Scientific African 15 (2022) e01103

Contents lists available at ScienceDirect

Scientific African

journal homepage: www.elsevier.com/locate/sciaf

# Assessment of the activities of *Chasmanthera dependens* Hochst. combined with other plants on chloroquine-sensitive and chloroquine-resistant *Plasmodium berghei*

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ARTICLE INFO

*Article history:*
Received 12 March 2021
Revised 26 October 2021
Accepted 25 January 2022

Editor: DR B Gyampoh

*Keywords:*
Repeated-dose toxicity
Herb-drug interaction
Chloroquine
*Vernonia amygdalina*
*Sphenocentrum jollyanum*

ABSTRACT

The prevalence of *Plasmodium* strains resistant to conventional monotherapy drugs has necessitated the need for more effective and affordable antimalarial agents. This study aimed at evaluating the antimalarial activities of some indigenous plants alone and in combination with chloroquine with a view to proffering herbal combination therapy as an alternative to the currently used antimalarial combination therapy. Extracts obtained separately from the decoctions of the roots of *Chasmanthera dependens* Hochst. (Menispermaceae), *Vernonia amygdalina* Delile (Asteraceae) and methanol extract of *Sphenocentrum jollyanum* Pierre (Menispermaceae) leaf were analysed by high resolution liquid chromatography-mass spectrometry and evaluated for repeated-dose oral toxicity in mice using standard protocols. Assessments of *C. dependens* and *V. amygdalina* with their combination were carried out on chloroquine-sensitive *Plasmodium*-infected mice with the 4-day chemosuppressive antimalarial test. *Sphenocentrum jollyanum, C. dependens* and their combinations with chloroquine (10 mg/kg) were assessed on chloroquine-resistant *Plasmodium* using the curative test model. The combination of *C. dependens* and *V. amygdalina* exhibited chemosuppression (71.1±3.7%) that was not significantly ($p>0.05$) different from chloroquine (80.1±1.9%). In the curative model, a significant parasite clearance (98.2%) was observed with the combination of *C. dependens* and chloroquine. The study concluded that the aqueous root extracts possessed potent antimalarial activity in chloroquine-sensitive malaria infection. It also revealed an additive interaction between *C. dependens* and chloroquine that could be further investigated as a herbal combination for chloroquine-resistant malaria.



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https://doi.org/10.1016/j.sciaf.2022.e01103

## Introduction

Malaria, endemic to Nigeria and other tropical countries, is caused by *Plasmodium* species and transmitted by the female *Anopheles* mosquito. Many plants have been found to be particularly of use in the chemotherapy of diseases and it is usual for local populations to utilise indigenous medicinal flora to treat ailments prevalent in that community [1]. *Chasmanthera dependens* stem bark and *Vernonia amgdalina* root were separately scientifically evaluated for antimalarial activity [2, 3]. Ethnomedicinally, a decoction of the mixture of the fresh roots of *C. dependens* and *V. amygdalina* is used in the treatment of malaria [4]. However, this traditional use is yet to be scientifically validated. *Sphenocentrum jollyanum* was pharmacologically verified for its antimalarial activity with the leaf having higher activity than the root, contrary to traditional claim [5]. However, the antimalarial activity of its combination with *C. dependens,* a plant in the plant family, has not been investigated. A number of indigenous plants has been scientifically validated especially on chloroquine-sensitive *P. berghei* but few reports on the justification of indigenous plants on chloroquine-resistant malaria [6]. Furthermore, some communities use medicinal plants with orthodox drugs in order to achieve enhanced activity and this has been scientifically validated [7, 8]. Therefore, this study investigated the antimalarial activity of the single and combined extracts of *C. dependens* and *V. amygdalina* in chloroquine-sensitive *Plasmodium*, single and combination of *C. dependens* and *S. jollyanum* extracts as well as possible potentiation with chloroquine in chloroquine-resistant *P. berghei*-infected mice.

