

Comparative antiplasmodial activities of the leaf extracts of *Parquetina nigrescens* and *Spathodea campanulata* in mice

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Abstract

Comparative antimalarial activity studies can complement the selection of ethnomedicinal plants for antimalarial drug development. The leaves of antimalarial ethnomedicinal *Spathodea campanulata* (SC) and *Parquetina nigrescence* (PN) were therefore chosen for comparison of their *in vivo* antimalarial activities against chloroquine-sensitive *Plasmodium berghei berghei* in mice using prophylactic, chemosuppressive and curative models. The methanol extracts of their separately air-dried and powdered leaves were obtained after cold maceration and evaporation *in vacuo*. Also, before both were investigated at 0-800 mg/kg, their preliminary toxicities were assessed using Lorke's method. Normal saline and pyrimethamine (1.2 mg/kg) or chloroquine (10 mg/kg) were negative and positive controls, respectively. All the results were subjected to statistical analysis using ANOVA with Student Newman Keul's *post hoc* test. Though both extracts were non-toxic at less than 5000 mg/kg, SC gave a significantly ($p < 0.05$) lower ED₉₀ (527.10 ± 15.27 mg/kg) in the prophylactic and PN, a significantly ($p < 0.05$) lower ED₉₀ (430.09 ± 5.64 , mg/kg) in the chemosuppressive and 582.38 ± 63.99 mg/kg in the curative model. The comparable ($p > 0.05$) survival times elicited by SC and PN at 200mg/kg and 800mg/kg respectively, to the positive control, after a 28-day observation for mortality confirmed SC as a better prophylactic, while PN at 800mg/kg confirmed chemosuppressive activities. Also, PN with lower effective doses, good survival times and survivor profile were better chemosuppressive and curative. The study concluded that *S. campanulata* is a better prophylactic and *P. nigrescens* also a better chemosuppressive and curative drug.

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1. Introduction

The central role of medicinal plants, among more than 12000 sources of natural remedies, in ameliorating several kinds of ailments since the existence of man is well known [1]. Medicinal plants which are prepared in crude forms as poultices, tinctures, decoctions, infusions, powders, teas and herbs, either singly or in combinations to treat various kinds of diseases, are also used as food, diets and spices also in perfumery [2-4]. In most countries in the world, traditional medicine has been integra-

ted into the health care delivery system for almost over 2500 years ago [5], with more than half of their population depending primarily on it. About 80 % of the African population uses plant based traditional medicine for their health care needs basically as a result of affordability and accessibility, especially for people in rural areas [6-8]. Also, the same traditional medicine, which has been the focus of a wider coverage of primary health care delivery around the world [9], has produced remedies employed in TM.



These are also included in the diet as recuperating items from certain ailments and diseases in Africa [10, 11]. Many industrialized civilizations such as India and China, use modern medicine, sometimes also obtained from plants, as a compliment to traditional practices [12]. Ethnomedicinal applications of plants have become a useful guide to the scientific evaluation of plants for the last 100 years [13]. However, out of about 250,000 plant species growing all over the world, only 1% have been thoroughly investigated for their pharmacological properties and potential chemotherapeutic values [14]. Some active principles such as, alkaloids, terpenoids, flavonoids, tannins, saponins, carotenoids, glycosides have been characterised in plants in order to justify their ethno-medicinal uses and claims [13, 15]. Medicinal plants have played an important role in curbing malarial disease, being the major documented and available option for its treatment in the world since ancient time [16]. This is because plants contain natural antiparasitic chemicals, which, if isolated, can be used in the management of resistant malaria, for which available orthodox antimalarial drugs are becoming ineffective.

