Research Article

Cytotoxic and Antioxidant Effects of Antimalarial Herbal Mixtures

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Many developing countries depend on herbal mixtures as the first line and cost-effective therapy for malaria. These mixtures with such curative tendencies may also be a source of toxicity to host cells. On the other hand, these mixtures may have anticancer potential activity characterized by cytotoxicity to cancer cells. The aim of the study was to determine the cytotoxic and antioxidant effects of five different antimalarial herbal mixtures. Five antimalarial herbal mixtures commonly used in Ghana (coded as STF, SMH, SMM, SGM, and STT) were purchased and freeze-dried. The dried samples were tested on human acute T-cell leukemia (Jurkat) and breast adenocarcinoma (MCF-7) cell lines. Cytotoxicity was assessed using the tetrazolium-based colorimetric (MTT) assay while antioxidant activity was determined using DPPH free-radical scavenging assay. Among the mixtures, SMM and SGM exhibited the strongest cytotoxicity towards Jurkat cells (IC_{50} values 59.17 μg/ml and 49.57 μg/ml, respectively), whereas STT showed the weakest cytotoxicity ($IC_{50} = 244.94$ μg/ml). Cytotoxic effect of SMM was also strongest towards MCF-7 cells whilst the least cytotoxic sample was SGM ($IC_{50} > 1000$ μg/ml). SMM had the highest antioxidant percentage ($EC_{50} = 1.05$ mg/ml). The increasing order of antioxidant percentage among the five herbal mixtures is SMM > SMH > STT > STF > SGM. The herbal mixtures may be potential sources of toxic agents to host cells. Therefore, further toxicity studies must be performed to safeguard health of the public. Interestingly, cytotoxicities exhibited by SMM and SGM suggest the presence of anticancer constituents in them which warrant further studies.

1. Introduction

Numerous strategies have been adopted to control the incidence of malaria; however, an estimated 216 million cases of malaria were recorded worldwide in 2017, with 445,000 deaths. Approximately 90% of all malaria deaths occur in Africa [1]. The resistance of the *Plasmodium* parasite to the available antimalarial drugs is a major challenge to the control of malaria [2]. Previously, chloroquine was the drug of choice in Ghana and other endemic African countries for treatment of uncomplicated malaria caused by *P. falciparum*, the parasite responsible for most malaria cases. Artemisinin combination therapies (ACTs) are currently the drugs of choice for the treatment of uncomplicated malaria in Ghana. The emergence of drug-resistant strains therefore necessitates intensive search for new drugs [3].

With the increasing levels of drug resistance and the high cost of treatment with conventional antimicrobial drugs, herbal mixtures readily serve as the most assessable and affordable means of treatment for many illnesses in many parts of the world [4, 5]. The majority of the population in many developing countries, where malaria is endemic, depends on herbal mixtures as therapy for malaria [6, 7]. Medicinal plants, such as *Uvaria chamae*, *Strophanthus hispidus*, *Psidium guajava*, *Cassia abbreviata*, *Aristolochia albida*, *Annona muricata*, *Mangifera indica*, and *Azadirachta indica* have been documented and are used in most African countries to treat malaria and other ailments, either alone or in combination with other plant medicines [6, 8–10].

The preference for herbal preparation has been partly informed by the fact that these herbs are of natural origin and are therefore considered safe to use as compared to the

synthetic or the orthodox drugs. However, not all of such natural products are safe. Herbal remedies associated with adverse effects have been reported and continue to engage the attention of researchers [11–14]. There are so many herbal mixtures available and sold in Ghana for treatment of malaria. However, safety profile of the majority of these antimalarial mixtures has not yet been scientifically investigated. Hence, this study sought to determine the cytotoxic effect of five different commonly used antimalarial herbal mixtures sold on the Ghanaian market on cultured human cell lines.

