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Research Article

Antimalarial and Antioxidant Activities of Sub-fractions of *Capsicum frutescens* L. in *Plasmodium berghei*-Infected Mice

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Abstract

Oxidative stress induced by malaria parasites significantly impacts the host microenvironment, exacerbated by resistance to existing antimalarial agents. This has prompted malaria-endemic settlements—especially those in sub-Saharan settlements—to adopt medicinal plants for the treatment of fever and malaria since they are readily available and affordable. This research evaluated the antimalarial and antioxidant activities of sub-fractions of *Capsicum frutescens* L. fruit (CFL) in *Plasmodium berghei* (NK65)-parasitized mice as potential leads for antimalarial drug development. Maintaining the same controls for the sub-fractions, combined sexes of 85 mice weighing 23.7±3.5 g were randomly grouped into seven (n=5) and inoculated intraperitoneally by 0.2 mL parasitized erythrocytes. Group A received 10 mL/kg normal saline (control), Group B received 10 mg/kg chloroquine, while Groups C to G received 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg body weight per sub-fraction, respectively. On 6 and 10 days post-inoculation (dpi), mice per group were euthanized. The *in vivo* test, selenium, vitamins C and E levels, 2,2-diphenyl-1-picrylhydrazyl (DPPH), superoxide ion, and peroxide ion-scavenging concentrations, as well as ferric-reducing antioxidant power (FRAP), were done using standard procedures. Treatment with CLF sub-fractions revealed low parasite clearance on 6 dpi but significantly suppressed parasites ($p<0.05$) on 10 dpi for the three sub-fractions and increased survival in mice. This was more pronounced in sub-fraction B (SFB). Out of the three sub-fractions, SFB compared favourably with butylated hydroxytoluene (BHT) for the scavenging activities of DPPH, peroxide ion, and superoxide ion, as well as the FRAP. This efficacy of SFB was also evident in the low EC₅₀ obtained. Therefore, CFL sub-fractions—SFB in particular—possess antiplasmodial and antioxidant activities against fever and malaria, suggesting its potential as a lead fraction for developing novel plant-derived antimalarial therapies.

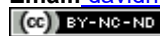
Keywords: Antioxidants, *Capsicum*, Erythrocytes, Medicinal Plants, *Plasmodium*.

Introduction

In spite of the advances made to control malaria, the disease still exist as a worldwide public health defier—particularly in sub-Saharan settlements—where millions of deaths are documented yearly [1]. With the inclusion of Nigeria, about 70% of the worldwide burden of malaria is concentrated in 11 African nations [2]. Despite efforts made by the Nigerian government and international

collaborators to target malaria control and reduce the disease in the region, it still weighs on orthodox healthcare services. Various strategies were deployed to promote the use of orthodox antimalarial remedies and, subsequently, the use of vaccines. However, the unaffordability of these products, compounded by economic challenges, coupled with resistance to the therapies, has caused a major setback. This setback, coupled with other factors like diverse cultural beliefs, perceived drug ineffectiveness, dissatisfaction, and adverse effects, has encouraged the adoption of traditional medicine (TM) for the management of several health challenges, including fever and

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malaria [3-6]. This is because TMs are often easily accessible, dependable, and affordable. Infection with malaria parasites usually stimulates oxidative stress by triggering reactive oxygen species (ROS) production during schizont eruptions, extending the length of damage in the host's microenvironment. The increasing production of the ROS with a concomitant reduction in antioxidants yields oxidative stress, thereby overloading the host's cells and complicating malaria infection [7]. Free radicals have been reported in biological systems to wield certain physiological actions like detoxification, biosynthesis, and clearance of microbes. Thus, the biological antioxidant defense system maintains equilibrium between the free radical generation and its removal [8]. This defense system comprises unique intricate antioxidant system, like vitamins C and E, which are known to influence the host's cellular and immunological actions. Natural antioxidants present in spices and medicinal plants have been confirmed to possess the capacity to combat the deleterious effects of oxidative stress [8]. However, resistance to conventional antimalarial drugs has driven several people in rural settlements to adopt medicinal plants such as *Capsicum* for their basic health needs. Bioactive agents derived from these plants have been in use for ages as a result of their abundant phytochemicals. As such, malaria-endemic settlements—like Nigeria—have relied on plants for ages for their medicinal value, either as herbal mixtures, concoctions, condiments, or as culinary ingredients. Although searches for more antimalarial remedies from plants are still ongoing, more than 160 families from over 1,200 plants have been documented from folkloric reports [9]. Out of this, the *Solanaceae* family consists of 27 *Capsicum* species, among which *Capsicum annuum* L., *Capsicum baccatum* L., *Capsicum chinense* J., *Capsicum frutescens* L. (CFL), and *Capsicum pubescens* R. are common. Pepper or *Capsicum* is a popular condiment commonly used in our diets, often added fresh, dried, powdered, or even refined. CFL, specifically known as chili or cayenne pepper, is the most pungent of all the five highlighted species, having its *in vivo* antimalarial properties confirmed in the crude extracts and solvent-partitioned fractions [9]. In Nigerian dialects, CFL is respectively called “ose-mkpe” and “ata-ijosi” in Igbo and Yoruba languages, where it is often used whenever body temperature rises. They also prepare a special diet with it—called “pepper soup”—for females after labour to aid in womb and body warmth, as stated in most rural communities visited. In other places, it is chewed raw with cassava flakes—called “garri”—for its pungency. The aqueous

extraction is the best possible mode of extraction, and it has been reported to possess antioxidants, minerals, and capsaicinoids, responsible for its nutritional and pharmacological efficacies [9, 10]. However, there is a dearth of information on the antimalarial potency of the sub-fraction.