## Methods

### *Plant collection*

The roots of *Chasmanthera dependens* Hochst (Menispermaceae) and *Vernonia amygdalina* Delile (Asteraceae) as well as the leaf of *Sphenocentrum jollyanum* Pierre (Menispermaceae) were collected in October 2015 on the campus of Obafemi Awolowo University (OAU), Ile-Ife. The plants were authenticated by Mr. I. I. Ogunlowo of the Faculty of Pharmacy Herbarium, OAU, and voucher specimens deposited with numbers: FPI 2070, FPI 2074 and FPI 2075, respectively. All the plant names were checked on www.theplantlist.org.

### *Extraction procedure*

Fresh roots of *V. amygdalina* and *C. dependens* (50 g) were cut into small pieces separately and extracted in water (0.35 L) in a decoction method. In addition, a combination (1:1) of the two plants (70 g each; 0.54 L water) was also prepared as a decoction and freeze-dried. *S. jollyanum* leaf (100 g) was macerated in methanol (2 $\times$ 0.8 L) for 48h and concentrated to give a yield of 8.0% (herbal drug extract ratio, 12.5:1).

The percentage yields of *V. amygdalina, C. dependens*, and their combination were 10.01, 5.82 and 4.48% corresponding to the herbal drug extract ratios of 10.1, 17.2 and 6.3, respectively.

### *Experimental animals*

Healthy albino mice of both sexes (mean weight, 20.5g) were kept in a well-ventilated environment and observed under 12 h light/dark cycles in standard cages in the animal facility of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. They were acclimatized for a period of two weeks and maintained on standard pellets (Capsfeed Limited, Osogbo, Nigeria) with free access to clean drinking water. The guidelines of the National Institutes of Health for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) were followed.

### *Repeated-dose toxicity study*

In the first phase, the single oral doses (400 mg/kg) of *C. dependens, V. amygdalina, S. jollyanum* and combinations of *C. dependens* with *V. amygdalina* and *C. dependens* with *S. jollyanum* were administered to healthy mice for five consecutive days while mice in the control group were given tween 80 (2.5%, 0.2 mL). In the second phase, only *C. dependens* extract (100 and 200 mg/kg) was given to the mice for five days. Signs of toxicity and any mortality were recorded. After the 5$^{th}$ day, blood was collected from the mice *via* cardiac puncture for analysis of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using standard protocols [9].

### HPLC-MS analysis of C. dependens, V. amygdalina *and* S. jollyanum

About 2mg of each extract was dissolved in methanol (1 mL) and centrifuged for 5 min (6600 rpm, 2200 g) before LC-MS analysis. High-resolution mass spectra (ESI-HRMS) were acquired on a LTQ Orbitrap spectrometer (Thermo Scientific, USA) equipped with a HESI-II source. The spectrometer was equipped with an Agilent1200 HPLC system (Santa Clara, USA) including pump, PDA detector, column oven (30°C) and auto-sampler (injection volume: 6$\mu$L). The spectrometer was operated in positive mode with a nominal mass resolving power of 60,000 at m/z 400 and a scan rate of 1 Hz using the following parameters: spray voltage 6 kV, capillary temperature 300°C, tube lens 100 V. Argon served as collision gas and $N_2$ was used as sheath gas (66 arbitrary units) and auxiliary gas [8 arbitrary units] [10].

*Plasmodium parasites*

Chloroquine-sensitive *Plasmodium berghei* NK 65 and chloroquine-resistant *P. berghei* ANKA strains were obtained from the Nigerian Institute of Medical Research, Lagos, and from the Institute of Advanced Medical Research and Training, Ibadan, respectively. The parasites were maintained by serial passage in albino mice. Each experimental mouse was injected via the peritoneum with 200 µL of blood suspension containing $1 \times 10^7$ *P. berghei* parasitized red blood cells.

*Antimalarial assays*

Evaluation of the chemosuppressive activity in chloroquine-sensitive *P. berghei* infection

About four hrs after inoculation, eight groups of chloroquine-sensitive *P. berghei*-infected mice were orally given separate doses of *C. dependens* and *V. amygdalina* (100, 200, 400 mg/kg/day), respectively, as well as chloroquine diphosphate solution (CQ base, 10mg/kg/day, Sigma, US) and tween 80 (200µL), respectively, as the positive and negative controls, for four days [11].

