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AN ANTIPLASMODIAL ACTIVITY OF LEAVES EXTRACTS OF *CASSIA OBTUSIFOLIA* AND *HYPTIS SUAVEOLENS* FROM GHANA

Addai-Mensah Donkor [*1], Tracey Amarquay [2], Eric Kyei-Baafour [3], Martin Ntiamoah Donkor [4], Benjamin Ahenkorah [5] and Kwadwo Asamoah Kusi [3]

Department of Pharmaceutics [1], School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana.
Department of Applied Chemistry [2], Faculty of Physical Sciences, University for Development Studies, Tamale, Ghana.
Department of Immunology [3], Noguchi Memorial Institute for Medical Research, College of Health Sciences, University of Ghana, Legon-Accra, Ghana.
Department of Biochemistry and Forensic Sciences [4], School Chemical and Biochemical Sciences, C. K. Tedam University of Technology and Applied Sciences, Navrongo, Ghana.
Department of Medical Laboratory Science [5], Bolgatanga Technical University, Bolgatanga, Ghana.

Keywords:

Hyptis suaveolens, *Cassia obtusifolia*, Antiplasmodial activity, Phytochemical

Correspondence to Author:
Addai-Mensah Donkor

Associate Professor,
Department of Pharmaceutics,
School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana.

E-mail: dr.adonkor@uds.edu.gh

ABSTRACT: A number of the population in developing countries use herbs and herbal products to treat various diseases including malaria. However, most of them have not been evaluated for their efficacy and toxicity and their use is not standardized in some instances. Poly-herbal extracts may yield better treatment outcomes compared to extracts from a single plant due to the combined effects of bioactive compounds from the multiple sources. *Cassia obtusifolia* and *Hyptis suaveolens* are two plants commonly used in herbal preparations for treating malaria in Ghana. This study was to investigate *Plasmodium falciparum* asexual growth inhibitory activities of the individual and combined leaf extracts of the two plants. Leaves of the *C. obtusifolia* and *H. suaveolens* were extracted separately with methanol, ethanol and water. The six extracts, as well as in combination were evaluated in a dose-dependent manner for their antiplasmodial activities against the chloroquine-sensitive *Plasmodium falciparum* 3D7. Aqueous extract of *C. obtusifolia*, including the mixture of the two plants were highly active with mean IC_{50} less than 4.0 µg/mL. Similarly, methanol extract of *H. suaveolens* and the mixture also had IC_{50} below 4 µg/mL. Ethanol extracts of the mixture was correspondingly active with IC_{50} less than 4.0 µg/ml. All the combined extracts inhibited parasite growth above 80% at concentration of 100.00 µg/mL. Our results showed good antiplasmodial activity of the two plants comparable to the standard drug, artesunate, and hence provides the basis for their continuous use as antimalarial therapy in Ghana, possibly in their combined form.

INTRODUCTION: Malaria remains a public health problem for resource-poor countries, especially in sub-Saharan African countries, with about 88% of cases occurring in these countries [1].

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Considering the six species [2, 3, 4] currently known to infect humans, *Plasmodium falciparum* is the most prevalent in Africa, contributing to about 90% of reported malaria cases [5].

In Ghana, malaria is a major cause of morbidity and mortality especially in children under five years and pregnant women, accounting for up to 30% of outpatient department (OPD) cases [6]. Resistance of *P. falciparum* to drugs such as chloroquine has caused a change in first line drug policy in many endemic countries and the

emergence of parasite resistance to recently introduced artemisinin-based drugs, especially in South-East Asia, poses a great danger to the malaria elimination agenda [7, 8, 9]. This, coupled with the unavailability of potent and effective vaccines against malaria, is very important to the search for new and effective antimalarial drugs to combat the disease.

Indigenous plants have been the main source of treatment for many ailments in most developing countries, where about 80% of the population depends on traditional medicine for their primary health care [10]. Extracts and decoctions of several plant species, either alone or in combination with other plants, have traditionally been used for treating malaria [11]. In Ghana, medicinal plant formulations for treating malaria and malaria-related symptoms account for about 6% of the total herbal medicines on the market [12, 13]. These herbal preparations range from very crude decoctions to preparations that have to some extent, been standardized through research and received some approval by standards authorities for use. In urban centers of Ghana where orthodox medicines are readily available, the use of herbal concoctions for treating malaria and many other infectious and non-infectious diseases is still very common [12, 14]. *Hyptis suaveolens* and *Cassia obtusifolia* are two plants that have been used in folkloric medicine to treat malaria in Ghana.

