Original article

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

Objectives: This study reported the antiplasmodial, anti-inflammatory and mito-protective effects of *Costus afer* (CA)*, Nauclea latifolia* (NA) and *Sphenocentrum jollyanum* (SJ) methanol extracts in *Plasmodium berghei*-infected mice.
Methods: Air-dried CA, NA and SJ were extracted with methanol. Antiplasmodial activity of these extracts were monitored using chloroquine-sensitive and resistant strains of *Plasmodium berghei*. Heme and hemozoin contents, interleukins and C-reactive protein as well as mitochondrial permeability transition (mPT) pore opening, lipid peroxidation (mLPO) and F_0F_1 ATPase activity were determined by spectrophotometry. Phytochemical constituents were determined using UPLC-QTOF-MS and NMR spectroscopy.
Results and Conclusions: CA, NL and SJ decreased percentage parasitemia to 0.25 ± 0.07; 0.30 ± 0.14 and $0.25 \pm 0.07\%$ relative to control ($8.60 \pm 0.15\%$) in the chloroquine-sensitive model and to 0.40 ± 0.14; 0.30 ± 0.14 and 0.45 ± 0.07, respectively as against $10.88 \pm 0.26\%$ of the infected control in the chloroquine-resistant model. In chloroquine-resistant model, NL decreased mLPO (0.41 ± 0.04) F_0F_1 ATPase (0.15 ± 0.02 mmol pi/mg protein /min) while CA enhanced mPT pore opening at 100 mg/kg,and SJ (50 mg/kg) reversed parasite-induced mPT pore opening (1.66 vs 9.4 folds). The NL increased heme, decreased hemozoin, IL-6, CRP, TNF-α, while SJ dose-dependently increased IL-10. UPLC-QTOF-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-epinau-cleidinal, strictosamide and quinovic acid 3-O-rhamnoside 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.

1. Introduction

Malaria has become a difficult disease to treat because of resistance to artemisinin-combinative therapy. It has also been difficult to design vaccine for the malaria because of the complex stages of the parasites' life cycle. The hope for drugs from medicinal plants is evident with the discovery of artemisinin from *Artemisia annua* (Menan et al., 2006). In ethno-medicine, *Costus afer's* rhizome, *Nauclea latifolia* and *Sphenocentrum jollyanum* parts have been used in the treatment of malaria either as single plant or in composite preparations (Omokhua, 2011). *C. afer* belongs to the Costaceae family formerly known as Zingiberaceae

(Aweke, 2007). They have inflammatory, and antiplasmodial potentials (Soladoye and Oyesika, 2008). Herbal remedies of *N. latifolia* (Smith) classified as Rubiaceae is used to treat malaria (Elujoba, 1995). Folk-lorically, parts of *Sphenocentrum jollyanum* can be prepared to treat malaria (Olorunnisola and Afolayan, 2011). Most plants with antima-larial properties equally have inflammatory potentials. Oxidative stress can affect mitochondrion in the host leading to its dysfunction via the irreversible permeabilization of its membranes (Kent et al., 2021). Malaria infection causes oxidative damage, affecting host and parasites. Malaria treatment, therefore, will prevent inflammation, bioenergetics stress and mitochondrial dysfunction. The folkloric treatment of

uncomplicated malaria using *C. afer, N. latifolia* and *S. jollyanum* has been well documented but their protective mechanism on host mitochondria has not been reported and information on the bioenergetics status of the infected host is lacking. This study reported the antiplasmodial, anti-inflammatory and mito-protective potentials of *C. afer, N. latifolia* and *S. jollyanum*.

2. Materials and methods

2.1. Plant sources and their extraction

Air-dried aerial parts of *C. afer,* stem bark of *Nauclea latifolia,* and root bark of *Sphenocentrum jollyanum* (1 kg each) were milled and separately soaked in 100% methanol,decanted, filtered and the filtrate was concentrated to solvent-free methanol extract.

2.2. Experimental animals and Research design

This study was divided into two phases. In Phase 1, mice were intraperitonealy infected with chloroquine-sensitive *P. berghei* (NK 65 strain) while in phase 2, chloroquine-resistant (ANKA strain) *P. berghei* was used. In Phase 1, fifty-five male Swiss mice were passaged with infected erythrocytes from a donor mouse. Five uninfected mice were classified as normal control. Parasitemia was confirmed after 72 h via microscopy. Similar procedure was repeated in phase 2 using resistant *Plasmodium berghei* (ANKA strain).

2.3. The design of the experiment

Mice in both phases received 10 mg/kg of Artemether-Lumefantrine in 5% DMSO as vehicle. The vehicle only was administered to the normal and infected control groups. Other test groups were treated with 50, 100 and 200 in mg/kg of the methanol extracts of the plant.for seven and five days in chloroquine-sensitive and resistant experiments, respectively.

2.4. Antimalarial effects of Costus afer, 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 modified established infection method described by Ryley and Peters (1970). Briefly, after the confirmation of parasitemia, mice were treated orally for seven days (chloroquine-sensitive model) and five consecutive days (choloroquine-resistant model). At forty-eight hours interval, blood was collected from the mouse tail for slide preparation, allowed to dry and fixed in methanol, dried and stained with Giemsa stain. The slides were rinsed in buffered water and air-dried. The slides were viewed on microscope using oil immersion objective. Parasitemia and chemo-suppression, were then calculated.

2.5. Heme and hemozoin determination

Blood sample (10 μL) was lysed in SDS and dissolved in 1 M NaOH followed by sonication for 10 min. The samples were then incubated for 2 h in a water bath. Heme content was determined by reading the absorbance of the samples at 404 nm and the total heme calculated using its molar absorption coefficient (9.08×10^4/M/cm) (Asakura et al., 1977). The hemozoin content was estimated from 10 μL of blood, lysed in 0.08% saponin and centrifuged at 18,000 rpm. The sediment was washed repeatedly using 250 μL of 25% SDS buffered with 25 mM Tris-HCl (pH 7.4), incubated at 37 °C overnight and washed at 18,000 rpm. The pellets were then dissolved in 1 M NaOH and the absorbance was read at 404 nm (Orjih and Fitch, 1993).

2.6. Preparation of serum

Withdrawn blood was allowed to clot and centrifuged at 3.500 rpm for 5 min to obtain the serum. Serum was aspirated into clean sample bottle and kept in the refrigerator.

2.7. Mitochondria isolation

The liver was removed, rinsed in isolation buffer (sucrose (70 mM), Hepes-KOH (5 mM, pH 7.4), mannitol (210 mM) and EGTA (1 mM)), weighed, chopped and a 10% in isolation buffer was homogenized. The homogenate was then spun twice at 2,500 rpm for 5 min each. The supernatant was further centrifuged at 13,000 rpm for 10 min to obtain mitochondria that was washed in washing buffer (Hepes-KOH (5 mM, pH 7.4), sucrose (70 mM), mannitol (210 mM) and BSA (0.5%)) twice at 12000 rpm for 10 min each time. Mitochondria were then suspended in buffer (Mannitol (210 mM), Sucrose (70 mM) and Hepes-KOH (5 mM, pH 7.4)), dispensed in Eppendorf vials and kept on ice (Johnson and Lardy,1967).

