moiety of 18,19-dihydroangustine. The rhamnosyl methyl resonance of quinovic acid 3-*O*-rhamnoside was observed as a doublet at δ_H 1.22 (J = 5.1 Hz)

Most of the chemical environments of *S. jollyanum* were observed in the other two extracts. However, chemical shift above δ_H 7.45 were very insignificant indicating that alkaloids with highly deshielded aromatic protons were either not present or in trace amounts in *S. jollyanum*, compared to *N. latifolia*, thus validating the result of the UPLC-QTOF-MS. The intensity of the anomeric protons at δ_H 4.50 and 5.13 with additional anomeric peaks at δ_H 4.60 and 4.72 suggest extended glycosylation when compared to *N. latifolia*.

The chemical structures of the identified compounds were further confirmed based on ^{13}C APT chemical shift assignments and relevant HMBC correlations of the diagnostic chemical shift. The APT other relevant 2D NMR spectra are available in the supplementary material.

Discussion

Resistance to major orthodox drugs has made the search for newer drugs with broad spectrum of potency inevitable. The sources of this search include synthesis of new drug candidates,

optimization of existing drugs for enhanced potency, repurposing existing drugs for multiple therapeutic functions, the design of drugs using medicinal chemistry, *in silico* approaches and bioinformatics and purification of drug candidates from medicinal plants. This latter source is a promising process by which drugs like artemisinin and quinine were discovered and optimized. Premised on this fact, this study investigated the potentials of *C. afer, N. latifolia* and *S. jollyanum* as sources of drug candidates for malarial chemotherapy. In this study, the methanol extracts of these plants decreased parasite load and suppresses parasite growth, thus substantiating the indigenous clams (Abbah et al., 2010; Iyamah and Idu, 2015). Moreover, medicinal plants used in this study gain acceptability in other parts of Africa including Benin Republic (Hermans et al., 2004), Cameroon (Taiwe et al., 2011) and Ivory Coast (Zirihi et al., 2005). Using established infection model, it was observed that extracts of these plants have curative potentials against both susceptible and resistant *Plasmodium berghei* infection. Phytochemicals from these plants possess antiplasmodial activity as seen in the decreased parasite load and chemosuppressive effects of these plants. Phytochemicals such as flavonoids, alkaloids, terpenes and saponins have curative potentials for *Plasmodium* infection. Flavonoids and phenolics are known to cure malaria via inhibition of hemolysis and parasite growth and in addition to this, they are good antioxidants and as such mitigate against oxidative stress, which is a known secondary side effects of infection (Lehane and Saliba, 2008; Gomes et al., 2022). Similarly too, alkaloids, which are sometimes secreted by plants as ecological survival agents, and repellents, possess curative potentials against malaria and this has been documented in animal models (Uzor, 2020). Terpenes are natural products with proven antimalarial properties. Terpenes such as limonene, linalool and perillyl alcohol possess antimalarial properties and their mechanism of action is via interference with *Plasmodium* protein isoprenylation (Rodriguez et al., 2017). There are plethora of scientific

evidences to show that indeed, the antiplasmodial potentials of medicinal herbs is traceable to the secondary metabolites secreted by these plants.

Heme, a macromolecular prosthetic group in some proteins is responsible for respiration in hemoglobin (Yuan et al., 2016). It is the major substance that is attacked by malarial parasite in the blood for its survival, polymerizing it to harmless hemozoin and make use of the amino acid constituents of globin for its salvage pathway of synthesis. In severe malaria, hemozoin is presented as malarial pigments. Preventing heme polymerization to hemozoin will further prevent parasite growth and survival and it is a mechanism of action of some orthodox antimalarial drugs. In this study, the three extracts supported increase in bound heme content of erythrocyte and prevented its degradation and polymerization to hemozoin. This way, parasites survival in both experimental models is very slim because the extracts deprived the parasites nutrient sources. Prevention of heme breakdown serves several purposes in malaria one of which is the prevention of hemolysis. Hemolytic anemia is a major secondary effects of *Plasmodium* infection (White, 2018). In this study, heme content decreased with a corresponding increase in hemozoin content in infected control. These findings are consistent with previous reports (Kotepui et al., 2015). Prevention of heme polymerization to form hemozoin by these extracts could be one of their mechanisms of action. Again, free heme, if present in the host system is toxic to both the host and the parasite because of its reactive iron content that can initiate free radical generation through the Fenton and Haber-Weiss reaction. If the extracts prevent the polymerization of free hene to hemozoin, the parasite cells can be commuted to death via ferroptosis pathway. However, the host has means to mitigate this through its enzymic and non-enzymic antioxidants.