Hyptis suaveolens is a branching pseudo-cereal plant that originates from tropic regions of America but can now be found in other areas of the tropical world, including Africa. *H. suaveolens* leaves have been reported to possess insecticidal and larvicidal properties and are therefore used as an insect repellent and insecticide, respectively [15]. Parts of the plant are used to cure cutaneous parasitic diseases, and the ethanol extract of the leaf has been reported to show wound-healing activity. *H. suaveolens* has also been reported to be effective against respiratory and gastrointestinal infections, indigestion, colds, pain, fever, skin diseases, gastric ulcer, and various inflammatory conditions [16]. The methanol leaf extract of *H. suaveolens* has been revealed to have antimicrobial and antibacterial activities [17]. *Cassia obtusifolia*, commonly called sicklepod, is a legume from the family Caesalpiniacea. It is found in North, Central, and South America, Asia, and Africa [16, 18]. Ethanol extract of the leaf was reported to show larvicidal activities on *Anopheles stephensi* [19], while the chloroform extract of the seeds has been shown to have an almost 100% mortality against common disease-transmitting mosquitoes at a concentration of 25 mg/L [20]. Decoctions of either the leaves, stem bark or roots, occasionally in combination with other plants decoctions, have been used to treat diseases such as diarrhea, stomach ache, malarial dysentery, rheumatism, back pain, and yellow fever. *C. obtusifolia* has been found to have anti-microbial[21] and anti-ulcer properties [22].

Based on the evidence of use of decoctions from these two plants for malaria treatment, this study aimed to investigate the antimalarial activities of the ethanol, methanol and aqueous extracts of the leaves of *H. suaveolens* and *C. obtusifolia* separately and as a combination, on the growth of the asexual stage *Plasmodium falciparum in-vitro*.

MATERIALS AND METHODS:

Collection and Authentication of Plant Materials: *H. Suaveolens* and *C. Obtusifolia* leaves were collected from Tono in the Kassena-Nankana District, Upper East Region, Ghana, during the rainy season. The plants were identified and authenticated by a plant taxonomist at the herbarium of Ghana Herbaria, Northern Savanna Biodiversity; Savanna Herbarium. The voucher specimen was deposited with numbers of SH 169 (*Cassia obtusifolia*) and SH 701 (*Hyptis suaveolens*) in the herbarium.

Preparation of Plant Materials for Extraction: Plant extracts were prepared using the cold maceration method described elsewhere with a little modification [23]. The leaves of plant samples were thoroughly washed with distilled water, dried under shade for ten days, and pulverized by blending. The powdered leaves of the two plants, 100 g each, were extracted separately by mixing with 2 L of methanol (70 %), ethanol (70 %), and distilled water with shaking at 4 h intervals for 24 h. The resulting extract in each case was filtered with Whatman No. 1 filter paper, and the filtrates were concentrated with a rotary evaporator at 45 °C.

The crude extracts were then freeze-dried and stored at -20 ℃ for further use. Regarding the extract combinations, equal quantity of 2 g each of the freeze-dried plant extracts were combined in sterile vials containing 10 mL concerning each solvent. The solvents were removed under rotary-evaporator and the samples were completely dried in a desiccator and subsequently stored at -20 ℃ until further use.

Phytochemical Screening: The two plants' aqueous, methanol, and ethanol extracts were subjected to phytochemical screening to identify major groups of chemical constituents using standard procedures already described elsewhere [24].

***Plasmodium falciparum* Culturing:** The 3D7 strain of *P. falciparum* was maintained in continuous culture by the modified method of Trager & Jensen [25], with RPMI 1640 medium containing 1% gentamycin and 1% L-glutamine, supplemented with 10% Albumax.

Parasites were maintained in “O" Rh "D” positive blood at 4% haematocrit and flushed with mixed gas (5.5% CO_2, 2% O_2 and 92.5% N_2) for 40s and incubated at 37 ℃. Monitoring parasites was done by preparing slides for microscopy and culture medium was changed daily. Synchronization of the culture (for parasitaemia above 5%) was performed using 5% sorbitol treatment and the ring stage of the parasites was harvested for the assay.