2.8. Determination of total protein in mitochondria

Mitochondria (10 μL) were made up to 1 mL and 3 mL of a 100:1:1 mixture of Na_2CO_3 (2%), Na + -K + tartarate (1%) and $CuSO_4·5H_2O$ (0.5%) in 100 mL of 0.1 M sodium hydroxide was added. After 10 min, 0.3 mL of Folin reagent was added and incubated for 30 min. The absorbance was read at 750 nm and mitochondria protein was quantified by using protein standard curve (Lowry et al., 1951).

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

Mitochondria (0.4 mg/mL protein) were incubated in the suspension buffer and rotenone (8 μM) for three and half min. Succinate (5 mM) was added and the absorbance read at 540 nm for 12 min at 30 s interval to ascertain mitochondrial quality. The same quantity of mitochondria was added to the suspension buffer, rotenone and the mixture was incubated for 3 min and then 3 μM calcium was added while succinate was added 30 s later and the absorbance read. Mitochondria with little decrease in absorbance in the absence of calcium but with decrease in absorbance in the presence of calcium are considered intact. Similar mitochondrial protein from the treated groups were subjected to the same mPT assay. The mPT opening effects of calcium, *Plasmodium* infection and the modulatory effect of *C. afer, N. latifolia* and *S. jollyanum* were then compared (Lapidus and Sokolove, 1993).

2.10. Assay of mitochondrial F_0F_1 ATPase activity

Sucrose, potassium chloride (25 and 0.5 mM, respectively), and Tris-HCl (65 mM) were added into the test tubes and made up to 1 mL. Mitochondrial protein (0.5 mg/mL) was added to their respective test tubes. ATP (1 mM) was added and the mixture was incubated for 30 min after which 10% SDS was added. The 25 μM of 2, 4-dinitrophenol was used as a standard uncoupler. One mL volume 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 after 30 min of incubation. Inorganic phosphate concentration was estimated from phosphate standard curve (Lardy and Wellman, 1953).

2.11. Assay of mitochondrial membrane lipids peroxidation

Mitochondria (0.4 mL) were added to 1.6 mL of Tris-KCl (0.15 M) 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 min, cooled, spun at 3000 rpm for 10 min and the absorbance

was read at 532 nm. TBARS was quantified by using its extinction co-efficient (0.156 $\mu M^{-1} cm^{-1}$) (Varshney and Kale, 1990; Adam-Vizi and Seregi (1982).

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)

Compounds separation and detection from the 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 2600 V and 2000 V, 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 4.1 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. The NMR spectra were recorded using deuterated methanol at room temperature on a Bruker Avance III, 400 MHz spectrometer. Raw NMR data were processed using Bruker Top Spin (version 3.6.4) software.

2.15. Statistical analysis of data

Representative profile was used for mPT assays. Other assays were analyzed by using descriptive statistics and multiple comparison
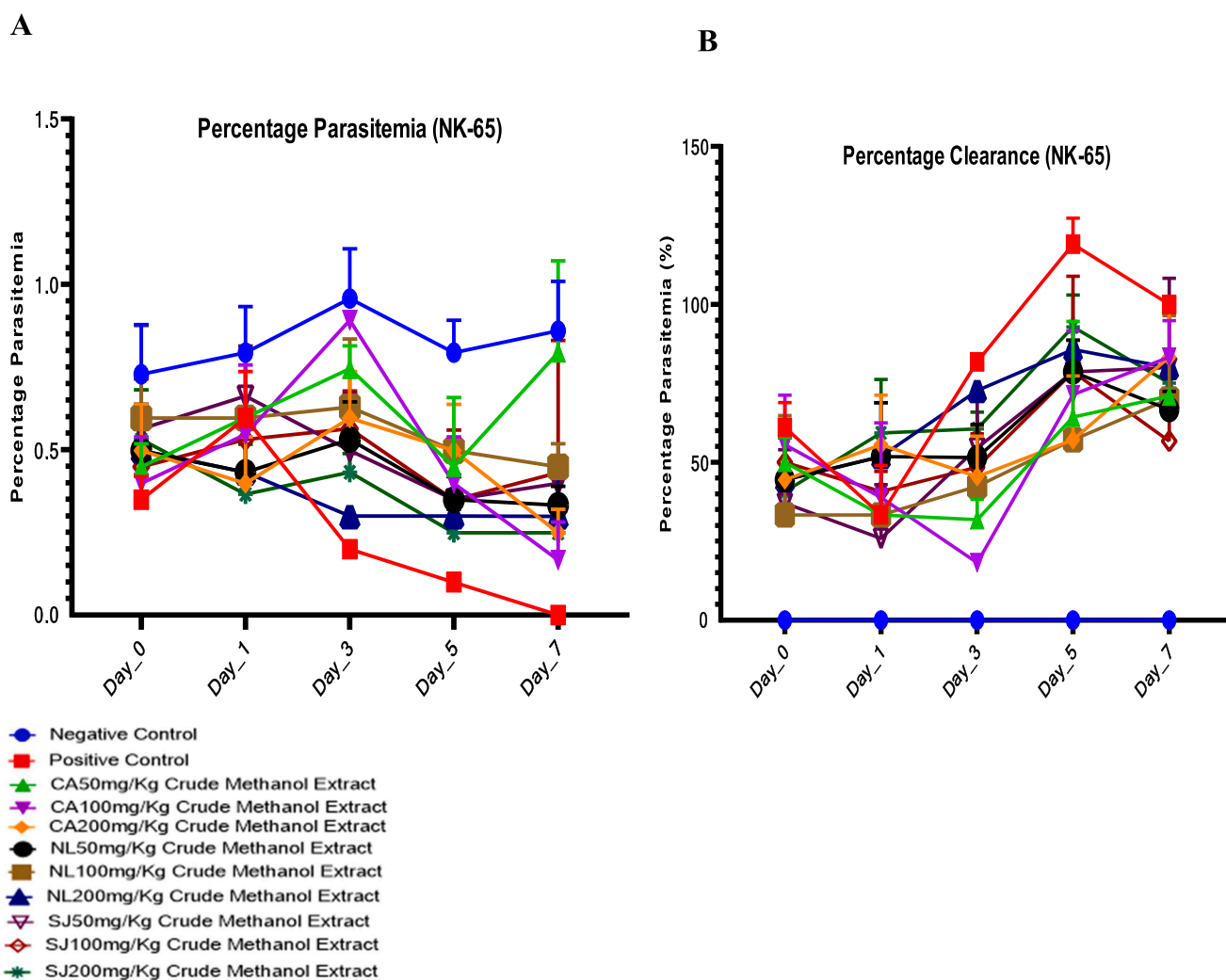

Fig. 1. Antiplasmodial potentials (A) and chermosuppression (parasite clearance) (B) of *C. afer, N. latifolia* and *S. jollyanum* methanol extracts in mice infected with chloroquine-sensitive *P. berghei.*

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)

Relative to the infected control (0.84 ± 0.09), 200 mg/kg dose of *C. afer* reduced the parasite burden to 0.42 ± 0.16, *N. latifolia* (0.35 ± 0.05) and 200 mg/kg *S. jollyanum* 0.38 ± 0.04 (Fig. 1a). Methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* significantly decreased the parasite burden in mice infected with *P. berghei* (chloroquine-sensitive strain) compared with the infected control (Fig. 1b).