The documented examples of plants investigated for antimalarial or antiparasitic activities encourage further attempts at the same for others yet to be investigated. The leaf and stem bark of *Spathodea campanulata* have been used in Ghana to treat malaria [17] while [18] reported the antimalarial activity of the chromatographic fractions of the leaf extract of *Parquetina nigrescence*. However, these two plants have never been compared in a full investigation of antimalarial activities using all models of testing. Moreover, this study can complement the usual arbitrary choice of ethnomedicinal plants for antimalarial drug development, hence this study.

2. Materials and methods

2.1 Material and equipment

Grinding machine, macerating flasks, round bottom flasks, glass funnels, rotary evaporator, oral cannula, digital thermometer, binocular light microscope, cotton wool, measuring cylinders, measuring scales, beakers, retort stand, spatula, heparinized bottles, dissecting set, glass slides, sterile disposable syringes (5.0 mL, 10 mL), and needles, petri dishes, Giemsa

stain, markers, paper tape, gloves, test-tubes, test-tube rack, aluminum foil, aluminum cages, feeding and water troughs, Albino mice.

2.2 Solvents and liquids

methanol, distilled water, normal saline, immersion oil, tween 80. Test drugs: chloroquine (10 mg/kg) and pyrimethamine (1.2 mg/kg).

2.3 Plant collection and authentication

The leaves of *Spathodea campanulata* and *Parquetina nigrescens* were collected from the premises of Oduduwa Hall near Hezekiah Oluwasanmi Library and the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife respectively. The leaves of both plants were identified and authenticated at the Faculty of Pharmacy Herbarium, Ife by Mr. I. I. Ogunlowo of the Pharmacognosy Department, Obafemi Awolowo University, Ile-Ife. Voucher specimen numbers, FPI2318 and FPI2317 were allocated, respectively for the leaf samples of *S. campanulata* and *P. nigrescens* deposited.

2.3.1 Plant material and preparation of plant extracts

The leaves were separately air-dried in the screen house of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife and powdered. About 600g each of the powdered plant materials were macerated separately in 2L methanol for 72 hours with intermittent shaking. The resultant extracts were filtered and concentrated *in vacuo* using the rotary evaporator. The percentage yields were calculated using the formula; $(100 \times W_1)/W_2$; where W_1 is the weight of dried extract; W_2 is the weight of plant material.

2.3.2 LD₅₀ Determination

Acute toxicity tests were carried out on the methanol extracts of *S. campanulata* and *P. nigrescens* using Lorke's method [19, 20]. These were carried out in two phases. Phase 1 requires nine animals; these animals are grouped into three (3) of three animals each. Each group of animals were administered with different doses (10, 100 and 1000 mg/kg) of the test extracts. The animals were observed for mortality for a period of 24 hours. Phase 2 requires three animals grouped into three (3) of one animal each, they were administered higher doses of 1600, 2900 and 5000 mg/kg body weight of the test extracts, equally observed for 24 hours for mortality [19].

The LD₅₀ was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality D₁₀₀ = Lowest dose that produced mortality.

2.3.3 Preparation of test extracts and standard drugs

The methanol extracts were prepared in doses 100, 200, 400 and 800 mg/ kg, which were obtained by dissolving 50, 100, 200 and 400 mg/ kg of the dried extract in 5.0 mL of normal saline respectively. Normal saline was administered to the negative control group while chloroquine (10 mg/kg) and pyrimethamine (1.2 mg/kg) were administered to the positive control groups as appropriate for each model.

2.4 Animals

A total of sixty (60) healthy, seven weeks old Swiss mice of either sex with weight ranging between 18 and 22g were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. They were randomly divided into groups of five animals each, then housed in aluminum cages with wood shavings as bedding and allowed free access to food and water *ad libitum*. They were allowed to acclimatize for a period of five to seven days before the experiment commenced.

2.4.1 Rodent parasite

Chloroquine sensitive *Plasmodium berghei berghei* NK65 with rising parasitaemia was obtained from the Institute of Advanced Medical Research and Training (IMRAT), University College Hospital, Ibadan. It was maintained by serial passaging in mice with close monitoring of the parasitaemia level. The parasitized animal (30% parasitaemia) was anaesthetized with dichloromethane. Blood was withdrawn by cardiac puncture into heparinised bottle and diluted with normal saline to contain 1×10^7 parasitized red blood cells in 0.2 mL of inoculum.