***In-vitro* Anti-plasmodial Assay:** The assay was performed in triplicate for each dilution of plant extract. A stock solution of 1000 µg/mL was made in RPMI 1640 for each freeze-dried plant material. All reconstituted extracts were sterile filtered using a 0.22 µm Millipore filter and for each extract, a three-fold serial dilution was made in triplicate wells of a 96-well tissue culture plate (Corning) for a total of six concentrations (1000 µg/mL, 333.30 µg/mL, 111.11 µg/mL, 37.04 µg/mL, 12.35 µg/mL and 4.12 µg/mL). Synchronized *P. falciparum* ring stages at 1% parasitemia and 2% hematocrit was added to wells in the plate, resulting in a final extract concentration of 100 µg/mL, 33.33 µg/mL, 11.11 µg/mL, 3.70 µg/mL, 12.35 µg/mL and 0.41 µg/mL in each well (10 µL of extract with 90 µL of culture). Artesunate was used as a positive control at concentrations 800.0 ng/mL, 266.7 ng/mL, 88.9 ng/mL, 29.6 ng/mL, 9.9 ng/mL and 3.3 ng/mL; negative control wells had parasites without any extract. After 48 h of incubation at 37 ℃, the contents of the wells were harvested, and thin blood films were made. After washing and drying, the films were fixed with methanol, stained for 10 min in 10% Giemsa and viewed under the microscope (× 100, oil immersion) to estimate the parasitemia. About 1000 RBCs per slide were counted to estimate parasitemia, and the percentage of parasite growth inhibition was calculated. Equation 1:

$$I\% = (Pc - Ps) / Pc) \times 100 \qquad \text{(Equation 1)}$$

Where, I = inhibition of parasite growth; Pc = mean parasitemia of negative control; Ps = mean parasitemia of extract or standard drug

Data and Statistical Analysis: The data presented is an average obtained from two independent assays. Antiplasmodial activities of the extracts were expressed as the concentration of the extract that inhibited 50% of parasites (IC_{50}) compared to the negative control (100% parasitaemia). IC_{50} values were calculated by plotting % inhibition against the logarithm to the base 10 of the extract concentrations (Log_{10} Conc.). Antiplasmodial activities of the extracts were characterized using previously described criteria [26]. Differences between inhibitions attributable to the different extracts were assessed by One-way ANOVA, followed by Bonferroni's Multiple Comparison test where necessary. All analyses were performed using the GraphPadPrism statistical tool (version 5, San Diego, USA), and statistical significance was set to an alpha level of 0.05.

RESULTS:

Phytochemical Screening: Results from the phytochemical screening of the six different extracts from the two plants are illustrated **in Table 1**. All six extracts exhibited positive for alkaloids, anthraquinones, cardiac glycosides, steroids, flavonoids, tannins, and terpenoids. Nonetheless, triterpenes were absent in the ethanol and aqueous extracts of *C. obtusifolia* and the aqueous extract of *H. suaveolens.* In addition, phenols were absent in aqueous extracts from the two plants, while saponins were absent in the ethanol extracts obtained from both plants.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE THREE EXTRACTS FROM THE TWO PLANTS

Secondary Metabolite	*Hyptis suaveolens*			*Cassia obtusifolia*		
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous
Alkaloids	+	+	+	+	+	+
Anthraquinone	+	+	+	+	+	+
Cardiac glycoside	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Glycoside	+	+	+	+	+	+
Phenols	+	+	-	+	+	-
Saponins	-	+	+	-	+	+
Steroids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Triterpenes	+	+	-	-	+	-

(+) positive result for metabolite; (-) negative result for metabolite.

Suppressive Effects of the Extracts on Parasite Growth *In-vitro*: All six extracts as well as the extract combination showed a dose-dependent inhibition of growth of 3D7 strain of *Plasmodium falciparum* parasite growth *in-vitro* **Table 2**. At the highest 100 µg /mL concentration, the ethanol extract of *C. obtusifolia* exhibited 76.87% inhibition of parasite growth. At the same concentration, the methanol extract exhibited 79.66% inhibition of parasites, while the aqueous extract exhibited 81.12% inhibition of parasites.