3.2. Therapeutic potentials of C. afer, N. Latifolia and S. Jollyanum against resistant (ANKA strain) P. Berghei infection in mice

Compared with the parasitemia of the infected control (1.01 ± 0.13), *N. latifolia* decreased the parasite burden (0.48 ± 0.13), followed by the *S. jollyanum* (0.52 ± 0.04) and lastly *C. afer* (0.57 ± 0.06) (Fig. 2a). The percentage clearance was corresponded with the decrease in the parasitemia (Fig. 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

In Fig. 3a, it was only the concentration of the bound heme of the test groups treated with *N. latifolia* that favourably compared with that of the normal and drug control groups. 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 that of the infected control (Fig. 3b). Hemozoin content of infected control increased while it was decreased by *C. afer, N. latifolia* and *S. jollyanum*, (200 mg/kg) (Fig. 3c).

3.4. Extracts of C. afer, N. Latifolia and S. Jollyanum reverse parasite-mediated mitochondrial pore opening, F_0F_1 ATPase and lipid peroxidation

Plasmodium berghei infection caused large amplitude swelling of host mitochondria (Fig. 4a). This occurs both in the chloroquine-sensitive (6 folds) and chloroquine-resistant models (9 folds) (Fig. 4b). In the susceptible model, *C. afer,* reversed the pore opening from 5 folds (50 mg/kg) through 4.7 folds (100 mg/kg) to 2.6 folds (200 mg/kg). *N. latifolia* decreased absorbance from 7 folds (50 mg/kg) through 3 folds (100 mg/kg) to 3.8 folds (200 mg/kg). The 50 mg/kg of *S. jollyanum* decreased the pore opening at 100 mg/kg to 4.6 folds. In the resistant model, *C. afer* reversed the pore opening at 200 mg/kg to 3 folds. *Sphenocentrum jollyanum* reversed the pore opening at 50 mg/kg. *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. N. latifolia* (200 mg/kg) further decreased ATP hydrolysis ($P < 0.05$) compared with the drug control (Fig. 4c). In Fig. 4d, the extracts decreased the ATP hydrolysis. *C. afer* (200 mg/kg) decreased ($P < 0.0001$) peroxidation compared with the drug control. *S. jollyanum* (200 mg/kg) decreased lipid peroxidation ($P < 0.0001$) more than *N. latifolia* (200 mg/kg) and is also preferred to *C. afer* at the same dose (Fig. 4e). In the resistant model, 200 mg/kg dose decreased ($P < 0.0001$) lipid peroxidation relative to the infected control (Fig. 4f).
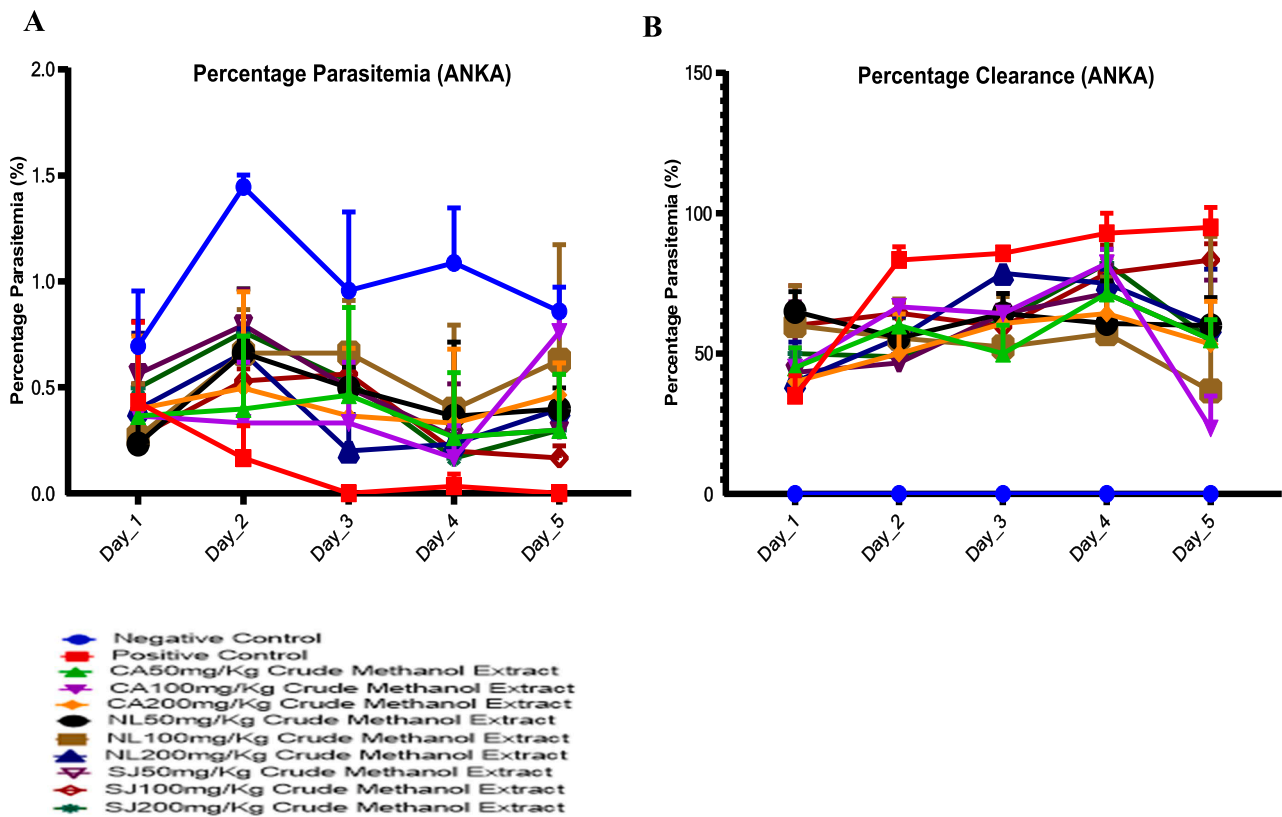