2. Materials and Methods

Fetal bovine serum (FBS), RPMI culture medium, penicillin-streptomycin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Human acute T-cell leukemia (Jurkat) and breast adenocarcinoma (MCF-7) cell lines were obtained from RIKEN BioResource Center Cell Bank (Japan). All other reagents and chemicals used for the work were of analytical grade and obtained from standard suppliers.

2.1. Sample Preparation.
Five (5) commonly used herbal antimalarial mixtures from different manufacturers were purchased from the market in Accra. These samples were coded (as STF, SMH, SMM, SGM, and STT), frozen at −20°C and freeze-dried.

2.2. Cell Culture.
Jurkat cells were cultured in RPMI medium supplemented with 10% FBS and 1% penicillin-streptomycin, whilst MCF-7 cells were cultured in DMEM with the same supplements. The cells were maintained in a humidified incubator with 5% CO_2 at 37°C and subcultured when they were about 80% confluent.

2.3. Cell Viability (MTT) Assay.
Determination of cytotoxicity of the herbal extracts was carried out *in vitro* using the MTT assay as described in [15, 16]. Five different concentrations (62.5 to 1000 μg/ml) of each of the herbal mixtures were prepared by serial dilution. Hundred microliters (100 μl) cell suspension was added into each well in a 96-well microtitre plate, and 10 μl of extract dilution was added. Triplicate experiments were performed. Curcumin was used as positive control. Negative control (vehicle, water) and extract control experiments were also set up. The plates were incubated in a humidified incubator with 5% CO_2 at 37°C for 72 h. Twenty microliters (20 μl) of 2.5 mg/ml MTT solution (in PBS) was added to each well of the plates, and incubation was continued for 4 h. After 4 hours, 150 μl of acidified isopropanol was added to each well, and the plates were incubated in the dark at room temperature overnight. The MTT assay spectrophotometrically measures the purple-coloured formazan resulting from the reduction of the yellow tetrazolium salt by metabolically active cells. Absorbance was read at the wavelength of 570 nm using a

microplate reader (Tecan Infinite M200, Austria). The effect of the herbal mixtures on cell viability was calculated using the following formula:

$$\text{viability}\,(\%) = \frac{A_0 - A_1}{A_0} - B \times 100, \tag{1}$$

where A_0 is the mean absorbance of wells with untreated cells (vehicle), A_1 is the absorbance of test wells, and B is the absorbance of blank wells (extract control, cell free). The concentration of the test sample that resulted in 50% decrease in the cell number, i.e., 50% inhibitory concentration (IC_{50}), as compared with that of the control cultures (untreated cells) was then determined.

2.4. Determination of Antioxidant Activity: DPPH Assay.
The scavenging activities of the powdered herbal mixtures on the stable free-radical DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) were assayed according to a method described, with slight modification [17, 18]. Serial dilutions of each sample were prepared (0.027–20 mg/ml). Each reaction mixture comprised 100 μl of 0.5 mM DPPH solution (in methanol) and 100 μl of the sample in 96-well plates. Butylated hydroxytoluene (BHT) was used as positive control. Triplicate experiments were performed. The plates were incubated for 20 min at room temperature in the dark, and the absorbance was read using a microplate reader (Tecan Infinite M200, Austria) at 517 nm. The percentage of inhibition was calculated using the equation:

$$\text{inhibition}\,(\%) = \frac{A_0 - A_1}{A_0} \times 100, \tag{2}$$

where A_0 is the absorbance of the control and A_i is the absorbance of the samples. EC_{50}, which is the concentration where 50% of the free-radical activity of DPPH is quenched, was extrapolated from a graph of percent antioxidant activity versus sample concentration [18].