Therefore, our study aimed to assess the *in vivo* antiplasmodial and *in vitro* antioxidant efficacy of CFL in *Plasmodium berghei*-infected mice, harnessing its potentials to combat malaria-related biochemical conditions and the resulting oxidative stress triggered by the disease with the target of getting a lead fraction for developing novel plant-derived antimalarial therapies.

Material and methods

Capsicum collection with extraction

Dried CFL fruits were bought at “Oja-Oba” market in Ilorin metropolis (8°29'46"N 4°32'46"E). It was authenticated by a taxonomist in Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where the voucher (UILH/004/2019/751) was deposited. The method of Nwikwe *et al.* [9] was adopted for the extraction of the solvent-partitioned extract. 364.10 g from the residual fraction of the aqueous extract was subjected to column chromatography and then thin-layer chromatography (TLC). Thereafter the prepared plate was developed inside a TLC chamber comprising methanol (partial polar), ethyl acetate (non-polar), and distilled water (polar) (50:30:20) mixed solvent system and viewed under ultraviolet light in an iodine vapor chamber at 254 nm. Fractions with close retention factor (R_f) values were combined to yield three different sub-fractions; sub-fraction A (SFA), sub-fraction B (SFB), and sub-fraction C (SFC) (data available on Table A in Appendix).

Experimental animals, handling and ethical clearance

Eighty-five adult albino mice (both sexes) weighing 23.7±3.5 g were collected from University of Ilorin Animal House, but were acclimatized at Institute of Advanced Medical Research and Training (IAMRAT), University of Ibadan, Ibadan, Nigeria (7°21'26"N 3°52'27"E/7.357133°N 3.874262°E). At IAMRAT, each mouse was weighed and placed in standard cages in conducive atmosphere with contact to clean water *ad libitum* and feed (Top Feeds Nigeria) for 2 weeks before commencing the experiment. Animal handling was carefully performed following the Guide [11] procedure. Ethical approval (UERC/ASN/2020/1996) was granted by the Unilorin Ethical Committee.

Parasite inoculation

Plasmodium berghei (NK65)-inoculated mice sensitive to chloroquine strains were acquired

from IAMRAT. These parasites were preserved using weekly blood serialized passages transferred to the uninfected mice through the infected ones, achieving $\geq 30\%$ parasitaemia, which was adopted as the donor mice model. The donors were euthanized via cardiac puncture, after which blood was obtained into heparinized tubes. The blood was thereafter diluted to an inoculum size (1×10^7) parasitized blood cells in 0.2 mL using citrate-glucose solution (0.5% glucose and 3.8% citrate). Finally, 0.2 mL of the diluted blood was injected intraperitoneally into each mouse.

Animal groupings for curative tests and parasite counts

The curative test for the three sub-fractions against chloroquine sensitive *P. berghei* (NK65) infection was done using 85 mice (75 for extracts, 5 for CQ, and 5 for the control), that is, the same control and CQ groups were maintained throughout. Per cage had 5 mice ($n=5$), using 0.9% normal saline as the vehicle. Each Mouse was inoculated with inoculum intraperitoneally. Group A = control (10 mL/kg vehicle), Group B received 10 mg/kg body weight CQ, while mice in Groups C to G received 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg body weight per sub-fraction, respectively. The choice of doses was from the previous study [9], while CQ was used as the standard drug due to its ability to concentrate in parasites-infected red blood cells [9].

$$\% \text{ Parasitaemia} = \frac{\text{Number of parasitized cells} - \text{erythrocytes}}{\text{Total number of erythrocytes}} \times 100\%$$

$$\% \text{ Suppression} = \frac{\% \text{ Parasitaemia for control} - \% \text{ Parasitaemia for treated}}{\% \text{ Parasitaemia for control}} \times 100\%$$

$$\text{Mean survival time (MST in days)} = \frac{\text{Total survival days of mice in each group (in days)}}{\text{Overall number of mice in a group}}$$

Determination of in vitro antioxidant assays

In the sub-fractions, the methods by Sutharsingh *et al.* [13] for 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity, Benzie and Strain [14] for ferric ion-reducing antioxidant power (FRAP), Robak and Gryglewski [15] for superoxide ion-scavenging activity, and Ruch *et al.* [16] for hydrogen peroxide-radical scavenging activity were used to determine their levels in triplicates.