Twenty-four hours after the last drug administration, a thin smear was made from the tail blood of each mouse. In addition, the combination (1:1) of *C. dependens* with *V. amygdalina* (100, 200, 400 mg/kg) was assessed every 12h for four days as earlier described.

*Evaluation of curative activity in chloroquine-resistant Plasmodium berghei-infection.* Treatment of nine groups of chloroquine-resistant *P. berghei*-infected mice was initiated 72 h after inoculation [12]. The mice were given oral doses of *S. jollyanum* (200 mg/kg), *C. dependens* (100 mg/kg) and the combinations of *S. jollyanum* (200 mg/kg) with *C. dependens* (25, 50 and 100 mg/kg each, every 12h) for five days. In addition, the plant combinations with chloroquine namely: *S. jollyanum* (200 mg/kg) with chloroquine (10 mg/kg) and *C. dependens* (50 mg/kg, every 12h) with chloroquine, were similarly evaluated.

After each daily drug administration, a thin film blood smear was prepared from each mouse, fixed with two drops of methanol and stained with giemsa (12.5%). Microscopic assessment ($\times$ 1000) of each blood smear for mean parasitaemia in ten different fields of view was carried out.

*Determination of the mean survival time*

The animals were observed for 28 days (from start of drug administration) and the mean survival was determined as the mean of the number of days each mouse survived in each group.

*Analysis of the result*

The mean parasitaemia was determined by calculating the ratio of parasitized RBC to the total number of RBC in percentage while the mean chemosuppression/parasite clearance (in %) was calculated as 100 x [A-B)/A], where A and B represent the mean parasitaemia of the negative control and test groups, respectively. Data was analysed and prepared with Microsoft® Office Excel (2010).

## Results

*Repeated-dose toxicity test*

In the repeated-dose toxicity study, two out of the five *C. dependens* (400 mg/kg)-treated mice died by the 5$^{th}$ day. Therefore, the dose was reduced to 100 and 200 mg/kg, which did not cause mortality in any of the experimental mice. There was no significant difference ($p>0.05$) in the ALT levels compared to the control while the AST values for *C. dependens* (100 and 200 mg/kg) was significantly lower than that of the control (Table 1).

*LC-MS analysis*

The fingerprints of the extracts of *C. dependens, V. amygdalina* and *S. jollyanum* were as shown in Fig 1. Six prominent peaks were seen in the profile of the *S. jollyanum* extract with retention times (mins) and molecular ion [M+, m/z] as follow: 12.23 [197.1172], 13.51 [352.1542], 14.97 [445.2951 & 481.3963], 15.33 [463.3056], 16.72 [495.3321] and 17.47 min [505.3165]. Two prominent peaks were observed in *C. dependens* at 8.59 [242.1752] and 12.05 [163.0496]. However, only one prominent peak at 10.84 min [223.0602] was obtained in the *V. amygdalina* extract.

*Antimalarial assays*

*Evaluation of chemosuppressive activity*

The chemosuppressive activities of *C. dependens* (50-200 mg/kg) and *V. amygdalina* (100-400 mg/kg) were between 17.3-60.0% and 56.6-58.5 %, respectively, while that of the combination was between 63.0-71.1%. Their activities were significantly ($p<0.05$) less than the activity of chloroquine (82.9%) [Table 2].

**Table 1**
Alanine and aspartate aminotransferase values of plasma from *S. jollyanum, V. amygdalina* and *C. dependens*-treated mice

| Extract (mg/kg) | ALT (mg/dl) | AST (mg/dl) |
|---|---|---|
| Tween 80 | 0.61± 0.00[a] | 483.86±54.70[b] |
| S 400 | 0.68±0.01[a] | 570.70±110.14[b] |
| C100 | 0.66±0.04[a] | 92.98±11.71[c] |
| C200 | 0.71±0.02[a] | 144.56±48.50[c] |
| C400 | 0.69±0.02[a] | 631.75±103.27[b] |
| V400 | 0.63±0.004 [a] | 562.98±124.67[b] |
| V+C (400) | 0.63±0.03 [a] | 292.11±21.96[b] |
| S+C (400) | 0.71±0.02[a] | 503.43±362.90[b] |

**Key**: S= *S. jollyanum* extract; C = *C. dependens* extract; V = *Vernonia amygdalina* extract. Values with the same superscript letters indicate the doses are not significantly different ($p>0.05$) from each other within a column only while different superscript letters are significantly different ($p<0.05$) from each other within the column.