3. Results

3.1. Cytotoxicity.
For evaluation of cytotoxicity, the cells were exposed to increasing concentrations of the herbal extracts with curcumin serving as positive control. Figures 1 and 2 show the dose-dependent effect of the herbal mixtures on Jurkat cell lines, with their corresponding IC_{50} values (Table 1). The antimalarial STT showed the least cytotoxic effect towards Jurkat cells with an IC_{50} value of 244.92 μg/ml, whilst SGM gave the strongest cytotoxic effects ($IC_{50} = 49.57$ mg/ml) on the cell lines. On the other hand, among the herbal mixtures, SGM showed the least cytotoxicity towards MCF-7 cell lines ($IC_{50} > 1000$ mg/ml), whereas SMM gave the strongest activity ($IC_{50} = 40.82$ mg/ml) on the cells. The herbal mixtures STF and SMH exhibited weak cytotoxic activities towards both human cell lines ($IC_{50} > 100$ μg/ml). Curcumin, which was used as the positive control exhibited a higher cytotoxicity against both cell lines as expected, with an IC_{50} value of 3.84 μg/ml and 7.45 μg/ml for Jurkat and MCF-7 cells, respectively.
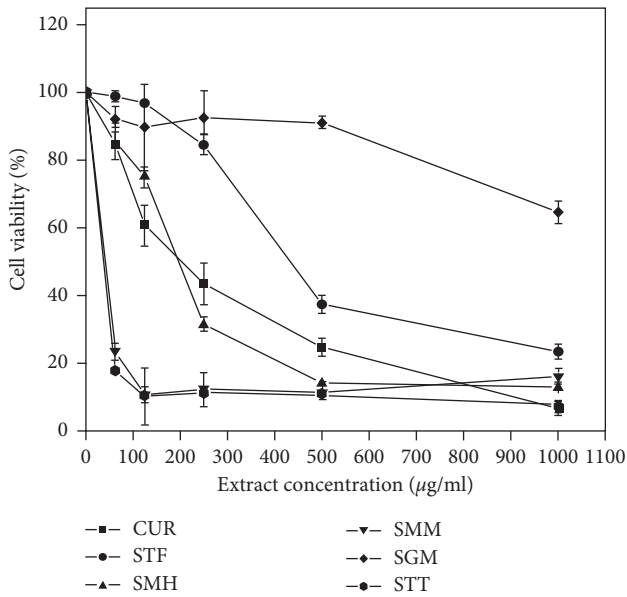
FIGURE 1: Cytotoxic effects of the herbal mixtures on Jurkat leukemia cells. Each plotted point represents the mean of three independent experiments, and the bars are standard deviations.
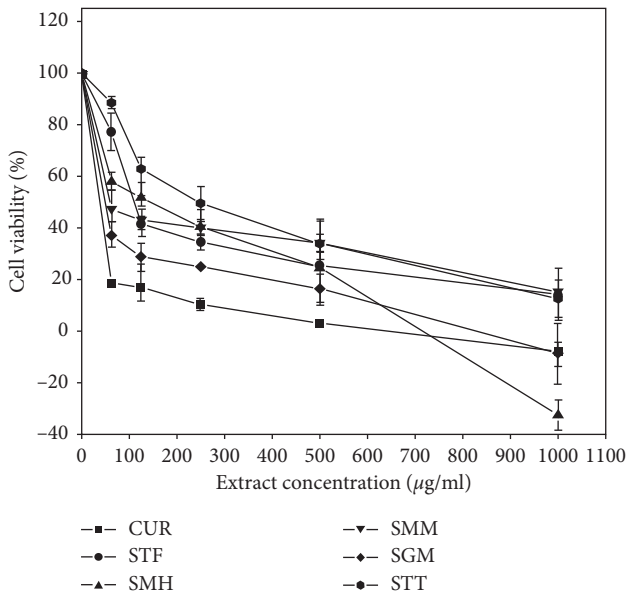

FIGURE 2: Cytotoxic effects of the herbal mixtures on MCF-7 breast cancer cells. Each plotted point represents the mean of three independent experiments, and the bars are standard deviations.