Fig. 2. Assessment of the percentage parasitemia (A) of resistant *P. berghei*-infected mice treated with methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* and the corresponding parasite clearance (B).
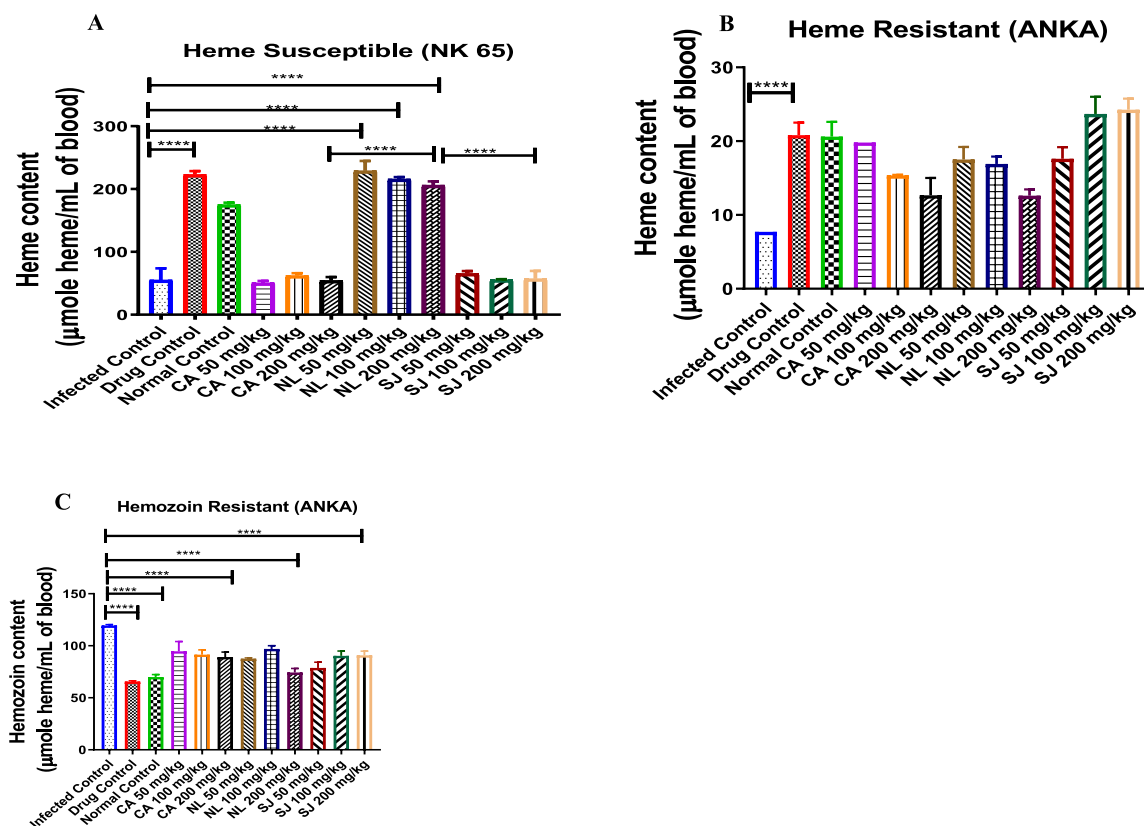
Fig. 3. The impact of *Plasmodium berghei* infection and treatment with methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* on bound heme in chloroquine-sensitive (A), chloroquine-resistant (B) as well as the hemozoin content. **** = $P < 0.001$.

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

The methanol extracts decreased the levels of IL-1β but 200 mg/kg of *S. jollyanum* had the highest inhibitory effects (Fig. 5a). In Fig. 5b, there was a significant ($P < 0.0001$) increase in IL-6 in infected mice. Similarly, *C. afer* and *N. latifolia* at 200 mg/kg significantly ($P < 0.0001$) decreased serum level of IL-6 in infected mice. Although, the serum level of IL-6 remains significantly ($P < 0.0001$) high in mice treated with 200 mg/kg of *S. jollyanum, C. afer* decreased ($P < 0.0001$) the serum IL-6 level relative to *S. jollyanum*. Serum level of interleukin-10 (IL-10) is higher ($P < 0.0001$) in normal controlmice and similar effect was observed in infected mice treated with 200 mg/kg *S. jollyanum* (Fig. 5c). Treatment of *Plasmodium* infection with higher doses of the extracts decreased serum CRP level (Fig. 5d). Serum level of TNF-α was significantly ($P < 0.0001$) decreased by *N. latifolia* (200 mg/kg) relative to the infected control (Fig. 5e),

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 (Fig. 6) present in the methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* was achieved in both positive and negative ionization modes. The MS data features such as quasimolecular ion, chemical formula and 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 revealed 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*.

4. Compounds profile by NMR analysis

The ^{1}H NMR fingerprints of the three extracts (Fig. 2) revealed common chemical shifts in most regions of the spectra.