Determination of selenium, vitamins C and E concentrations

The methods described by Tee *et al.* [17] for vitamin C, Contreras-Guzma and Strong [18] for vitamin E, and Ishizaki [19] were used to determine their concentrations in triplicates in the sub-fractions.

Analysis of data

All data are presented as mean \pm SEM ($n=5$). Statistical comparisons were made using one-way

The method developed by Ryley and Peters [12] was used against the parasite-infected mice in each sub-fraction. Each mouse per cage was spotted using picric acid and inoculated with the inoculum on Day 0 (D_0). Treatment began 72 h afterwards and continued for 4 successive days (D_3 to D_6) per 24 h interval. On daily basis, oral administration was done once, however, two smears—thin and thick—were prepared from blood obtained from the tail of each subject on clean and greaseless slides on the 6th day (D_6). On Day 10 (D_{10}) post-inoculation (dpi), more smears were prepared. The resulting films were thereafter fixed in methanol and stained in 10% Giemsa stain for 15 min. The stains on each slide was cleansed in running water, dried in air, and visualized under microscope ($\times 1000$ magnification). After random fields were used for parasite count, parasitaemia suppression in percentage was determined for each group by comparing the control to the parasitaemia status in the treated. The slides per mouse per sub-fraction were visualized; three fields with 200 to 500 cells were noted per slide. Sub-fraction producing parasitaemia that is $< 30\%$ was taken as inactive, while those that caused suppression of 30 to 40% were taken as partially active, and those $> 40\%$ were taken as active [9]. Likewise, the mice were observed for 28 days to obtain the mean survival period in days.

ANOVA followed by Tukey's post-hoc test ($p < 0.05$ considered significant).

Results and discussion

Curative test for sub-fractions of C. frutescens L. fruit

The curative tests on Table 1 showed that aqueous extract of CFL displayed partial activity at 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg on 6 dpi for SFA. This activity improved on 10 dpi with active parasite suppression across all doses administered. On 6 dpi for the SFB, partial parasite suppression was observed at all doses; however, on 10 dpi, they were actively suppressed at all doses. In SFC likewise, the parasites were partially suppressed at all doses on 6 dpi, but on 10 dpi, they were actively suppressed at all doses. Conversely, the CQ-administered group suppressed the parasites

actively above the extract-treated mice on 6 and 10 dpi. Nevertheless, after observing for 28 days at 0.5, 1.0, 2.0, 4.0, and 8.0 mg/kg, the group that received SFA recorded survival rate of 13, 14, 14, 15, and 15 days; the SFB-treated group recorded 17, 17, 18, 19, and 20 days survival rate; while the SFC-treated group recorded 16, 16, 17, 18, and 18 days survival, respectively. Equally, the CQ-treated group recorded 28 days survival, while the control mice survived for 5 days (Figure 1). Unlike the *in vivo* suppressive test in mice, which evaluates the ability of an extract to prevent parasite infection from developing, *in vivo* curative screening provides viable information on the ability of the extract to reduce or eliminate the parasites after establishing the parasites. Curative screening of the sub-fractions of CFL was experimented on to ascertain the ability of CFL to reduce or eliminate malaria parasites in mice models. The crude extracts have been reported to reduce parasite infection *in vivo* and prolong the survival of the subjects [9]. This plant is used indigenously for its medicinal value in managing fever and malaria since it is readily available. However, there is a dearth of information on the bio-constituents responsible for the antioxidant and antiplasmodial potentials in late establishment of malaria parasites in *Plasmodium berghei* (NK65)-infected mice.

From this study, SFA, SFB, and SFC resulting from the pooled residual fraction of the aqueous

extract revealed that they eradicated the parasites on 10 dpi. SFB showed the greatest parasite clearance across all doses, indicating that it produced more significant or desirable effects (Table 1). It may be attributed to the fact that SFB is more effective at eliminating the parasite load in the infected mice due to its improved bioavailability in the cells. It may also be caused by stronger direct killing of the parasites, faster recovery (improved health with less weight loss), longer half-life (that is, the sample remains active in the bloodstream for a long time), better schizonticidal activity (that is, enhanced killing of blood-stage parasites), etc. The superior suppression of SFB (especially at 8.0 mg/kg) may also be due to the abundant antioxidant concentrations present in the phytoconstituents, which prevent the parasites from depleting and exploiting the host's antioxidant microenvironment for their survival. The clearance of parasites in the sub-fractions was observed to be slow on the first day of administration, but subsequent oral administration produced a better response in a dose-dependent pattern. The delay in effective response could be as a result of the slow action of the extracts in parasite clearance. It validates the findings of Ofeniforo *et al.* [20] that SFC suppressed *Plasmodium* parasites more than SFA and SFB in *Spilanthes filicaulis*.