3.2. Antioxidant Activity. Protection against oxidative damage is one of the most widely described attributes of plant extracts and relates to their radical scavenging activity. The scavenging properties of the herbal mixtures were evaluated by the DPPH radical scavenging assay. The EC_{50} values are a parameter widely used to measure antioxidant activity [19]. The lower the EC_{50} value, the higher the antioxidant activity. Table 2 shows the EC_{50} values of the herbal mixtures and BHT, which was used as positive control. Among the mixtures, SMM gave the lowest EC_{50} value (1.05 mg/ml) which indicates the highest antioxidant

TABLE 1: *In vitro* cytotoxic effects of the herbal mixtures on human cells.

Herbal mixtures	IC50 values of JURKAT cells and MCF-7 cells (μg/ml)	
STF	110.6 ± 8.46	432.78 ± 5.77
SMH	143.99 ± 13.22	196.55 ± 2.88
SMM	59.17 ± 11.54	40.82 ± 1.52
SGM	49.57 ± 4.10	1000 ± 4.35
STT	244.94 ± 10.41	97.95 ± 1.15
CUR	3.80 ± 0.15	7.45 ± 0.11

The values represent the mean of three independent experiments. CUR: curcumin, positive control.

TABLE 2: Antioxidant activity of the herbal mixtures.

Herbal mixtures	EC50 (mg/ml)
STF	2.69 ± 0.35
SMH	1.45 ± 0.23
SMM	1.05 ± 0.21
SGM	3.58 ± 0.31
STT	1.65 ± 0.38
BHT	0.23 ± 0.02

BHT, butylated hydroxytoluene, a known potent antioxidant was used as positive control.

activity. This was followed by SMH and STT which gave similar activities (EC_{50} values of 1.45 mg/ml and 1.65 mg/ml, respectively). The highest EC_{50} value was found for SGM.

4. Discussion

Traditional herbal medicines have been used to treat malaria for years, and they are the source of major groups (artemisinin and quinine derivatives) of potent modern antimalarial drugs [20]. Herbal preparations may achieve the desired curative purpose when administered but may also contain other ingredients that may have toxic effects on human. We assessed the cytotoxic effects of five known potent antimalarial herbal mixtures on human cell lines Jurkat and MCF-7. Antioxidant activities of the mixtures were also assessed to determine the potential of the products to protect the body against oxidative stress. The herbal mixtures that were considered in this study are commonly used mixtures claimed to be very effective antiplasmodial agents.

Results obtained show that the five antimalarial herbal mixtures tested had varying cytotoxic activities toward the human cell lines compared to the positive control (curcumin), which was highly cytotoxic to both cells. Possibly, the cytotoxic effect of individual components has been diluted out in the mixture. Alternatively, the observed cytotoxicity could be an additive or synergistic effect from the various components of the mixtures. *In vitro* analysis of potential toxic, mutagenic, and carcinogenic effects of herbal medicines has been established on both normal and cancer cells by many scientific studies [21–25]. These medicines have been reported to have therapeutic synergistic effect or may also be antagonistic for the side effects observed [26, 27]. Assessment of the effects of other commercial herbal

preparations has been done in South Africa, where 6 commonly used preparations were tested for their effects on isolated human platelets [28]. Similar studies by Mothibe et al. on a herbal body-healing mixture observed variable stimulatory and inhibitory effects on human neutrophils [29].

The inhibitory effects of a herbal mixture in general have been attributed to the total activity of the crude extract, rather than that of a single major component of the mixtures [30], although this may not always be the case. Cancer cells are frequently used for cytotoxicity studies because of the rapid growth rate compared to normal cells, thus facilitating quick acquisition of *in vitro* data on test samples. More so, whatever harms cancer cells will also usually harm normal healthy cells in the body.

Figures 1 and 2 show the inhibitory effect of the herbal mixtures on the two cell lines. In all instances, the percentage cell viability decreased in a dose-dependent manner with increasing concentrations of the mixture. It could also be seen that toxic effect of mixtures on cells varied depending on the cell line used. Although both SMH and SMM were produced from the same plant component (*Cryptolepis sanguinolenta*) (Table 3), they showed different degrees of cytotoxicity on the cells tested. This difference may possibly be due to different extraction methods employed by the two different manufacturers. The activities may also partly be due to the fact that these two mixtures have other plant components with cytotoxic effect which have not been stated on their respective labels.