**Table 2**
Antimalarial activity and mean survival values of the mice treated with *Chasmanthera dependens* and *Vernonia amygdalina* roots and their combinations using the chemosuppressive model

| Extract/Dose (mg/kg) | Parasitaemia (%±SEM) | Chemosuppression (%±SEM) | Mean survival (Days±SEM) |
|---|---|---|---|
| C50 | 7.90±0.91[a] | 17.27±9.58 [c] | 18.75±3.20 [e] |
| C100 | 4.30±0.42 [a] | 54.99±4.38 [c] | 17.20±0.86 [e] |
| C200 | 3.84±0.51 [b] | 59.74±5.36 [c] | 17.00±3.16 [e] |
| V100 | 4.15±0.32 [a] | 56.55±3.34 [c] | 19.50±1.19 [e] |
| V200 | 4.13±0.38 [a] | 56.69±3.99 [c] | 20.50±1.44 [e] |
| V400 | 3.96±0.68 [b] | 58.47±7.17 [c] | 15.60±2.69 [e] |
| V+C-100 | 3.34±0.54 [b] | 64.99±4.23 [c] | 14.26±1.69 [e] |
| V+C-200 | 3.53±0.70 [b] | 63.00±5.55 [c] | 14.43±1.94 [e] |
| V+C-400 | 2.76±0.47 [b] | 71.07±3.69 [d] | 14.60±1.47 [e] |
| Tween 80 | 9.54±0.99 [a] | 0.00±0.00 [c] | 13.20±3.37 [e] |
| CQ-10 | 1.63±0.13 [b] | 82.90±1.35 [d] | 22.00±6.00 [e] |

Key: SEM- Standard error of the mean; C = *Chasmanthera dependens;* V=*Vernonia amygdalina* V+C= combination of *Vernonia amygdalina* and *Chasmanthera dependens*; CQ-Chloroquine (10 mg/kg); Values with the same superscript letters indicate the doses are not significantly different ($p>0.05$) from each other within a column only while different superscript letters are significantly different ($p<0.05$) from each other within the column.

## Evaluation of the curative treatment

The parasite clearance values of the combinations of *C. dependens* (25, 50 and 100 mg/kg) with *S. jollyanum* (200 mg/kg) reduced significantly from 44.4-49.6% on Day 1 to 0.0% on Day 5 of treatment while *S. jollyanum* (200 mg/kg) with chloroquine (10 mg/kg) gave curative activity from 39.8 to 90.7 % which was not significantly ($p>0.05$) different from the activity of chloroquine (Fig. 2). Furthermore, the combination of *C. dependens* with chloroquine effected a significant parasite clearance of 98.2±0.39 % that was significantly ($p<0.05$) higher than the activity of chloroquine alone (85.78±4.34%).

## Mean survival time of *P. berghei*- infected mice using the curative test

The survival of the chloroquine-sensitive *P. berghei*-infected mice, given both the single and combined extracts of *C. dependens* and *V. amygdalina,* were not dose-dependent using the chemosuppression model. The survival times were between 15.60±2.69 and 20.50±1.44 days while the chloroquine-treated mice lived for 22.00±3.00 days (Table 2).

In the curative tests, mice treated with the combinations of *S. jollyanum* with CQ and *C. dependens* with CQ survived comparably with ($p>0.05$) chloroquine while other mice survived for significantly less than the chloroquine-treated mice (Fig 3).