Table 1. Curative test for sub-fractions of aqueous extract of *Capsicum frutescens* L. fruit on *Plasmodium berghei*-infected mice against control

Treatment	SFA 6 dpi	SFA 10 dpi	SFB 6 dpi	SFB 10 dpi	SFC 6 dpi	SFC 10 dpi
	Percentage Parasitaemia (Percentage Suppression)					
Control	2.82 ± 0.12 (0)	6.77 ± 0.15 (0)	2.82 ± 0.12 (0)	6.77 ± 0.15 (0)	2.82 ± 0.12 (0)	6.77 ± 0.15 (0)
10 mg/kg body weight CQ	0.28 ± 0.32 (90.07)	0.20 ± 0.21 (97.05)	0.28 ± 0.32 (90.07)	0.20 ± 0.21 (97.05)	0.28 ± 0.32 (90.07)	0.20 ± 0.21 (97.05)
0.5 mg/kg body weight extract	1.92 ± 0.19 (31.91)	4.00 ± 0.28 (40.92)	1.79 ± 0.21 (36.52)	3.08 ± 0.11 (54.50)	1.91 ± 0.23 (32.27)	3.55 ± 0.35 (47.57)
1.0 mg/kg body weight extract	1.91 ± 0.14 (32.27)	3.88 ± 0.11 (42.69)	1.75 ± 0.12 (37.94)	3.07 ± 0.28 (54.67)	1.85 ± 0.32 (34.40)	3.52 ± 0.12 (48.01)
2.0 mg/kg body weight extract	1.79 ± 0.22 (36.52)	3.74 ± 0.41 (44.76)	1.70 ± 0.10 (39.72)	2.62 ± 0.16 (61.30)	1.80 ± 0.13 (36.17)	3.40 ± 0.52 (49.78)
4.0 mg/kg body weight extract	1.72 ± 0.15 (39.01)	3.70 ± 0.21 (45.35)	1.70 ± 0.14 (39.72)	2.34 ± 0.25 (65.44)	1.78 ± 0.11 (36.88)	3.29 ± 0.17 (51.40)
8.0 mg/kg body weight extract	1.70 ± 0.22 (39.72)	3.40 ± 0.13 (49.79)	1.72 ± 0.11 (39.01)	2.22 ± 0.17 (67.21)	1.70 ± 0.16 (39.72)	3.08 ± 0.21 (54.51)

dpi-day post-inoculation. Values are shown as means ± S.E.M (n=5)

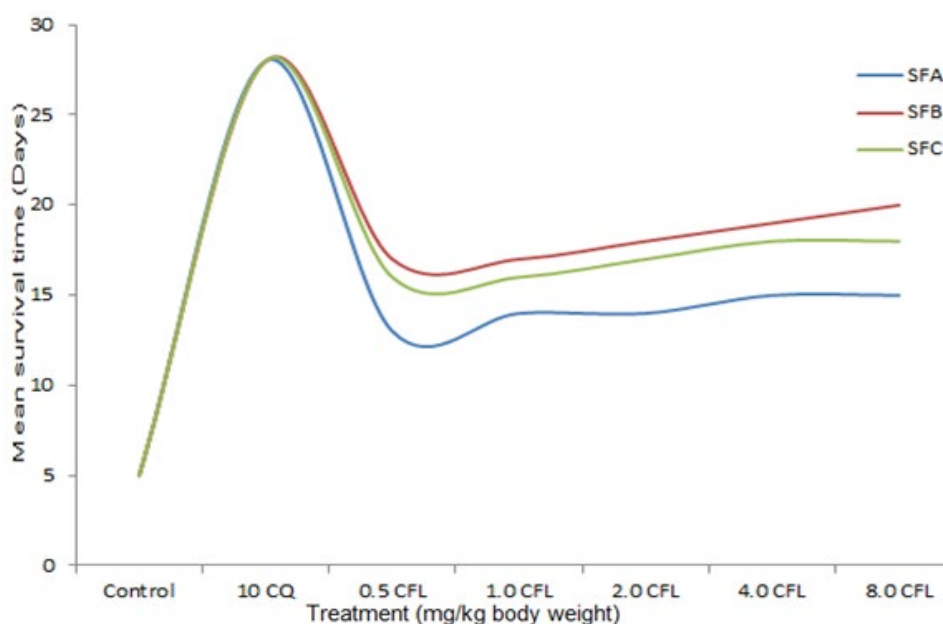


Figure 1. Mean survival time (days) from curative test for sub-fractions of aqueous extract of *Capsicum frutescens* L. fruit on *P. berghei*-infected mice against control

The mean survival time (MST), which is a measure of the mean period that the mice survived during the 28 days of monitoring, revealed that SFB survived longer than SFA and SFC. This further lends credence to the improved parasitaemia reduction compared to SFA and SFC (Figure 1). The antimalarial effects of CFL have been attributed to some of their active bioactive agents [9]; therefore, the low curative effects in SFA and SFC may be ascribed to differential distributions of the bioactive agents in these sub-fractions that act singly or combined [21, 22].

***In vitro* antioxidant studies**

Table 2 revealed that SFA had the highest concentrations of vitamin C (103.38 mg/100 g), but less in SFC (79.27 mg/100 g) and the least in SFB (43.22 mg/100 g). Vitamin E concentrations were also higher in SFA (87.67 µg/100 g), but lesser in SFC (44.52 µg/100 g) and least in SFB (11.37 µg/100 g). Similarly, selenium concentration was highest in SFB (0.12 µg/kg), lesser in SFC (0.09 µg/kg), and least in SFA (0.05 µg/kg).