2.4.2 Inoculation of mice

Each of the mice was intraperitoneally inoculated with 0.2 mL of the diluted blood containing 1×10^7 infected red blood cells for the antimalarial test in all the three models used.

2.5 Antimalarial activities of the test extracts

For the purpose of this study, the three models of the antimalarial study were used to test the activities of

the extracts of leaves of *Spathodea campanulata* and *Parquetina nigrescens* on chloroquine-sensitive *Plasmodium berghei berghei* NK65 strain separately. The prophylactic, chemosuppressive and curative antimalarial models were carried out on both plant extracts using standard procedures [18].

2.5.1 Prophylactic anti-malarial test model

The prophylactic activities of the test extracts were carried out in acclimatized mice as follows: Thirty (30) mice were arranged into six groups (I-VI) of five animals each. Group I-IV were administered orally with the methanol extract of *S. campanulata* at the selected doses of 100, 200, 400 and 800 mg/kg respectively for three consecutive days. Groups V and VI received normal saline and 1.2 mg/kg Pyrimethamine, to serve as negative and positive controls respectively. On the fourth day, each mouse received an intraperitoneal inoculation of 0.2 mL diluted blood containing 1×10^7 of *Plasmodium berghei berghei* parasitized red blood cells. The rectal temperature of each mouse was recorded daily and the blood smear was taken on the sixth day (72 hours after inoculation). The blood smear was fixed with methanol, stained with Giemsa and dried. The same procedure was carried out on similar doses of the methanol leaf extract of *P. nigrescens*.

2.5.2 Chemosuppressive antimalarial test model

The chemosuppressive test was carried out using the method described by Arya *et al.*, 2022. Thirty (30) acclimatized animals were divided into six groups (I-VI) of five animals each and inoculated with 0.2ml of diluted blood containing 1×10^7 of *Plasmodium berghei berghei* parasitized red blood cells. Two hours post inoculation, treatment of the test animals was initiated by oral administration of the test extracts of *Spathodea campanulata* at selected doses of 100, 200, 400 and 800 mg/kg to Groups I-IV while groups V and VI were administered with normal saline and chloroquine, 10mg/kg to serve as negative and positive controls respectively. The administration of drugs was done for four consecutive days after recording their rectal temperature daily. The blood smear was taken on the fifth day by withdrawing blood from the tail of each animal, using it to make a thin smear, fixing with methanol and staining with Giemsa to determine their parasitaemia level. The same procedure was carried out on the methanol leaf

extract of *P. nigrescens*.

2.5.3 Curative antimalarial test models

The curative antimalarial test was carried out using the method described by Arya *et al.*, 2022. Thirty mice were divided into six groups (I-VI) of five animals each and they were intraperitoneally inoculated with 0.2mL of diluted blood containing 1×10^7 parasitized red blood cells of *P. berghei berghei* NK65 strain. Seventy two (72) hours post inoculation, the mice in Groups I-IV were administered orally with selected doses of 100, 200, 400 and 800 mg/kg of *Spathodea campanulata*. Groups (V and VI) were administered with normal saline and chloroquine 10mg/kg as negative control and positive control respectively. This was repeated for five consecutive days after measuring their rectal temperature. The blood smear was taken on a daily basis to determine the parasitaemia level. The same procedure was carried out on the methanol leaf extract of *Parquetina nigrescens*.

2.6 Preparation of blood smears

The tip of the tail of each mouse was gently excised to get drops of blood. The blood was collected on the surface of a clean glass slide, which had been carefully labeled on the other side with paper tape. The blood was carefully spread across the slide using another slide placed on the spot at an angle of 45°. The blood was allowed to dry and fixed with a few drops of methanol and air-dried.