TABLE 2: DOSE-DEPENDENT SUPPRESSIVE EFFECTS OF THE SIX PLANT EXTRACTS AND COMBINATIONS

Concentration (µg /mL)	Ethanol		Methanol		Aqueous	
	Parasitaemia	Inhibition (%)	Parasitaemia	Inhibition (%)	Parasitaemia	Inhibition (%)
Cassia obtusifolia						
100.00	0.49±0.09	76.87	0.45±0.14	79.66	0.44±0.18	81.12
33.33	0.67±0.11	66.56	0.60±0.17	72.94	0.61±0.26	73.80
11.11	0.84±0.07	59.31	0.76±0.24	66.03	0.73±0.30	68.25
3.70	1.08±0.01	45.94	0.92±0.28	58.69	0.88±0.31	60.94
1.23	1.25±0.07	36.64	1.08±0.30	50.88	1.01±0.33	55.01
0.41	1.41±0.01	29.84	1.30±0.33	40.30	1.16±0.31	46.95
Hyptis suaveolens						
100.00	0.45±0.19	84.22	0.48±0.19	83.11	0.51±0.25	82.16
33.33	0.67±0.29	77.21	0.65±0.24	77.51	0.71±0.33	75.85
11.11	0.75±0.32	73.31	0.81±0.32	71.26	0.84±0.40	70.48
3.70	0.94±0.39	66.56	0.97±0.41	65.50	0.96±0.39	65.93
1.23	1.10±0.49	61.16	1.12±0.45	60.30	1.11±0.42	60.44
0.41	1.28±0.57	54.82	1.35±0.51	51.53	1.33±0.50	52..51
Combination#						
100.00	0.43±0.18	80.75	0.48±0.22	78.92	0.45±0.18	79..95
33.33	0.57±0.22	74.23	0.66±0.31	71.47	0.70±0.26	68.56
11.11	0.76±0.29	66.10	0.83±0.40	63.08	0.83±0.31	62.43
3.70	0.95±0.31	6.54	1.01±0.49	56.12	0.99±0.31	54.48
1.23	1.15±0.29	45.78	1.18±0.52	48..11	1.19±0.34	44.35
0.41	1.39±0.35	34.27	1.45±0.58	35.51	1.34±0.40	37.76

#:combination of equimolar quantities (by mass) of freeze-dried material from the two plants, originally extracted using the same solvent.

At the lowest concentration of 0.41 µg/mL, the ethanol, methanol and aqueous extracts of *C. obtusifolia* inhibited 29.84%, 40.30%, and 46.95% of parasites, respectively. Considering *H. suaveolens*, the ethanol, methanol and aqueous extracts at the highest concentration of 100 µg/mL inhibited the parasite growth by 84.22 %, 83.11 % and 82.16 %, respectively. On the other hand, at the lowest concentration of 0.41 µg/mL, the ethanol, methanol and aqueous extracts of *H. suaveolens* inhibited 54.82%, 51.53% and 52.54% of parasites, respectively. Concerning the combination of the two plants, the ethanol, methanol, and aqueous extracts at the highest concentration of 100 µg/mL

inhibited parasite growth by 80.76%, 78.92%, and 79.95%, respectively **Table 2**. At the lowest concentration of 0.41 µg/mL, the ethanol, methanol, and aqueous extracts inhibited 34.27 %, 35.51 %, and 37.76 %, respectively. The reference drug (Artesunate) inhibited 83.13% at the highest tested concentration (800 ng/mL) and 51.29 % at the least 3.29 ng/mL concentration **Table 3**.

TABLE 3: DOSE-DEPENDENT SUPPRESSIVE EFFECT OF ARTESUNATE

Concentration (ng/mL)	Parasitaemia	Inhibition (%)
800.00	0.52±0.09	83.13
266.67	0.66±0.12	78.39
88.89	0.85±0.16	72.22
29.63	1.01±0.18	66.74
9.88	1.24±0.15	59.17
3.29	1.48±0.11	51.30

Antimalarial Activity: *In-vitro* antiplasmodial activities of the six extracts from the two plants were studied and are presented in **Table 4**. The plants' extracts were tested individually and then as mixtures or combinations per solvent for original extraction. Two independent assays were done in triplicate, and the average activities of these independent assays are reported here.

All individual extracts and their combinations showed very good antiplasmodial activities with all IC_{50} values less than 5 µg/mL **Table 4**.