Four out of the five herbal mixtures considered in this study were either made from *Cryptolepis sanguinolenta* or had the plant as a component of the mixture. Indeed, antimalarial activity of extracts of this plant has been long established in Africa [31–33]. Cytotoxicity of aqueous extract of *Cryptolepis sanguinolenta* on different cell lines has also been reported by Ansah and Goodman [34]. Except for SMM which was cytotoxic even at a lower concentration, cytotoxicity increased with increasing concentrations of the mixtures. In most rural communities, extract of these plants may be the only alternative for the treatment of malaria. This raises the concern on how much of these mixtures one needs to consume to achieve the desired curative purpose without experiencing significant toxic effect.

Plant-based medicines generally contain a significant amount of phyto-antioxidants which prevent oxidative damage to the hosts such as that which is caused by the *Plasmodium* parasite from malaria infection. We also tested whether these mixtures have antioxidant effects. All the herbal mixtures had high antioxidant activities compared to the positive control, with SMM recording the strongest activity ($EC_{50} = 1.05$ mg/ml). The importance of antioxidant activity is to slow down or prevent the oxidative damage to the host cells caused by oxidation reactions that produce free radicals [35, 36]. Malaria infection induces the generation of hydroxyl radicals in the liver, which can lead to the induction of oxidative stress and apoptosis [37]. These stresses are managed through various cellular enzymatic and nonenzymatic systems enhanced by certain compounds, most of which are found in plants. Thus, the curative ability of the

TABLE 3: Herbal antimalarial mixtures and their plant components.

Herbal mixtures	Mass of dried extract (mg)*	Plant component
STF	0.34	*Azadirachta indica, Alstonia boonei*
SMH	0.69	*Cryptolepis sanguinolenta*
SMM	0.17	*Cryptolepis sanguinolenta*
SGM	0.07	*Cryptolepis sanguinolenta, Morinda lucida, Nauclea latifolia*
STT	0.03	*Carapa procera, Cryptolepis sanguinolenta*

*Mass of the extract obtained after freeze drying 30 ml of the herbal mixtures.

herbal mixtures, as testified by consumers, could partly be attributed to the relatively high antioxidant activity of these mixtures.

It is worth noting that SMM had the highest percentage antioxidant activity among the five herbal medicines as well as have the highest cytotoxic effect on the two cancer cells that we used. The high antioxidant activity of SMM, coupled with its strong effect on cancer cells, could be explored in the treatment of cancer. The active components of this herbal preparation (*Cryptolepis sanguinolenta*) could be a subject for further investigation as far as treatment of cancer is concerned.

In conclusion, although these herbal mixtures are claimed to be potent antimalarial agents they could be potentially toxic to host cells. Therefore, comprehensive toxicity studies are warranted to safeguard public health. Also, close attention must be paid to the dosage and the patient's health history since certain diseases could be secondary factors in the generation of toxicities in the presence of these herbal mixtures.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

The research was performed as part of the employment of the authors from the University of Cape Coast.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

[1] WHO, *World Malaria Report 2017*, World Health Organization, Geneva, Switzerland, 2017.

[2] P. Guyant, V. Corbel, P. J. Guérin et al., "Past and new challenges for malaria control and elimination: the role of operational research for innovation in designing interventions," *Malaria Journal*, vol. 14, no. 1, p. 279, 2015.

[3] D. C. Warhurst, "Drug-resistant malaria: laboratory and field investigations," *Journal of Pharmacy and Pharmacology*, vol. 49, no. 2, pp. 3–7, 1997.

[4] WHO, *WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems*, World Health Organization, Geneva, Switzerland, 2004.