## Discussion

The priority in herbal medicinal research is the assessment of safety before the efficacy of the products is established. It is common in indigenous communities to find plants used in malaria therapy in the form of decoctions or infusions without any formal knowledge on the possible toxicity of such preparations. Studies previously carried out on these plants revealed no acute toxicity [13–15]. In this study, the repeated-dose toxicity study was carried out for five days to simulate

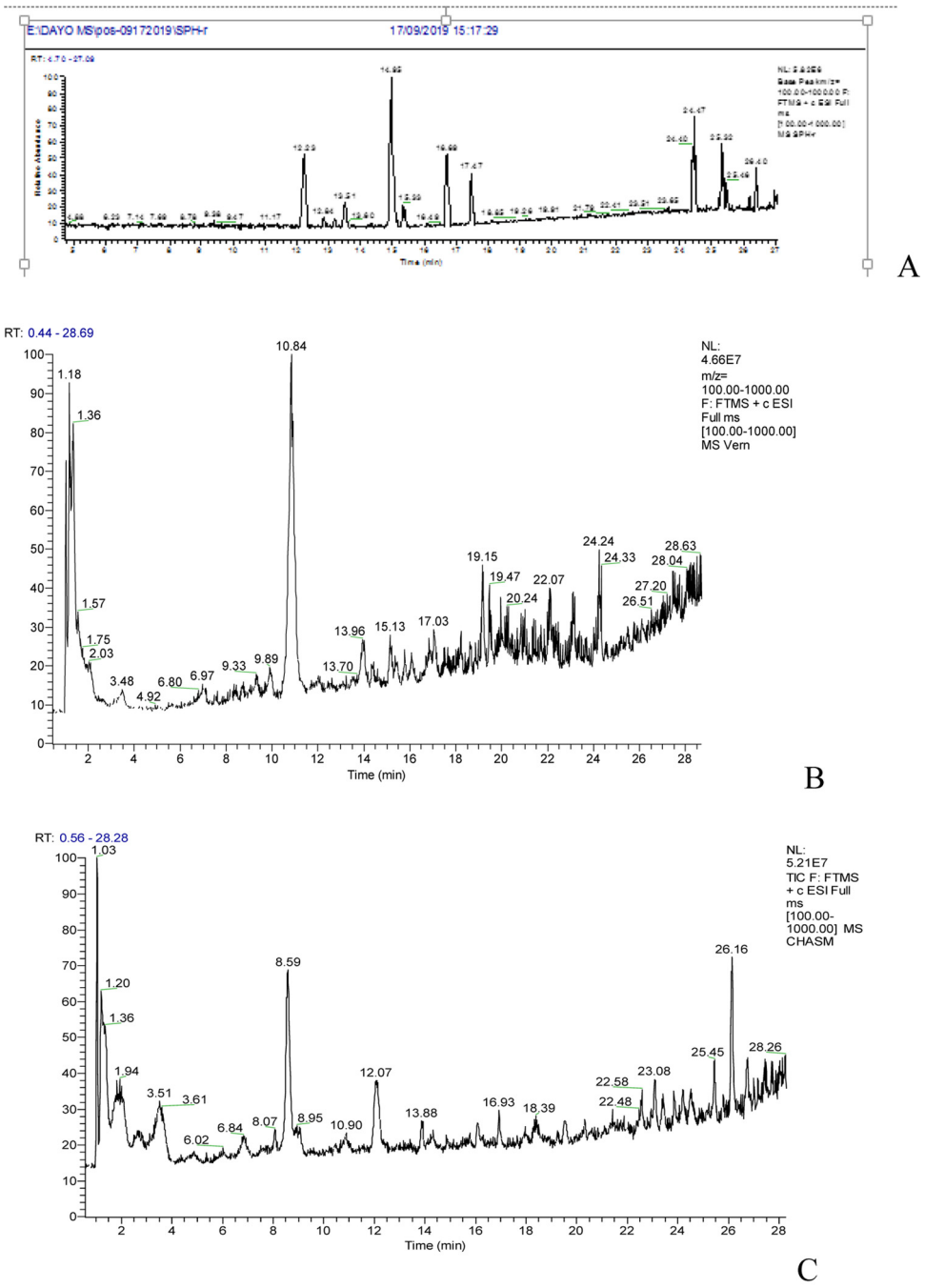


**Fig 1.** LC-MS fingerprints of *Sphenocentrum jollyanum* (A), *Vernonia amygdalina* (B) and *Chasmanthera dependens* (C) extracts