2.6.1 Staining of blood smear

The fixed blood films were stained using a 4% Giemsa diluted with water at a ratio 1:9. The stained slides were allowed to stand for about 30 minutes, rinsed with water and allowed to dry.

2.6.2 Assessment of parasitaemia level and percentage chemosuppression

Each of the glass slides containing the stained blood film was mounted on the microscope and examined using immersion oil ($\times 100$) objective to observe the distribution of red blood cells. Ten fields of view were counted for each slide. Each view was observed for the number of parasitized red blood cells (PRBC) as well as the unparasitized red blood cells (UPRBC). The percentage parasitaemia for each field of view was calculated as follows:

$$\text{Percentage parasitaemia} = \frac{PRBC \times 100}{PRBC + UPRBC}$$

Where,

PRBC = Number of parasitized red blood cells

UPRBC = Number of unparasitized red blood cells

Also, the percentage reduction in parasitaemia or chemosuppression or clearance for prophylactic, chemosuppressive and curative tests respectively was calculated as follows:

$$\text{PNC} - \text{PTD} \times 100 / \text{PNC}$$

Where,

PNC= Average percentage Parasitaemia in the negative control

PTD= Average percentage parasitaemia in the test dose.

The values were recorded as % parasitaemia \pm SEM and % reduction or chemo suppression or clearance \pm SEM [19].

2.6.3 Determination of survival time and percentage survivor

The mice in each of the above experiments were observed for 28 days post drug administration to determine their survival time and percentage of survivor. The survival times and percentage survivor of each of the groups were determined and recorded in days \pm SEM and percentages respectively [19].

2.6.4 Determination of median effective doses: ED₅₀ and ED₉₀

The effective dose ED₅₀ is the dose of a drug that is pharmacologically effective in 50% of the population exposed to the drug [21]. In order to determine the ED₅₀ and ED₉₀, a graph of test doses against percentage reduction in parasitaemia, chemosuppression or clearance was plotted using Microsoft Excel 2017 application.

2.7 Statistical analysis

All the values obtained in all the experiments were expressed as mean \pm SEM and analyzed statistically using One-way Analysis of Variance (ANOVA) followed by Student Newman Keul's *post-hoc* test for comparisons to determine the source of significant difference for all values. Values of P < 0.05 were considered to be of statistical significance.

3. Results and discussion

The methanol extracts of *S. campanulata* and *P. nigrescens* leaf were evaluated in a LD₅₀ determination using Swiss mice at increasing dose of 100-5000

mg/kg [22], the estimated lethal dose showed that the extracts were safe up to 5000 mg/kg when taken orally [23, 22]. The estimated LD₅₀ for *P. nigrescens* is less than 5000 mg/kg [24].

The use of medicinal plants for the treatment of various kinds of diseases dates back to the existence of man [25-27]. Such plants which are usually prepared in crude forms, as poultices, tinctures, decoctions, infusions, powders, teas and herbs, either singly or in combinations have afforded many herbal remedies. Medicinal plants and herbal recipes have claims for use in the treatment of malaria and other parasitic diseases. They are usually validated through various relevant pharmacological tests in order to justify these claims, the end of which has afforded many drugs which are on the shelves of pharmacies today [14]. Despite the various evaluations of about 250,000 plant species growing all over the world, only 1% have been thoroughly subjected to scientific evaluations for their pharmacological properties and potential chemotherapeutic values [28, 29]. It is therefore important that more such tests be conducted on available and effective recipes or known medicinal plants so that more effective drugs can be available now and for generations to come. A deliberate evaluation of the highly effective ones has the potential of affording potent antimalarial drugs which can combat resistant strains of malarial parasites which develop daily [30].