[5] S. Chatterjee, A. Banerjee, and I. Chandra, "Hemidesmus indicus: a rich source of herbal medicine," *Medicinal & Aromatic Plants*, vol. 3, no. 4, p. 155, 2014.

[6] A. Asase, A. A. Oteng-Yeboah, G. T. Odamtten, and M. S. J. Simmonds, "Ethnobotanical study of some Ghanaian anti-malarial plants," *Journal of Ethnopharmacology*, vol. 99, no. 2, pp. 273–279, 2005.

[7] O. Idowu, O. Soniran, O. Ajana, and D. Aworinde, "Ethnobotanical survey of antimalarial plants used in Ogun state, Southwest Nigeria," *African Journal of Pharmacy and Pharmacology*, vol. 4, no. 2, pp. 055–060, 2010.

[8] C. Ukaga, B. Nwoke, P. Onyeka et al., "The use of herbs in malaria treatment in parts of Imo state, Nigeria," *Tanzania Journal of Health Research*, vol. 8, no. 3, 2009.

[9] T. Ngarivhume, C. I. E. A. van't Klooster, J. T. V. M. de Jong, and J. H. Van der Westhuizen, "Medicinal plants used by traditional healers for the treatment of malaria in the Chipinge district in Zimbabwe," *Journal of Ethnopharmacology*, vol. 159, pp. 224–237, 2015.

[10] M. L. Willcox and G. Bodeker, "Traditional herbal medicines for malaria," *BMJ*, vol. 329, no. 7475, pp. 1156–1159, 2004.

[11] E. Ernst, "Harmless herbs? a review of the recent literature," *The American Journal of Medicine*, vol. 104, no. 2, pp. 170–178, 1998.

[12] P. D'Arcy, "Adverse reactions and interactions with herbal medicines. Part 1: adverse reactions," *Adverse Drug Reactions and Toxicological Reviews*, vol. 10, no. 10, pp. 189–208, 1991.

[13] M. Ekor, "The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety," *Frontiers in Pharmacology*, vol. 4, p. 177, 2014.

[14] P. A. G. M. De Smet, "Herbal remedies," *New England Journal of Medicine*, vol. 347, no. 25, pp. 2046–2056, 2002.

[15] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[16] R. Appiah-Opong, I. K. Asante, D. O. Safo et al., "Cytotoxic effects of Albizia zygia (DC) JF Macbr, a Ghanaian medicinal plant, against human *t*-lymphoblast-like leukemia, prostate and breast cancer cell lines," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 8, pp. 392–396, 2016.

[17] M. S. Blois, "Antioxidant determinations by the use of a stable free radical," *Nature*, vol. 181, no. 4617, pp. 1199-1200, 1958.

[18] R. Appiah-Opong, I. Tuffour, G. K. Annor et al., "Antiproliferative, antioxidant activities and apoptosis induction by Morinda lucida AND *Taraxacum officinale* in human HL-60 leukemia cells," *Journal of Global Biosciences*, vol. 5, no. 7, pp. 4281–4291, 2016.

[19] V. Bondet, W. Brand-Williams, and C. Berset, "Kinetics and mechanisms of antioxidant activity using the DPPH.free radical method," *LWT-Food Science and Technology*, vol. 30, no. 6, pp. 609–615, 1997.

[20] L. Cui and X.-z. Su, "Discovery, mechanisms of action and combination therapy of artemisinin," *Expert Review of Anti-Infective Therapy*, vol. 7, no. 8, pp. 999–1013, 2009.

[21] S. C. Ferreira-Machado, M. P. Rodrigues, A. P. M. Nunes et al., "Genotoxic potentiality of aqueous extract prepared from *Chrysobalanus icaco* L. leaves," *Toxicology Letters*, vol. 151, no. 3, pp. 481–487, 2004.

[22] S. Machana, N. Weerapreeyakul, S. Barusrux, A. Nonpunya, B. Sripanidkulchai, and T. Thitimetharoch, "Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line," *Chinese Medicine*, vol. 6, no. 1, p. 39, 2011.