the duration of drug administration in the curative antimalarial rodent model. No sign of toxicity was observed in the mice treated with extracts of *S. jollyanum* and *V. amygdalina*. However, mortality was observed in the mice given the highest dose (400mg/kg) of *C. dependens* and this necessitated a reduction of the dose of *C. dependens* for the antimalarial investigations. The levels of the liver marker enzymes were not significantly altered so it is possible that the mortality observed may not be a result of hepatic injury. Consequently, the detailed safety assessment of *C. dependens* would need to be carried out.

In this study, the LC-MS fingerprint of each of the extracts revealed characteristic peaks. In medicinal plant research, the establishment of the identity, authenticity and consistency of plant (s) is of primary importance. Apart from the sensory and microscopical inspection of plants, analytical techniques such as chromatographic analysis is incorporated in the quality control of medicinal plants [16]. Therefore, chromatographic fingerprinting helps to identify the single herbs added

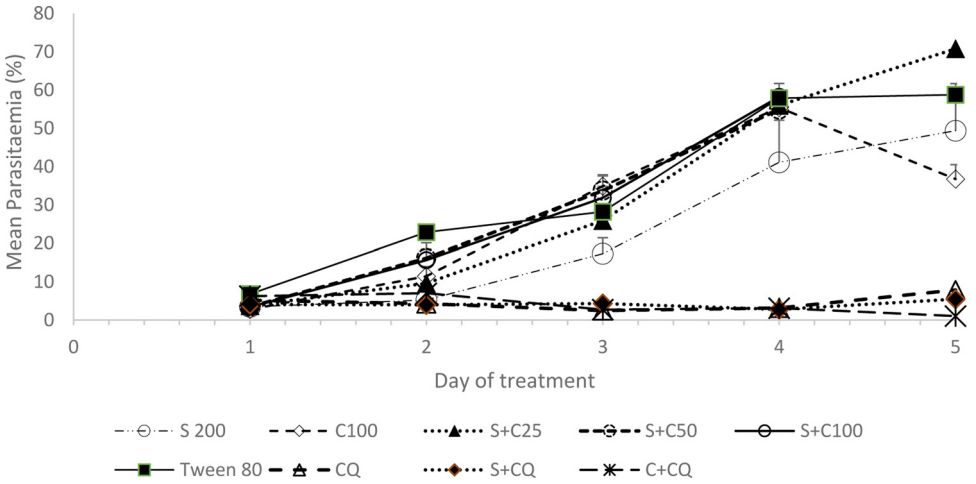


**Fig 2.** Effects of *S. jollyanum* and *C. dependens* on chloroquine-resistant *P. berghei* ANKA using the curative antimalarial model

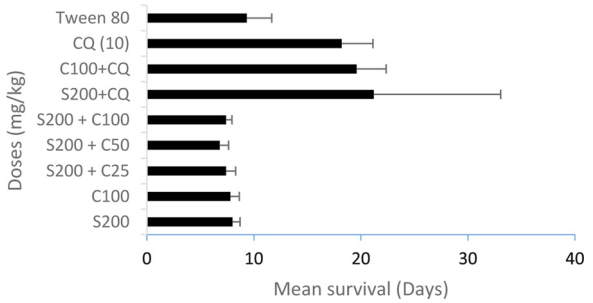


**Fig 3.** Mean survival time of chloroquine-resistant *Plasmodium berghei*-infected mice treated with *Sphenocentrum jollyanum, Chasmanthera dependens*, chloroquine and their combinations using the curative test model
* S= Sphenocentrum jollyanum; C= Chasmanthera dependens, CQ - chloroquine