Comparing the activities of medicinal plants either in the three or same models of malarial treatment affords the discovery of plants that have better activities for each of the models [31, 32]. Comparison in this way would also enable scientists to make decisions on the model that can be used for the evaluation or the plants of choice in the ultimate search for potential antimalarial constituents. It could also assist in identifying potential plants that can be combined together in making standardized herbal recipes.

Spathodea campanulata (leaf and stem bark extracts) have been used in Ghana to treat malaria [17] and its leaf reported to possess antimalarial activities. Also, the aqueous leaf extract of *Parquetina nigrescens* possesses similar antimalarial profile [17, 33-35]. However, there has not been a documented report on

the evaluation of their antiplasmodial activities using the three models of antimalarial studies neither their comparative activities. In this work, both plants were compared using prophylactic, chemosuppressive and curative activity models with a view to maximizing their use in the treatment of malaria. It may also enable a better appreciation of the usefulness of each of the plant extracts in the preparation of herbal antimalarial remedies.

The percentages of parasitaemia and reduction in parasitaemia at the tested doses of 100-800 mg/kg in the prophylactic tests, the median effective doses (ED₅₀ and ED₉₀), survival times and percentage survivor elicited by each of the extracts of *Parquetina nigrescens* and *Spathodea campanulata* leaf showed a thin line of difference in the activities the extracts. The comparable median effective doses (ED₅₀) given by both plants and the significantly different ED₉₀ in the prophylactic test are a confirmation of the little difference in their activities. Plants with similar activities or little difference in activities may complement each other additively or synergistically when combined together in making herbal recipes. The combined extracts of the leaves of *Syzygium cumini* and *Psidium guajava* belonging to the family Myrtaceae were found to be more effective than individual plant extracts against diabetes [36]. This may suggest a possibility of combining both plants in an herbal antimalarial remedy.

The significantly different parasitaemia level elicited in mice by the methanol extract of *S. campanulata* from that of the negative and positive control at all the tested doses (Table 1) implied an intrinsic prophylactic activity. In essence, the comparable activities at lower doses (100 and 200 mg/kg) to each other and at higher doses (400 and 800 mg/kg) to each other and to the positive control indicated better prospect for use. Comparing both extracts, at relatively lower (100 and 200 mg/kg) doses and higher doses (400 and 800 mg/kg), showed that both plants exhibited comparably lower and higher percentage of reduction in parasitaemia respectively (Table 1). The highest reduction was 72% and 71% at 400 mg/kg for both extracts respectively and 71.71 and 70% at 800 mg/kg (Table 1) is noteworthy. However, though both elicited comparable ED₅₀, *Spathodea campanulata* presented a significantly lower ED₉₀ over

Table 1. Percentage parasitaemia and reduction in parasitaemia in the prophylactic model

Doses (mg/kg)	<i>Spathodea campanulata</i>		<i>Parquetina nigrescens</i>	
	% P	% RP	% P	% RP
0	6.48±0.40 ^c	0.0±0.00 ^a	6.48±0.40 ^d	0.0±0.00 ^a
100	2.41±0.14 ^b	62.86±3.2 ^b	2.87±0.00 ^c	55.08±0.1 ^b
200	2.32±0.13 ^b	64.16±2.9 ^b	2.69±0.23 ^{b, c}	58.56±6.4 ^b
400	1.82±0.24 ^a	71.13±5.6 ^b	1.91±0.19 ^b	70.55±5.2 ^b
800	1.89±0.13 ^a	70.83±3.0 ^b	1.97±0.15 ^b	69.56±4.3 ^b
Pyrimethamine (1.2mg/kg)	0.99±0.11 ^a	84.72±3.98 ^c	0.99±0.11 ^a	84.72±3.98 ^c
ED ₅₀ (mg/kg)		275.71±7.75 ^a		295.60±9.85 ^a
ED ₉₀ (mg/kg)		527.10±13.27 ^a		579.97±25.02 ^b