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,
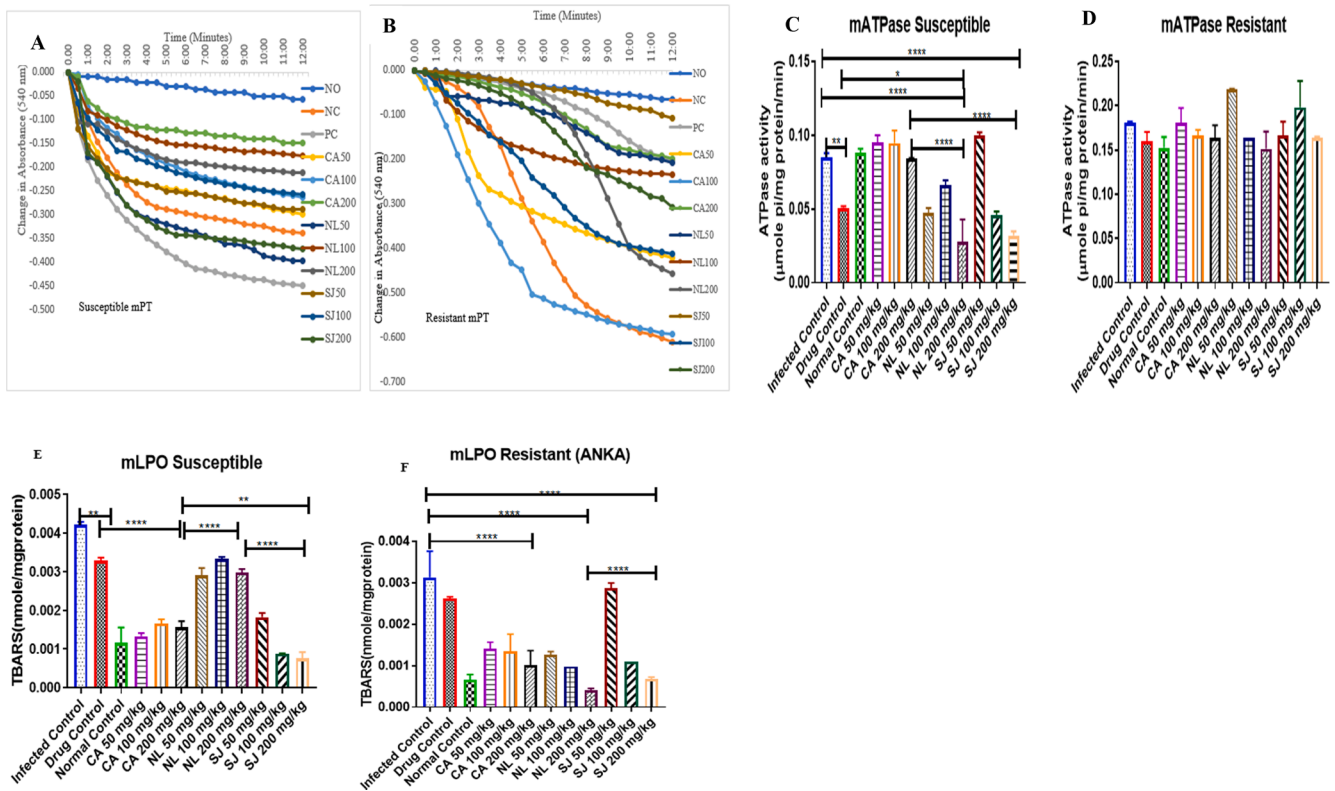
Fig. 4. Modulatory effects of *Plasmodium berghei* infection on some mitochondrial parameters: reversal effects of *C. afer, N. latifolia* and *S. jollyanum* extracts on mitochondrial permeabilisation in susceptible (A) and resistant (B) models. These extracts modulated ATPase activity in susceptible (C) and resistant (D) models. They also inhibited lipid peroxidation in susceptible (E) and and resistant (F) models. * = $P < 0.05$; ** = $P < 0.01$; **** = $P < 0.0001$.
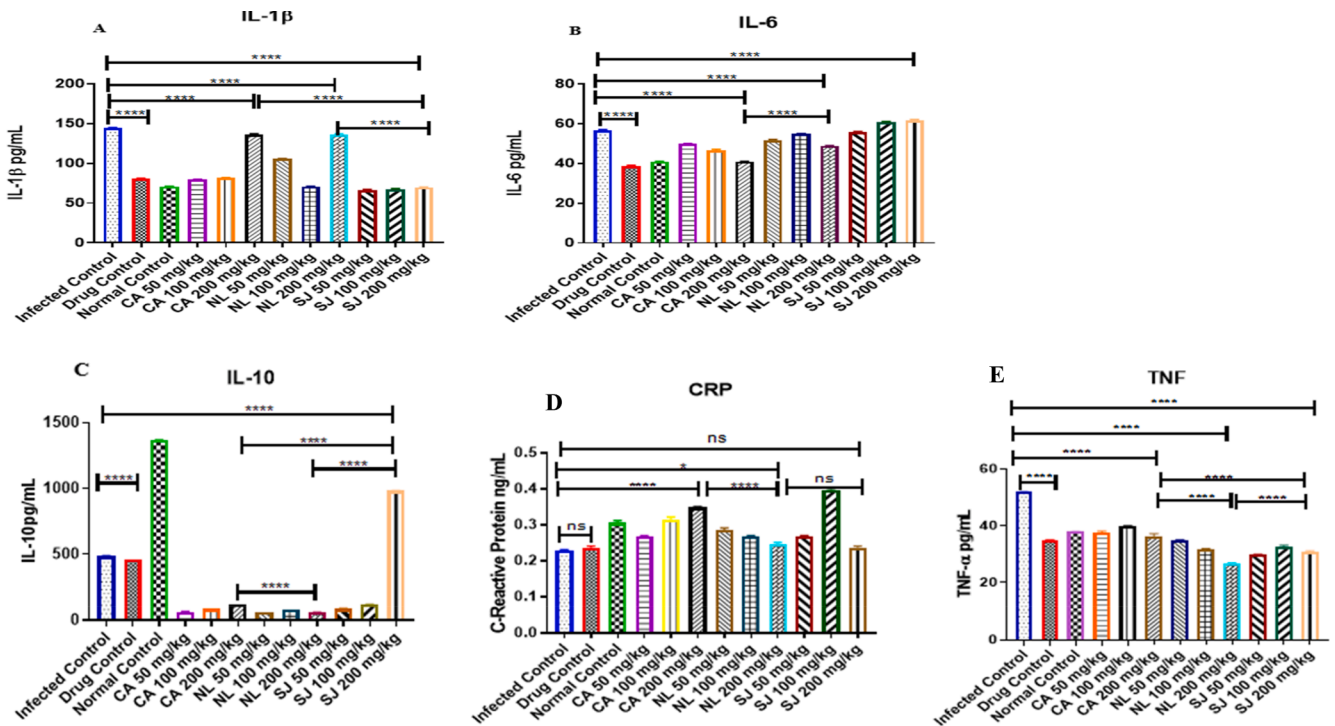