together as a herbal combination. Although none of the peaks corresponded to previously-reported compounds, nonetheless, each profile afforded a pattern for the identity of the extract under the conditions stated. In ethnomedicine, a decoction of freshly-chopped root of *C. dependens* with the root of *Vernonia* spp. is usually taken to treat malaria [4]. In the present study, it was observed that the two plants exhibited considerable antimalarial activity when evaluated separately, which was in agreement with earlier reports [2, 3]. The combination of the two plants, also exhibited significant activity (71 %) which, therefore, justified their ethnomedicinal use. *C. dependens* possessed analgesic, anti-inflammatory, anti-ulcerogenic, aphrodisiac and fertility-enhancing activities [17–20]. The stem bark of the plant possessed furanoditerpenes: columbin, hydroxycolumbin, and alkaloids such as glaucine, tetrahydropalmatine, jatrorrhizine and govanine, all of which do not have masses that correspond to any of the peaks identified in this study [21–23]. Berberine, reported to be the major constituent of the root [24] neither detected by Ohiri and co-workers [20] not was its peak detected in this study.

The aqueous root extract of *V. amgdalina* showed moderate chemosuppressive antimalarial activity against the chloroquine-sensitive *P. berghei*. In mice, the root exhibited analgesic and antipyretic activities [15]. In a clinical trial in Uganda, the aqueous infusion of the leaf reduced parasitaemia, although with recrudescence in humans with *P. falciparum* infection [25]. The only prominent peak identified in the fingerprint of *V. amygdalina* root appeared as a moderately polar major chemical constituent of the extract. Anthraquinone glycosides such as emodin, physcion, erythroglaucin and stigmastane derivatives such as vernoniosides, vernoniacums, vernoguinosides have been reported from the root of *Vernonia* spp. [26–29].

In this study, *S. jollyanum* lacked significant activity against chloroquine-resistant *Plasmodium* parasites in contrast to an earlier report [5]. In that study, the methanol extract (200 mg/kg) of the leaf exhibited inhibition (75 %) contrary to what was obtained in this study (15 %). This variation may be due to different *Plasmodium* strains or geographical variation in plant collection or other experimental conditions. *S. jollyanum* possessed alkaloids such as palmatine, jatrorrhizine, tetrahydrojatrorrhizine, columbamine and sterols such as sitosterol, campestrol, stigmasterol as well as the furanoditerpenes: columbin, isocolumbin and fibleucin, isolated from the fruit [30]. Ecdysteroids, with antiulcer activity, were reported from the seeds while ecdysteroidal glycosides were reported from the root [31, 32].

Contrary to our hypothesis, there was no improved activity with the combination of *Chasmanthera* and *Sphenocentrum* compared to the activity of each plant. It was thought that the combination of the two Menispermaceae plants might reveal a potentiating antimalarial activity such as was observed with the stem bark of *Alstonia boonei* and seed of *Picralima nitida*, both of the Apocynaceae family. Their combination resulted into a higher antimalarial activity suggestive of synergism of similar chemical constituents in both plants [33]. Interestingly, the present study demonstrated an additive interaction between the herb-drug combination of *C. dependens* and chloroquine in mice infected with chloroquine-resistant *P. berghei*. The additive effect implies that the combination would be more beneficial in treating CQ-resistant malaria than a monotherapy of either agent.

The failure of antimalarials in many communities has generated public health concerns necessitating more exploration of plants or optimising those with existing antimalarial activity when used alone or in combination with orthodox medicines. Similar studies had been earlier reported with *Curcuma longa, Morinda lucida* and *Uvaria chamae* [8, 34]. As the field of herb-drug interaction continues to emerge to provide an alternative to the presently recommended artemisinin-combination therapy, it is believed that an effective and affordable remedy will soon be available in malaria-endemic regions of Africa and the world.

## Conclusion

The study reports the activity of *C. dependens* root on chloroquine-resistant *Plasmodium* strain for the first time. The study also revealed an additive antimalarial activity of *C. dependens* with chloroquine. Further studies may be needed to understand the mechanism of action of the interaction.

## Author contribution

AOA conceived, supervised the study and drafted the first manuscript; T-TR, IDO and KFA were involved in the bench work and data analysis. All authors contributed to the writing of the manuscript and all approved the final version.

## Declaration of Competing Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Acknowledgement

AOA is grateful to Prof M. Spiteller, INFU, Technical University, Dortmund, Germany, for access to the HPLC-HRMS facility.

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