Mitochondria metabolism occupies a vital position in the pathogenesis and outcome of malaria infection in the host. The opening of the mitochondrial pore in infected host causes bioenergetics

stress, affects mitochondrial dynamics, turnover, homeostasis and cell death. The reversal of parasite-induced mitochondrial permeabilisation by methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* will improve host energetics by increasing ATP synthesis via oxidative phosphorylation and prevents cell death via mitochondrial-mediated pathway. Previous study has shown that artemisinin affects *Plasmodium* mitochondria and it could be the drug's mechanism of action (Hou et al., 2020). Interestingly, our previous studies on *Diospyros mespiliformis* (David et al., 2020), *Mondia whitei* (Olanlokun et al., 2018, 2019,), *Phyllanthus amarus* (Olanlokun et al., 2020) and *Alstonia boonei* (Olanlokun et al., 2013) showed that although, orthodox antimalarial drugs can initiate the opening of mitochondrial pore and at the same time are potent against parasite load, the antimalarial potency of medicinal plants coupled with their mito-protective effects is preferred because of their reversal effects on parasite-induced mitochondrial pore opening that could lead to cell death (Olanlokun et al., 2021). While artemisinin combinative therapy remains the drug of choice for malaria, the effect of the drug on oxidative phosphorylation is a major concern in malaria chemotherapy. The therapeutic dose of this drug (arthemether-lumefanthrine) did not stimulate mitochondrial respiration but rather uncouples oxidative phosphorylation as observed in this study. However, the discovery that *C. afer* and *S. jollyanum* reversed parasite-induced uncoupling of mitochondria showed that their administration favours ATP synthesis via stimulation of oxidative phosphorylation. Furthermore, stimulation of ATPase activity of the enzyme by 50 mg/kg of *C. afer,* and *N. latifolia* as well as 100 mg/kg of *S. jollyanum* in the resistant model of the experiment showed that the effects of these medicinal plants are bimodal. Although, the stimulating effect of *C. afer* and *N. latifolia* was found to be dose-dependent, the effect of *S. jollyanum* did not show linear correlation. Previous study on primaquine showed similar effect (Baker et al., 1986). In this study, peroxidation of mitochondrial lipids was observed as one of the

secondary effects of *Plasmodium* infection and this was prominent in the infected control. While varied doses of *C. afer* and *S. jollyanum* significantly decreased the total peroxidative products, an observation that such was not observed in the *N. latifolia*-treated mice showed that although, the parasite clearance effect of these medicinal plants may not differ significantly with respect to the doses used, doses that are safe for an extract may not be safe for another. Again, *N. latifolia* that showed peroxidative effect in the susceptible model of the experiment was found to be safe in the resistant model without causing oxidative damage. Taken together, we found that extracts of these plants mitigate oxidative damage as an additive effect to their antimalarial potentials. This indicates that extracts of these plants may have cardio-protective effect against cardiac complication which is a residual effect of *Plasmodium* infection. Mitochondrial lipids are indispensable for the proper maintenance of their functions and integrity and dysregulation in their constituent can cause serious deleterious effects. Among others, peroxidation of cardiolipin, a unique mitochondrial phospholipid in the inner mitochondrial membrane can cause re-orientation of the backbone and head groups of the fatty acids of the oxidized molecules and therefore affect cellular processes that are dependent on cardiolipin functionality such as electron and proton transport system (Vähäheikkilä et al., 2018). Previous study has found that loss of cardiolipin content, changes in its fatty acyl chain composition, as well as peroxidation of cardiolipin has been associated with mitochondrial dysfunction in a variety of pathological conditions such as ischemia, hypothyroidism, aging, and heart failure (Chicco and Sporagna, 2007). Both complicated and uncomplicated malaria has been linked with cardiac problems and the reasons may not be too far from peroxidation of mitochondrial lipids.

The pathogenesis and complications of malaria are dependable on the response of the disease treatment and biomedical factors such as the inflammatory outcome of the disease and treatment.

Normally, body response to infection via inflammation is a welcome host response to infection. However, excessive or rather chronic inflammatory response may mediate another secondary disease in *Plasmodium* infection. Because of this, a balance in pro-inflammatory and anti-inflammatory markers is necessary in malaria disease. Interleukin-1 regulates inflammation because it controls a variety of innate immune responses. Resistant *Plasmodium* infection are usually associated with complicated malaria where elevated serum level of IL-1β are usually significant as observed in this study where resistant model of *Plasmodium berghei* (ANKA strain) were used (Mahittikorn et al., 2022). Apart from the fact that it shows increased severity of the disease, increased IL-1β level in *Plasmodium*-infected patients is also responsible for pathological changes and symptoms observed in malaria disease. Their elevated level in the peripheral blood is also linked to parasite clearance. This is one of the adaptive response noticed in the infected individuals shortly before chemotherapy (Farrington et al., 2017). Similarly, serum elevation of IL-6 is an indication for severe malaria and it can be used as its marker. Contrarily, being a pleiotropic cytokine, and because of its immune capability, IL-6 can perform defensive role in the host. Therefore, this study shows that the increase in the levels of this cytokine in infected mice treated with *N. latifolia* and *S. jollyanum* may be as a result of the healing processes as previous observed by other study (Simpson et al., 1997). The TNF-α mediates inflammation and its effects on various cell types makes it a major regulator of inflammation in the pathogenesis of malaria. High levels of TNF-α in the infected control may indicate severity of hyper-parasitemia (as observed in this study), severe anemia and hypoglycemia (Shaffer et al., 1991). Elevated levels of TNF-α may indicate prognosis of malaria disease and the outcome of treatment. The C reactive protein performs pathogenic role in malaria disease. It binds to infected erythrocytes and assists in the clearance of same. Therefore, its serum level may indicate parasite burden and as a result may