Figure 2 revealed that the sub-fractions at 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 1.2, 1.4, 1.8, and 2.0 mg/mL, respectively, had DPPH radical-scavenging activities of 0, 4, 45, 55, 60, 68, 74, 77, 77, 77, and 75% for SFA; 0, 9, 57, 67, 72, 76, 82, 86, 86, 86.5, and 85% for SFB; and 0, 7, 50, 55, 60, 68, 74, 80, 80, 80, and 79% for SFC against the synthetic antioxidant (BHT) of 0, 9, 60, 70, 75, 80, 85, 88, 89, 89, and 88%. Thus, the DPPH radical-scavenging activity of SFB at all concentrations compared favourably to BHT than to SFA and SFC, which had lower activities. At the same doses, the sub-fractions respectively had hydrogen peroxide-scavenging activities of 0, 3, 6.8, 30, 46.75, 60.45, 64.08, 67.38, 67.25, 65.95, and 62.92% for SFA; 0, 5.5, 19.27, 42.5, 60.05, 70.36, 73.46, 74.05, 75.84, 72.28, and 70.57% for SFB; and 0, 4, 11.8, 35.5, 53.85, 65.27, 67.88, 70.35, 67.25, 67.28, and 65.17% for SFC against BHT of 0, 10, 30.64, 65, 72.68, 77.54, 78.92, 78.98, 83.74, 81.51, and 80.38%. Thus, the peroxide-scavenging activity of SFB at all concentrations compared favourably to BHT than SFC and SFA, which had lower activities (Figure 3).

Table 2. Effects of sub-fractions of aqueous extract of *Capsicum frutescens* L. fruit on vitamins C and E and selenium concentrations

Sub-fraction	Vitamin E (µg/100 g)	Vitamin C (mg/100 g)	Selenium (µg/kg)
A	87.67 ± 0.30	103.38 ± 0.24	0.05 ± 0.02
B	11.37 ± 0.25	43.22 ± 0.20	0.12 ± 0.04
C	44.52 ± 0.17	79.27 ± 0.18	0.09 ± 0.02

Values represent means of triplicates determinations

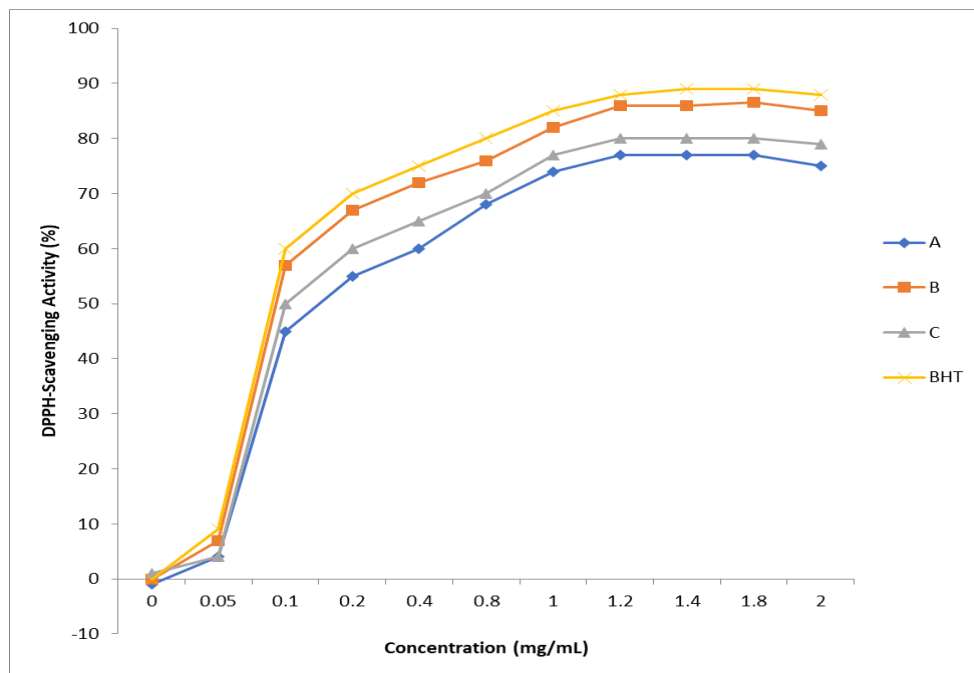


Figure 2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities of sub-fractions of CFL. *BHT*-butylated hydroxytoluene. Values represent means of triplicates determinations.