[23] A. Radovanovic, "Evaluation of potential cytotoxic effects of herbal extracts," *Serbian Journal of Experimental and Clinical Research*, vol. 16, no. 4, pp. 333–342, 2015.

[24] D. d. L. Moreira, S. S. Teixeira, M. H. D. Monteiro, A. C. A. X. De-Oliveira, and F. J. R. Paumgartten, "Traditional use and safety of herbal medicines," *Revista Brasileira de Farmacognosia*, vol. 24, no. 2, pp. 248–257, 2014.

[25] A. Sharif, M. F. Akhtar, B. Akhtar et al., "Genotoxic and cytotoxic potential of whole plant extracts of Kalanchoe laciniata by Ames and MTT assay," *EXCLI Journal*, vol. 16, p. 593, 2017.

[26] A. Koul, S. C. Gangar, and V. Sandhir, "Pitfalls in journey from traditional to modern medicine," *Natural Product Radiance*, vol. 4, no. 1, 2005.

[27] S. Bent, "Herbal medicine in the United States: review of efficacy, safety, and regulation," *Journal of General Internal Medicine*, vol. 23, no. 6, pp. 854–859, 2008.

[28] M. E. Mothibe, C. P. Kahler-Venter, and E. Osuch, "Evaluation of the in vitro effects of commercial herbal preparations significant in African traditional medicine on platelets," *BMC Complementary and Alternative Medicine*, vol. 19, no. 1, p. 224, 2019.

[29] M. E. Mothibe, C. P. Kahler-Venter, and E. Osuch, "In vitro effects of a commercial herbal medicine used as African traditional medicine on human neutrophils," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 14, no. 3, pp. 51–50, 2017.

[30] D. Sarkar, A. Sharma, and G. Talukder, "Plant extracts as modulators of genotoxic effects," *The Botanical Review*, vol. 62, no. 4, pp. 275–300, 1996.

[31] P. Grellier, L. Ramiaramanana, V. Millerioux et al., "Antimalarial activity of cryptolepine and isocryptolepine, alkaloids isolated from Cryptolepis sanguinolenta," *Phytotherapy Research*, vol. 10, no. 4, pp. 317–321, 1996.

[32] G. C. Kirby, A. Paine, D. C. Warhurst, B. K. Noamese, and J. D. Phillipson, "In vitro and in vivo antimalarial activity of cryptolepine, a plant-derived indoloquinoline," *Phytotherapy Research*, vol. 9, no. 5, pp. 359–363, 1995.

[33] B. Noamesi, A. Paine, G. Kirby, D. Warhurst, and J. Phillipson, "In vitro antimalarial activity of cryptolepine, an indoquinoline," *Transactions of the Royal Society of Tropical Medicine and Hygiene*, vol. 85, p. 315, 1991.

[34] C. Ansah and N. J. Gooderham, "The popular herbal antimalarial, extract of Cryptolepis sanguinolenta, is potently cytotoxic," *Toxicological Sciences*, vol. 70, no. 2, pp. 245–251, 2002.

[35] B. Greve, L. G. Lehman, B. Lell, D. Luckner, R. Schmidt-Ott, and P. G. Kremsner, "High oxygen radical production is associated with fast parasite clearance in children with *Plasmodium falciparum* malaria," *The Journal of Infectious Diseases*, vol. 179, no. 6, pp. 1584–1586, 1999.

[36] M. H. Yang, K. D. Yoon, Y.-W. Chin, J. H. Park, and J. Kim, "Phenolic compounds with radical scavenging and cyclo-oxygenase-2 (COX-2) inhibitory activities from *Dioscorea opposita*," *Bioorganic & Medicinal Chemistry*, vol. 17, no. 7, pp. 2689–2694, 2009.

[37] S. Percário, D. Moreira, B. Gomes et al., "Oxidative stress in malaria," *International Journal of Molecular Sciences*, vol. 13, no. 12, pp. 16346–16372, 2012.