be used as a prognostic marker for the disease. A balance in the inflammatory cytokines plays significant roles in the outcome of *Plasmodium* infection and the pathogenesis of the disease (Rovira-Vallbona et al., 2012). Increased serum IL-10 as observed in the group treated with *S. jollyanum* in this study is profitable because of its anti-inflammatory role. It limits the extent at which host immune system responds to pathogens and as a result prevent damage to the host cell and normal tissue homeostasis (Iyer and Cheng, 2012).

Like artemisinin, phytochemicals are promising drug leads for the treatment and prevention of diseases. The curative and chemo-preventive potentials of *C. afer, N. latifolia* and *S. jollyanum* depends solely on the phytochemicals they contain. Through the chemical profiling of these medicinal plants, important phytochemicals were identified. Sonibare et al., 2023, identified fatty acids and their methyl esters in the leaf of *C. afer* using the GC-MS; Boison et al., 2019 identified steroidal saponins from the plant. Since the total phytochemicals in plants cannot be profiled in a single study, we identified diocin and divaricatinic acid in the plant for the first time in addition to other steroidal saponins and faty acids earlier profiled from the plant. Diocin is a nitrofuran derivative and it has been found to possess antiprotozoal and antibacterial activity and it is also a monoamine oxidase inhibitor. Although, divaricatinic acid has been identified and purified previously from other plants (Lomchid et al., 2016; Nguyen et al., 2019), this compound of the hopane family is identified in *C. afer* for the first time. Previous study has shown that this compound has antiproliferative and antimalarial property (Lomchid et al., 2016). Chlorogenic acid, quercetin and its derivatives and caffeic acids have been isolated from *N. latifolea* and their pharmacological properties have been stated in previous study (Ajayi et al., 2020). In another study by Ata et al., (2009), quinovic acid and its derivatives, strictosamide and various structural isomers of naucleamide were identified. In addition to some of this, we have identified

Tetrahydropalmatine and Jatrorrhizine in the plant for the first time. Pharmacological usefulness of tetrahydropalmatine includes analgesic, neuroprotection and anti-inflammatory effects (Du et al., 2022). Jatrorrhizine is an isoquinoline alkaloid with anti-inflammatory effects (Arens et al., 1985). Mabioside B and a sugar derivative of cucurbitacin, 16-(2-Prop-1-en- yl)-2-O-*β*-D-glucopyranosyl cucurbitacin, in addition to other compounds previously identified from the plant, were identified for the first time in *S. jollyanum*. Mabioside B belongs to the group of compounds referred to as dammarane-type triterpenoids and they have anti-inflammatory and immunomodulatory activity (Ruan et al., 2016). Cucurbitacins have anti-inflammatory, antioxidant, antimalarial antimicrobial and hepatoprotective effects (Varela et al., 2022).

In summary, this study shows how medicinal plants, through their phytochemicals, possess antimalarial potentials against chloroquine-susceptible and resistant parasites, have anti-inflammatory, analgesic and immunomodulatory potentials. Different phytochemicals with varying pharmacological potentials are present in the medicinal plant and they occur as composite medicines in the form of polypharmacy for synergistic and combinative therapy purposes. This may be the reason why medicinal plants are prepared as decoctions. The purification and formulation of relevant phytochemicals from this plant for the treatment of diseases will have beneficial purposes for the treatment of malaria and prevention of side effects of monotherapy that is common to orthodox medicines.

Ethical statement

The conduct of this study follows the ARRIVE guidelines. Moreover this study was revised and approved by University of Ibadan Animal Care and Use Research Ethics Committee and approval number UI-ACUREC/17/0092 was assigned to this study.

Conflict of Interest

Authors state that no conflict of interest exists

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This study was self-sponsored by all the authors

Authors contributions

JOO conceived and designed the study, performed experiments and wrote the draft manuscript. SOO perfomed experiments, AO performed experiments, BO performed experiments, OB did the LC-MS and NMR study, VM corrected the manuscript, OOO corrected the draft manuscript.

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