When tested as separate products, the methanolic extracts of *C. obtusifolia* and ethanolic extract of *H. suaveolens* gave the lowest IC_{50} values (0.64 for *C. obtusifolia* and 0.85 µg/mL for *H. suaveolens*) while the ethanolic extracts of *C. obtusifulia* gave the highest IC_{50} value of 4.18 µg/mL, with IC_{50} values of the methanolic extract of *C. obtusifolia* being lowest compared with those of the other two solvents extracts. When tested as a mixture, however, the methanol extract combination had IC_{50} value in **Table 4** that was slightly higher than those of the individual plant extracts were, suggesting the possibility of antagonism between components of the two different plants against the parasites. These notwithstanding, there were no statistically significant differences amongst IC_{50} values for all the extracts. The IC_{50} value for the standard control drug artesunate was 0.002 µg/mL, and this was significantly lower compared to IC_{50} values for all plant extracts tested **Table 4**.

TABLE 4: IC_{50} VALUES OF THE PLANT EXTRACTS ON THE 3D7 STRAIN OF *P. FALCIPARUM*

Treatment	IC_{50} (95% CI) µg/mL		
	Ethanol	Methanol	Aqueous
Cassia obtusifolia	4.18 (2.73-9.37)	0.64 (0.19-4.40)	2.22 (1.80-8.47)
Hyptis suaveolens	0.85(0.25-21.90)	1.33 (0.54-10.63)	1.31(0.58-19.16)
Combination[#]	1.98 (1.59-8.12)	1.85 (0.76-17.19)	2.21 (2.12-14.51)
Artesunate		0.002 (0.015-0.05)	

Data shown is the mean IC_{50} values with 95% confidence intervals of the two plants, their mixture and the reference drug, artesunate, using the three extraction methods. #:combination of equimolar quantities (by mass) of freeze-dried material from the two plants, originally extracted using the same solvent.

DISCUSSION: Medicinal plants, over centuries, have been used as sources of chemotherapeutic agents. Phytochemicals constitute an integral part of medicinal plants and are the source of their numerous bioactivities. Numerous plants containing a wide range of phytochemicals as their bioactive constituents have antiplasmodial activities [27]. The antimalarial drug quinine isolated from the bark of the *Cinchona* species (Rubiaceae) was the first drug used to treat malaria [28]. The use of plants as primary healthcare medicines in developing countries is still ongoing, although many of these countries have improved their healthcare systems over the decade. Furthermore, almost 80% of the populations in these countries still depend on plants and plant products to cure diseases, including malaria [27, 29]. In Ghana, malaria treatment is mainly by using traditional herbal medicines, even in urban and peri-urban centers [30, 31]. However, there is a paucity of data on the efficacy and safety of these medicinal plants. The study therefore aimed at evaluating and comparing the antiplasmodial activity of ethanol, methanol, and aqueous extracts of *C. obtusifolia* and *H. suaveolens*, two plants used to treat malaria in most locations of the northern part of Ghana. The antiplasmodial activity of the extracts was categorized using criterion [26] where ($IC_{50} \leq 5$ µg/mL) was considered highly active; ($5 < IC_{50} \leq 15$ µg /mL) moderately active; ($15 < IC_{50} \leq 50$ µg/mL)

weakly active and (IC_{50} > 50 µg/mL) inactive. Based on the classification above, all the extracts obtained from the various extract ants, ethanol, methanol, and aqueous of the two plants (*H. suaveolens* and *C. obtusifolia*), and also their mixtures were considered highly active with mean IC_{50} less than 5 µg/mL, against the chloroquine-sensitive strain 3D7 *P. falciparum*. The artesunate control was comparably highly active with IC_{50} of 0.002 µg/mL. *H. suaveolens* has been reported elsewhere to show good antiplasmodial activity both *in-vitro* and *in-vivo* [32, 33]. Ethanol and hydroethanol leaf extracts of *H. suaveolens* showed good antiplasmodial activity with an IC_{50}< 6.25 µg/mL on the chloroquine-resistant (Dd2) strain of *P. falciparum* [34]. In addition, petroleum ether extract of *H. suaveolens* has also shown a good antiplasmodial activity with an IC_{50} value of 2µg/mL [35]. Thus, our results from the methanol and ethanol extracts confirm the findings of other authors. The antiplasmodial activity shown by various extracts of *H. suaveolens* from our study and those from literature, could be credited to the various phytochemicals present. Consistent with our study findings, various extracts *of C. obtusifolia* have also been reported to have considerable antiplasmodial activity [36].