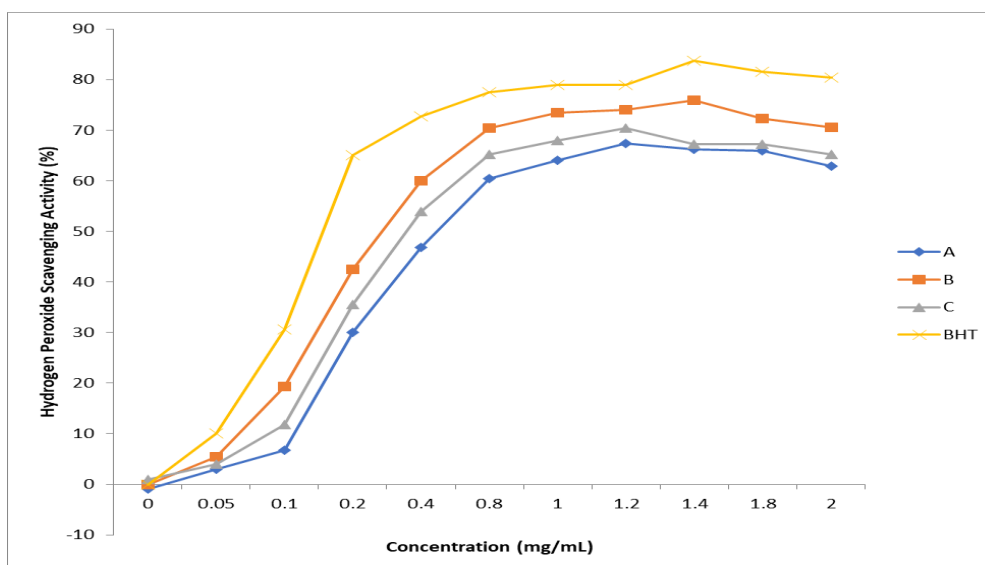


Figure 3. Hydrogen peroxide-scavenging activities of sub-fractions of CFL. *BHT*-butylated hydroxytoluene. Values represent means of triplicates determinations.

In similar trends, Figure 4 revealed that the sub-fractions at 0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 1.2, 1.4, 1.8, and 2.0 mg/mL, respectively, had superoxide radical-scavenging activities of 0, 2.85, 8.58, 20.5, 26.15, 37.47, 44.82, 53.38, 56.28, 56.45, and 55.42% for SFA; 0, 8.24, 19.27, 40.5, 50.01, 55.36, 59.68, 62.75, 62.94, 65.1, and 63.61% for SFB; and 0, 5.07, 15.37, 33.5, 43.85, 52.7, 55.68, 58.82, 60.39, 60.81, and 61.25% for SFC against

0, 9.68, 26.68, 49.25, 58.68, 65.54, 69.22, 70.58, 71.7, 72.88, and 73.46% for BHT. Thus, the superoxide radical-scavenging activity of SFB at all concentrations compared favourably to BHT than SFA and SFC, which had low activities. At the same doses, the sub-fractions respectively had a ferric-reducing effect of 0, 0.15, 0.21, 0.22, 0.23, 0.26, 0.24, 0.25, 0.27, 0.28, and 0.32% for SFA; 0, 0.15, 0.38, 0.78, 0.92, 1.12, 1.4, 1.58,

1.83, 2.06, and 2.01% for SFB; and 0, 0.11, 0.21, 0.34, 0.65, 0.69, 0.67, 0.68, 0.8, 0.84, and 0.79% for SFC against BHT of 0, 0.15, 0.38, 0.78, 0.94, 1.25, 1.5, 1.68, 1.92, 2.08, and 2.05%. Thus, the

ferric-reducing effect of SFB at all levels compared favourably to BHT than to SFA and SFC, which had lower activities (Figure 5).

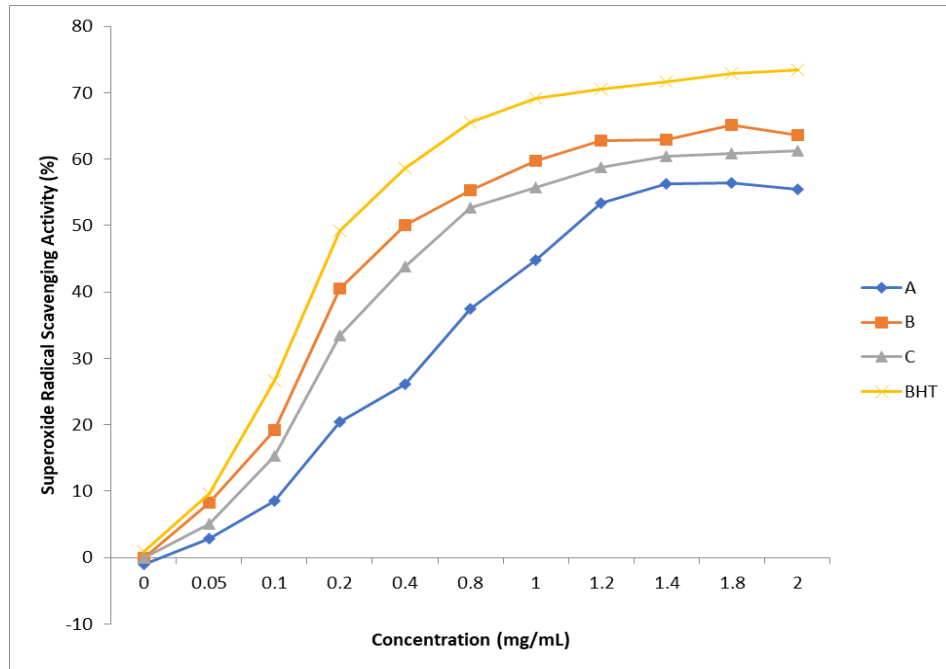


Figure 4. Superoxide radical-scavenging activities of sub-fractions of CFL. *BHT*-butylated hydroxytoluene. Values represent means of triplicates determinations