Fig. 5. Anti-inflammatory potentials of the methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* via the determination of serum interleukins 1β, (A), interleukin 6 (B) interleukin 10 (C), C-reactive protein (D) and Tumour Necrosis Factor alpha (E) in mice infected with resistant (ANKA strain) *Plasmodium berghei*. Ns = not significant, * = $P < 0.05$; **** = $P < 0.0001$.
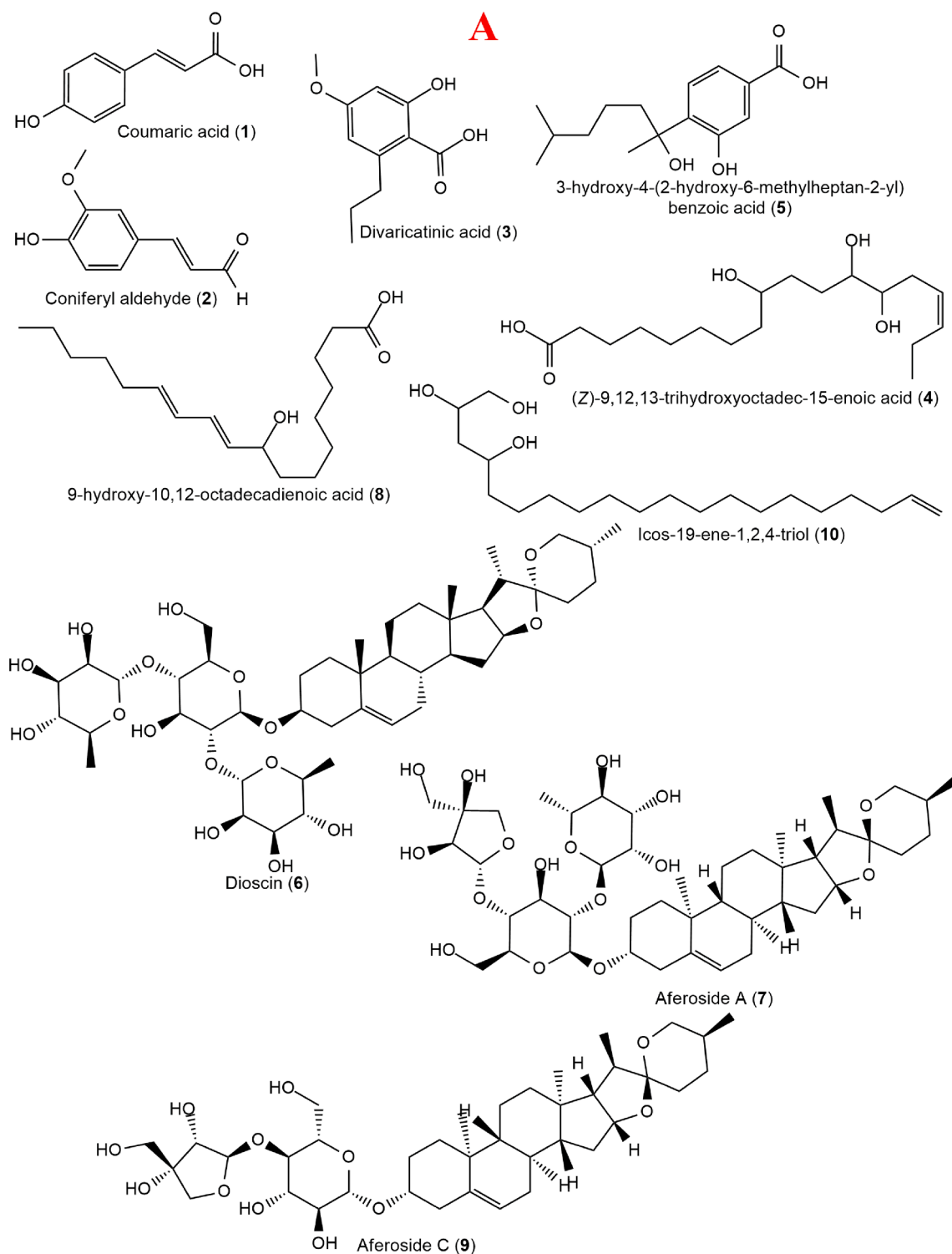
Fig. 6. Structures of compounds identified in *Costus afer* (A), *Nauclea latifolia* (B) and *Sphenocentrum jollyanum* (C) contd. Structures of compounds identified in *Costus afer* (A), *Nauclea latifolia* (B) and *Sphenocentrum jollyanum* (C).

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 (Table 1). 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

B

3-O-Caffeoylquinic acid (**11**)

Tetrahydropalmatine (**12**)

18,19-Dihydroangustine (**13**)

Jatrorrhizine (**14**)

Palmatine (**15**)

17-*epi*-Naucleidinal (**16**)

Strictosamide (**17**)

Quinovic acid 3-O-rhamnoside (**18**)

C

Quinic acid (**19**)   Protocatehuic acid (**20**)

3-O-Caffeoylquinic acid (**11**)

3,4-Dicaffeoylquinic acid (**21**)

Jatrorrhizine (**14**)

16-(2-Prop-1-en- yl)-2-O-*β*-D-glucopyranosyl cucurbitacin I (**23**)
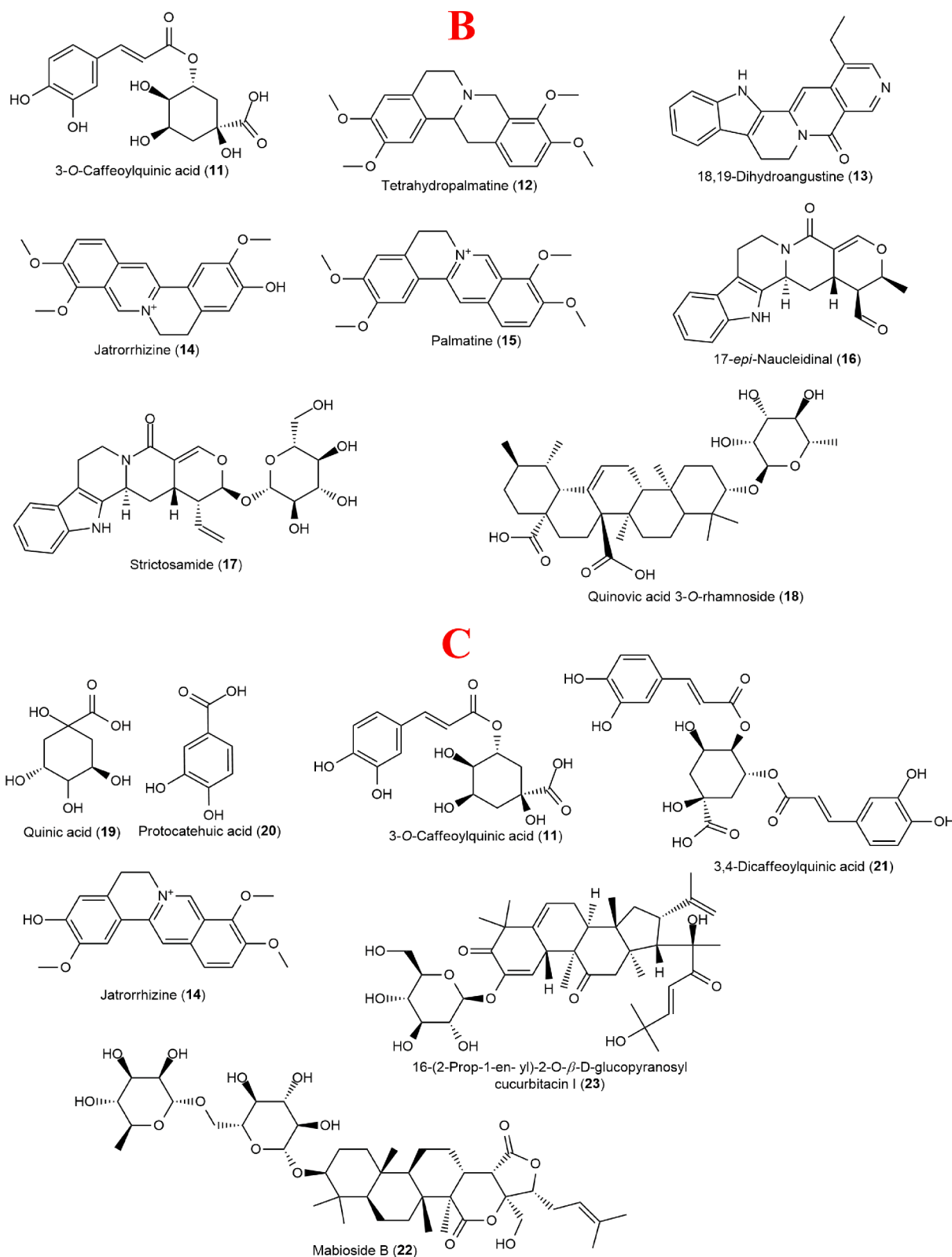
Mabioside B (**22**)

Fig. 6. (*continued*).

glycosylation when compared to *N. latifolia* (Fig. 7).