Additionally, ethanolic leave extract from *C. occidentalis* was very active with IC_{50} of 3 µg/mL [37]. Phytochemical test results indicated the presence of steroids, alkaloids, cardiac glycosides, glycosides, tannins, flavonoids, terpenoids, and anthraquinones in all three solvent extracts of *H. suaveolens* except for phenol which was absent from only the aqueous extract; saponins were absent in only the ethanol extract, and triterpenes were absent from the aqueous extract but present in the remaining extracts of *H. suaveolens*. The variations of these phytochemicals in extracts of the three different extractants could be the reason for the high IC_{50} value for the ethanol, followed by methanol extracts of *H. suaveolens*. Similarly, the ethanol, methanol, and aqueous extracts of *C. obtusifolia* contained steroids, alkaloids, flavonoids, cardiac glycoside, glycosides, tannins, phenolic compounds, terpenoids, and antraquinones; except for phenols that were also absent in the aqueous extract. The absence of phenols in the aqueous extracts of both plants could be because the distilled water used could not extract this compound properly. The absence of saponins in the ethanol extract of both plants and triterpenes in both the aqueous and ethanolic extracts of *C. obtusifolia* could have accounted for the differences in the IC_{50} values observed for the extracts from the three different extractants. It was also observed that the methanolic extracts of both plants contained all the phytochemicals tested and possibly afforded its low IC_{50} value with strong antiplasmodial activity comparable to that of the aqueous extract. Nafiu *et al.* [38] implicated alkaloids, phenols, tannins, saponins, and anthraquinones in the antimalarial activity of *Lecaniodiscus cupanioides*. In a review evaluating the potentials of antimalarial compounds derived from African medicinal plants from 2013 to 2019, Bekono *et al.* [39] reported that naphthoisoquinoline alkaloids isolated from the leaves of *Ancistrocladus sp.* displayed significant inhibitory activities against the NF54 strain of *P. falciparum* with IC_{50} values between 0.043 and 0.055 µM. Furthermore, aporphine alkaloids identified from the leaves of *Annickiakummeriae* (Annonaceae) exhibited antiplasmodial activities against the CQ-resistant K1 strain of *P. falciparum* [39].

The authors also deduced that oleanane triterpenes isolated from the leaves of *Ekebergia capensis* potently inhibited the D6 and W2 strains of *P. falciparum* with relatively low IC_{50} values. Douanla *et al.* [40] isolated novel steroids along with known steroids from the stem bark of *Antrocaryonklaineanum (*Anacardiaceae). Upon evaluation of the crude extracts and the isolated compounds in *vitro* against the 3D7 and W2 strains of *P. falciparum*, the crude extracts displayed moderate activity against 3D7 whereas the steroids exhibited strong activity against both strains. Simelane *et al.* [41] recounted isolation of known ursolic acid and oleanolic acid triterpenoids from the twigs of *Keetialeucantha* (Rubiaceae). The authors deduced that the compounds had *in vitro* activities on the 3D7 strain of *P. falciparum* with IC_{50} values between 32.4 and 59.4 µM. Ellagic acid obtained from the stem bark of *Terminalia brownii* (Combretaceae) to be active against chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum* with IC_{50} value against both strains comparable to 8.01 µM [42]. Additionally, Tshitenge *et al.* [43] identified two phenolic glycosides as antimalarial. The differences

observed between the IC_{50} values obtained for the current work and that of published data could be due to the extractants used, the process by which the plant materials were collected, the nutrients in the soil at the time, the geographical area where the plant was collected, the season, and the vegetative stage of the plant [44] which might have influenced the activities of the extracts.

CONCLUSIONS: The current data of leaf extracts of the two medicinal plants and their combination demonstrated good antiplasmodial activity comparable to the reference drug, artesunate. There was a dose-dependent inhibition of parasites and lower IC_{50} values comparable to the reference drug, artesunate.

The IC_{50} values and percentage of parasite inhibition of the decoction made from the two plants did not differ from the individual plants. The results provide scientific reasoning for using *Hyptis suaveolens* and *Cassia obtusifolia* leaves in traditional medicine as antimalarial agents. However, an *in-vivo* efficacy and safety study in experimental animals is warranted to confidently extrapolate the findings into humans.

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Data Availability: The corresponding author can obtain the data upon reasonable request.

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Author Contributions: AMD, TA conceived and designed the study; TA collected the plant samples; TA, EKB cultured parasites and performed the experiments; AMD, KAK, EKB, TA performed statistical/data analysis; KAK, AMD, EKB, TA, MND and Bawrote paper.

CONFLICTS OF INTEREST: The authors declare that they have no competing interests.

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