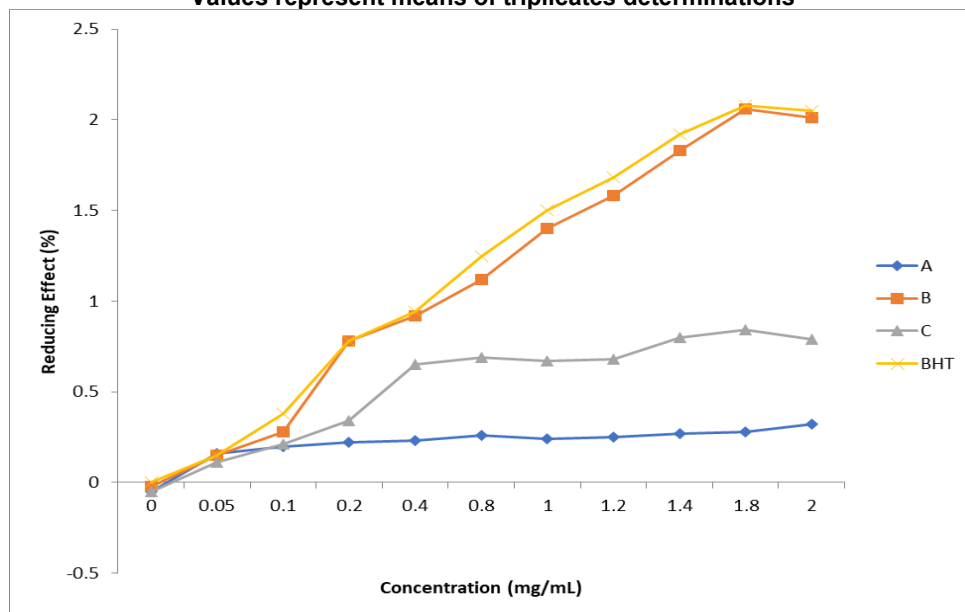


Figure 5. Ferric ion reducing antioxidant power (FRAP) effects of sub-fractions of CFL. *BHT*-butylated hydroxytoluene. Values represent means of triplicates determinations

The data on Table 3 extrapolated from the *in vitro* antioxidant activity for the EC_{50} revealed that SFB had the lowest DPPH scavenging activity of 0.06 compared to 0.04 of BHT, while SFC and SFA had 0.10 and 0.12, respectively. In similar trends, SFB had hydrogen peroxide-scavenging activity of 0.20, comparable to 0.11 of BHT, while SFA and

SFC had 0.52 and 0.39, respectively. The superoxide scavenging activity of SFB (0.24) also compared favourably to that of BHT (0.15), while SFA and SFC had 1.13 and 0.52, respectively. In metabolic processes, during oxidative phosphorylation, electrons do leak from the electron transport chain. These electrons interact

with molecular oxygen to generate radical oxygen, which further generates ROS—like hydroxyl radical, superoxide anion radical, and peroxy radical—which can prompt oxidative damage on biomolecules like proteins, DNA, and lipids [23]. Though biological systems have ways of rendering reactive species harmless and have necessary mechanisms to prevent or repair damages resulting from these reactive species, they are sometimes overwhelmed by the rate of production of additional reactive species arising from pathological conditions such as malaria [21, 24]. This results in systemic complications, which could be salvaged by the antioxidant capacity of administered compounds. Depending on which chemical reaction is involved, the *in vitro* antioxidant tests tend to measure the radical-scavenging status, rather than the preventive antioxidant capacity, of a sample and can loosely be categorised as hydrogen transfer reaction-based and electron transfer reaction-based [25]. In this study, the sub-fractions exhibited *in vitro* antioxidant effectiveness, including a ferric ion-reducing effect at varying degrees (Figures 2-5). SFB had higher concentrations of DPPH, peroxide ion, superoxide ion scavenging activities, and ferric ion reducing potential, which compared favourably with BHT. It validates the study of Balogun *et al.* [8], who reported a similar trend in *Clerodendrum violaceum* leaf extracts. Thus, this efficacy of the sub-fractions may be credited to the rich phytochemicals, particularly alkaloids and flavonoids, embedded in CFL [9], as well as the significant amount of vitamins C and E and selenium, capable of overcoming any resulting free radicals. These antioxidants may inhibit or deter cellular damage through their free radical-scavenging activity [8].