5. Discussion

This study investigated the potentials of *C. afer, N. latifolia* and *S. jollyanum* as sources of drug candidates for malarial chemotherapy.

The methanol extracts of these plants decreased parasite load and suppresses parasite growth, thus substantiating the indigenous clams (Iyamah and Idu, 2015). The three extracts supported increase in bound heme content of erythrocyte and prevented its degradation and polymerization to hemozoin in order to decrease parasite survival. Heme content decreased with a corresponding increase in hemozoin content in

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

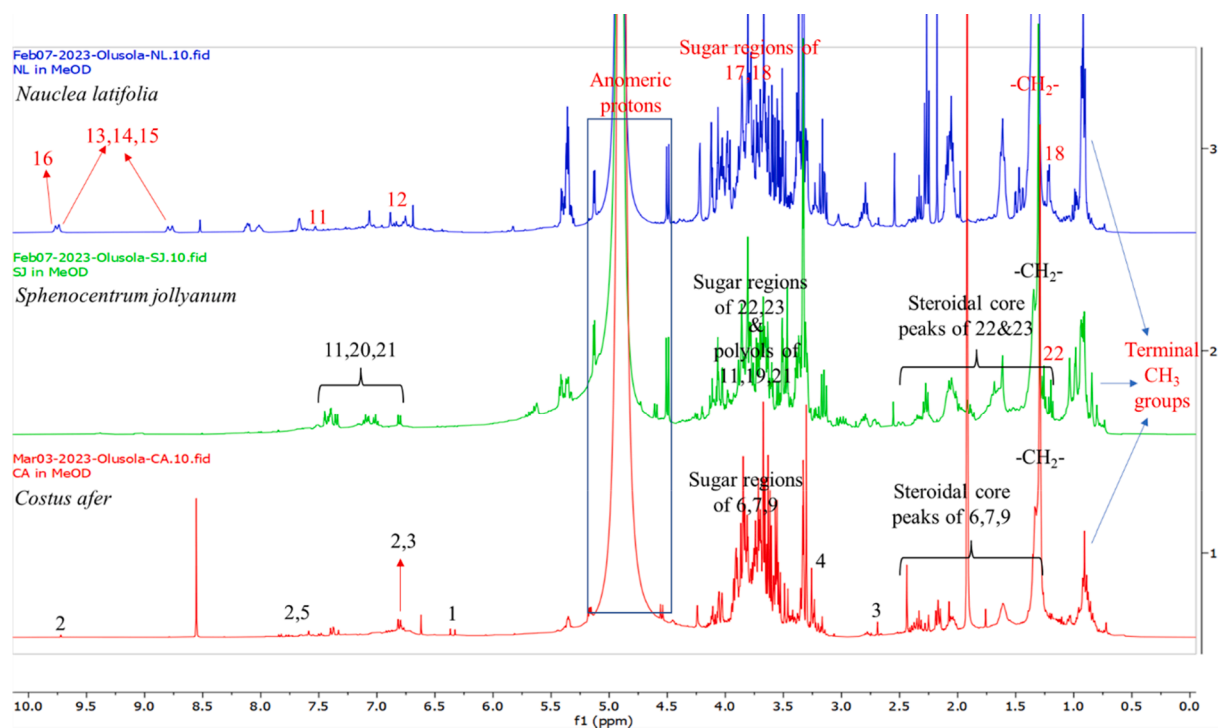**Fig. 7.** ^{1}H NMR spectra of the methanol extracts of *Costus afer* (red fingerprint), *Nauclea latifolia* (blue fingerprint) and *Sphenocentrum jollyanum* (green fingerprint).

infected control, thus acting as one of their mechanisms of action (Kotepui et al., 2015; White, 2018).

The reversal of parasite-induced mitochondrial permeabilisation by methanol extracts of *C. afer, N. latifolia* and *S. jollyanum* will increase ATP synthesis and prevents cell death. Our previous studies on *Diospyros mespiliformis* (David et al., 2021), *Mondia whitei* (Olanlokun et al., 2018, Olanlokun et al., 2019), *Phyllanthus amarus* (Olanlokun et al., 2020) and *Alstonia boonei* (Olanlokun et al., 2013) showed that the antimalarial potency of medicinal plants coupled with their mito-protective effects will improve host survival (Olanlokun et al., 2021). Stimulation of ATPase activity by the extracts showed that the effects of these medicinal plants may be bidirectional (Baker et al., 1986). Also, *C. afer* and *S. jollyanum* significantly decreased the total peroxidative products, thus preventing oxidative damage (Chicco and Sporagna, 2007; Vähäheikkilä et al., 2018).

Increased IL-1β level in *Plasmodium*-infected patients is 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 responses noticed in the infected individuals shortly before chemotherapy (Farrington et al., 2017). Pleiotropic IL-6 can perform defensive role in the host, showing that the increase in the levels of IL-6 in infected mice treated with *N. latifolia* and *S. jollyanum* may be because of the healing processes (Simpson et al., 1997). 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). The C reactive protein performs pathological role in malaria disease. It binds to infected erythrocytes and assists in the clearance of same, indicating parasite burden. Increased serum IL-10 observed in *S. jollyanum*-treated mice is profitable because it prevents damage to the host cell and normal tissue homeostasis (Iyer and Cheng, 2012).

We identified diocin and divaricatinic acid in *C. afer* for the first time in addition to other phytochemicals earlier profiled. Previous study has shown that this compound has antiproliferative and antimalarial property (Lomchid et al., 2016). We also identified tetrahydropalmatine and jatrorrhizine in *N. latifolia* for the first time. Pharmacological usefulness of tetrahydropalmatine includes analgesic, neuro-protection and anti-inflammatory effects (Du et al., 2022). Mabioside B and a sugar derivative of cucurbitacin, 16-(2-Prop-1-en- yl)-2-O-β-D-glucopyranosyl cucurbitacin, were identified for the first time in *S. jollyanum*. Mabioside B has anti-inflammatory and immunomodulatory activity (Ruan et al., 2016).

In summary, different phytochemicals with varying pharmacological potentials are present in the medicinal plants 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 and composite preparations. The purification and formulation of relevant phytochemicals from this plant for the treatment of diseases will have beneficial purposes for the treatment of malaria.

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/073-0122/03 was assigned to this study.

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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.

Declaration of Competing Interest

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.103065.