DPPH is a known parameter which measures the tendency of the extract to act as a free-radical scavenger for its antioxidant activity. The higher activities of DPPH shown in SFB may be as a result of its higher antioxidant concentrations compared to SFA and SFC. This may imply that SFB may be more effective in stabilizing the adverse effect of ROS formed *in vivo* through parasite infections, which may supplement the endogenous antioxidant systems. This finding is similar to those of Sutharsingh *et al.* [13] and Balogun *et al.* [8], who respectively reported the DPPH radical scavenging capacity of chloroform and ethanol extract of *Naravelia zeylanica* and methanol extract of *C. violaceum* leaf extract in mice. They reported a linear increase in reducing power over a range of concentrations and attributed the antioxidant activity to various mechanisms and also concluded that the reductive ability of a compound might be an

important indicator of its possible antioxidant activity.

In the Fenton reaction, cellular hydrogen peroxide is converted to the hydroxyl radical that can lead to oxidative damage to macromolecule; hence, the ability of the sub-fractions to scavenge hydrogen peroxide, may suggest their possible ability to also scavenge hydrogen peroxide *in vivo*. Ferric ion in cells takes part in the formation of hydroxyl radical in the Fenton reaction, which can also cause oxidative damage of macromolecules [26]. Therefore, ferric ion reduction in SFB—and SFC to a lesser extent—may indicate their ability to reduce ferric ion *in vivo*, thereby protecting cellular macromolecules from the influx of ferric ion during malaria infections. Therefore, it may be suggested that the antioxidant molecules in the sub-fractions might play significant roles in scavenging the ROS generated during parasite infections either singly or jointly via mechanisms that may comprise the interference of lipid peroxidation, since it has been implicated in malaria, and disruption of the free radical chain systems [8, 27].

Vitamin C (ascorbic acid) is the primary circulating water-soluble antioxidant, which functions as scavengers of free radicals by donating electrons to oxidants, thereby forming a stable dehydroascorbate [28]. Vitamin E (tocopherol) is better regarded as an antioxidant than as a vitamin since, unlike many vitamins, tocopherol does not function as a cofactor in enzymic reactions. Its primary role is to inhibit membrane phospholipid peroxidation and cell damage by acting as an antioxidant [29]. Tocopherol-OH is capable of transferring an atom from hydrogen with a single electron to a free radical, consequently eliminating the radical before it interacts with cell membrane proteins and cause damage. Selenium plays vital a role in the glutathione enzyme process and also preserves the cells by preventing free oxygen radical production [30]. The low levels of vitamins C and E in SFB compared to SFC and SFA may imply that SFB possesses an appreciable amount of the antioxidants to combat threats posed by oxidative stress generated by the parasites. This is because reduced levels of vitamins C and E have been reported to decrease in Plasmodium-infected subjects [7, 31, 32]. Since treatment with CFL produced low levels of these vitamins, thus high efficacy of SFB may be driven by other potent compounds (like its high selenium content or other phytochemicals) that are powerful enough to overcome its relatively lower levels of vitamins C and E. This also supports the finding of Olatunji and Afolayan [33], who stated that *Capsicum* fruits contain low amounts of vitamin C. Conversely,

high amounts of selenium have been reported to suppress malaria by inducing apoptosis-like cell death in *Plasmodium*, thereby enhancing the antioxidant capacity of the infected mice [34]. This

implies that the high levels of selenium in SFB compared to SFC and SFA are capable of enhancing the *in vitro* antioxidant capacity of the infected mice.

Table 3. EC₅₀ values for *in vitro* antioxidant activity on sub-fractions of *C. frutescens* L. Fruit against standard

Sub-fractions/BHT	EC ₅₀ (mg/mL)		
	DPPH Scavenging Activity	Hydrogen Peroxide Scavenging Activity	Superoxide Scavenging Activity
A	0.12	0.52	1.13
B	0.06	0.20	0.24
C	0.10	0.39	0.52
BHT	0.04	0.11	0.15

EC₅₀-half maximum effective concentration; BHT-butylated hydroxytoluene; DPPH-2,2-diphenyl-1-picrylhydrazyl

Furthermore, SFB recorded the lowest EC₅₀ values for the free radical-scavenging activities, which compares favourably to BHT (Table 3), since low EC₅₀ values indicate higher antioxidant action. This implies that a lesser amount of the extract is required to achieve an equal level of antioxidant capacity, indicating higher effectiveness among the three sub-fractions in mice. Therefore, this *in vitro* antioxidant status displayed by SFB may be responsible for the high *in vivo* parasite reduction and prolonged lifespan compared to SFC and SFA.

Conclusions

The *in vivo* curative studies of the sub-fractions of aqueous extract of CFL revealed that the sub-fractions possess antiplasmodial potency—particularly in SFB—and prolonged the lifespan of *Plasmodium*-infected subjects in dose-dependent patterns. The sub-fractions also possess a substantial amount of *in vitro* antioxidants comparable to BHT to combat emanating free radicals in mice. However, SFB demonstrated the highest antiplasmodial and antioxidant efficacy, suggesting its potential as a lead fraction for developing novel plant-derived antimalarial therapies. Hence, further research should be conducted to determine those metabolites responsible for its antiplasmodial and antioxidant efficacy, using SFB in particular.

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