References

Adam-Vizi, V., Seregi, M., 1982. Receptor Dependent Stimulatory Effect of Noradrenaline on Na+/K+ ATPase in Rat Brain Homogenate. Role of Lipid Peroxidation. Biochem. Pharmacol. 31, 2231–2236.

Asakura, T., Minakata, K., Adachi, K., Russell, M.O., Schwartz, E., et al., 1977. Denatured hemoglobin in sickle erythrocytes. J. Clin. Investig. 59, 633–640.

Aweke *Costus afer* Ker Gawl, G. 2007. In: Schmelzer GH, Gurib-Fakim, A. Prota. medicinal plants/plantes médicinales. Wageningen, Netherlands: PROTA.

Baker, J.K., Hullihen, J.M., Pedersen, P.L., et al., 1986. Selective toxicity of the antimalarial primaquine-evidence for both uncoupling and inhibitory effects of a metabolite on the energetics of mitochondria and its ATP synthase complex. Pharm. Res. 3, 290–293.

Chicco, A.J., Sporagna, G.C., 2007. Role of cardiolipin alterations in mitochondrial dysfunction and disease. Am. J. Physiol-Cell. Physiology 292, C33–C44.

David, O.M., Olanlokun, J.O., Owoniyi, B.E., Ayeni, M., Ebenezer, O., Koorbanally, N., et al., 2021. Studies on the mitochondrial, immunological and inflammatory effects of solvent fractions of *Diospyros mespiliformis* Hochst in *Plasmodium berghei* infected mice. Sci. Reports 11, 6941.

Du, Q., Meng, X., Wang, S., et al., 2022. A Comprehensive review on the chemical properties, plant sources, pharmacological activities, pharmacokinetic and toxicological characteristics of tetrahydropalmatine. Front. Pharmacol. 13, 890078.

Elujoba, A.A.A., 1995. Elujoba Female Infertility in the Hands of Traditional Birth Attendants in South-West Nigeria. Fitoterapia 66 (1995), 239–248.

Farrington, L., Vance, H., Rek, J., Prahl, M., Jagannathan, P., Katureebe, A., Arinaitwe, E., Kamya, M.R., Dorsey, G., Feeney, M.E., et al., 2017. Both inflammatory and regulatory cytokine responses to malaria are blunted with increasing age in highly exposed children. Malar. J. 16, 499.

Iver, S.S., Cheng, G., 2012. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit. Rev. Immunol. 32, 23–63.

Iyamah, P.C., Idu, M., 2015. Ethnomedicinal survey of plants used in the treatment of malaria in Southern Nigeria. J. Ethnopharmacol. 173, 287–302.

Johnson, D, Lardy, H, 1967. Isolation of liver or kidney mitochondria. Methods Enzymol 10, 94–96.

Kent, A.C., El Baradie, K.B.Y., Hamrick, M.W., et al., 2021. Targeting the mitochondrial permeability transition pore to prevent age-associated cell damage and neurodegeneration. Oxid. Medi. Cellular. Long. 2021.

Lapidus, R.G., Sokolove, P.M., 1993. Spermine inhibition of the permeability transition of isolated rat liver mitochondria: an investigation of mechanism. Ach. Biochem. Biophy. 306, 246–253.

Lardy, H.A., Wellman, H., 1953. The catalytic effect of 2,4-dinitrophenol on adenosine triphosphate hydrolysis by cell particles and soluble enzymes. J. Biol. Chem. 201, 357–370.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., et al., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 262–275.

Menan, H., Banzouzi, J.T., Hocquette, A., Pelissier, Y., Blache, Y., Kone, M., Mallie, M., Assi, L.A., Valentin, A., et al., 2006. Antiplasmodial activity and cytotoxicity of plants used in West African traditional medicine for the treatment of malaria. J. Ethnopharmacol. 106, 131–136.

Olanlokun, J.O., Bakare, I., Ofoegbu, B., Uleh, P., Olorunsogo, O.O., et al., 2018. *Mondia whitei*, an African spice inhibits mitochondrial permeability transition in rat liver. Prev. Nutri. Food. Sci 23, 206–213.

Olanlokun, J.O., Adetutu, A., Olorunsogo, O.O., et al., 2019. *In vivo* antiplasmodial effects of *Diospyros mespiliformis* and *Mondia whitei* methanol extracts on *Plasmodium berghei*-induced malaria in mice. Interv. Medi. Applied. Sci 11, 197–206.

Olanlokun, J.O., Babarinde, C.O., Olorunsogo, O.O., et al., 2020. Antimalarial properties and preventive effects on mitochondrial dysfunction by extract and fractions of Phyllanthus amarus (Schum. And Thonn) in Plasmodium berghei-infected mice. J. Basic. Clin. Physiol. Pharmacol 32, 255–266.

Olanlokun, J.O., Bodede, O., Prinsloo, G., Olorunsogo, O.O., 2021. Comparative antimalarial, toxicity and mito-protective effects of *Diospyros mespiliformis* Hochst. ex A. DC. and *Mondia whitei* (Hook.f.) Skeels on *Plasmodium berghei* infection in mice. J. Ethnopharmacol. 268, 113585.

Olorunnisola, O.S., Afolayan, A.J., 2011. In Vivo antimalaria of methanol leaf and root extracts Sphenocentrum jollyanum. Afr. J. Pharm. Pharmacol. 5, 1669–1673.

Orjih, A.U., Fitch, C.D., 1993. Hemozoin production by Plasmodium falciparum: variation with strain and exposure to chloroquine. Biochim. Biophys. Acta. 1157, 270–274.

Ruan, J., Zheng, C., Qu, L., Liu, Y., Han, L., Yu, H., Zhang, Y., Wang, T., et al., 2016. Plant Resources, 13C-NMR Spectral Characteristic and Pharmacological Activities of Dammarane-Type Triterpenoids. Molecules 21, 1047.

Ryle, J.F., Peters, W., 1970. The antimalarial activity of some quinolone esters. Am. Trop. Med. Parasitol. 84, 209–222.

Shaffer, N., Grau, G.E., Hedberg, K., Davachi, F., Lyamba, B., Hightower, A.W., Breman, J.G., Phuc, N.D., 1991. Tumor necrosis factor and severe malaria. J. Infect. Dis. 163, 96–101.

Simpson, R.J., Hammacher, A., Smith, D.K., Matthews, J.M., Ward, L.D., et al., 1997. Interleukin-6: structure-function relationships. Protein. Sci. 6, 929–955.

Vähäheikkilä, M., Peltomaa, T., Róg, T., Vazdar, M., Pöyry, S., Vattulainen, I., et al., 2018. How cardiolipin peroxidation alters the properties of the inner mitochondrial membrane? Chem. Phy. Lipids 214, 15–23.

Varsey, R., Kale, R.K., 1990. Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. Int. J. Radiat. Biol. 58, 733–743.

White, N.J., 2018. Anaemia and malaria. Malar. J. 17, 371.