Keys: Data show mean ± SEM, n=5. 0 mg/kg: NC (Negative control); Normal saline, PC (Positive control); 10 mg/kg body weight Chloroquine. % P: percentage parasitaemia; % RP: percentage reduction in parasitaemia; ED₅₀: Effective dose in mg/kg that produced 50% reduction in parasitaemia; ED₉₀: Effective dose in mg/kg that produced 90% reduction in parasitaemia. Only values with different superscripts (a or b) within columns are significantly different (p < 0.05, one –way analysis of variance followed by the Student Newman Keuls' *post hoc* test, Superscripts a or b are statistical notations to depict significantly different activities in the values).

Table 2. Average survival time and percentage survivor of mice in the prophylactic model

Doses (mg/kg)	<i>Parquetina nigrescens</i>		<i>Spathodea campanulata</i>	
	AST	PS	AST	PS
0	19.2±0.37 ^a	20	19.2±0.37 ^{a, b}	20
100	21.6±1.94 ^a	40	14.8±4.59 ^a	60
200	19.4±0.60 ^a	80	25.6±1.60 ^b	60
400	19.2±4.64 ^a	60	24.0±2.53 ^{a, b}	60
800	26.2±1.11 ^b	60	18.4±2.54 ^{a, b}	60
PYR	28±0.00 ^b	100	28.0±0.00 ^b	100

Keys: Data show mean ± SEM, n=5. 0 mg/kg: NC (Negative control); Normal saline, PYR (Positive control) Pyrimethamine (1.2 mg/kg). AST; Average survival time. PS: Percentage survivor. Only values with different superscripts a or b within columns are significantly different (p < 0.05, one –way analysis of variance followed by the Student Newman Keuls' *post hoc* test, Superscripts a or b are statistical notations to depict significantly different activities in the values).

Parquetina nigrescens leaf extract (Table 1). Adetutu reported the poor activity of *P. nigrescens* at a lower dose of 100 mg/kg in the prophylactic model [37]. The activity of *Morinda lucida* in a similar experiment was comparable at a lower dose of 100 and 200 mg/kg and also at a higher dose of 400 and 800 mg/kg [38]. Though, prophylactic activities of both plants were lower than those of pyrimethamine [38] they can still be used as prophylactic drugs against malaria. Likewise, in a similar experiment, *C. albidum* leaf and stem bark gave comparable activities to the positive control drug whereas *C. aurantifolia* leaf and fruit gave lower activities than the positive control [39]. Also, at 800 mg/kg dose, *Mangifera indica* leaf extract gave 71% reduction in parasitaemia which was comparable to that of pyrimethamine [40], while other MAMA Decoction plants gave lower prophylactic activities at similar doses.

Also, in the prophylactic model (Table 2), the survival time elicited by *P. nigrescens* at all the tested doses except the highest (26.2 ± 1.11days) was comparable to that of the negative control, but the survival time of 25.6 ± 1.60 days elicited at the dose of 200 mg/kg by *S. campanulata* was also comparable to the positive control. When this is compared with 18.4 ± 2.54 days given by *P. nigrescens* at the same dose and also considering the percentage survivor of 80 and 60 given by both plants respectively (Table 2), it is safe to conclude that both plants possess some potential for malarial prophylaxis. In the chemosuppressive model, *Parquetina nigrescens* presented a relatively lower value of percentage parasitaemia at all the tested doses of 100-800 mg/kg than *Spathodea campanulata* (Table 2). Both extracts gave percentage chemosuppression that were comparable at all doses to the positive control thus

Table 3. Percentage parasitaemia and chemo suppression in the chemo suppressive model

Doses (mg/kg)	<i>Spathodea campanulata</i>		<i>Parquetina nigrescens</i>	
	% P	% CS	% P	% CS
0	8.56±0.68 ^c	0±0.00 ^a	8.56±0.68 ^c	0±0.00 ^a
100	2.19±0.14 ^b	70.39±5.5 ^b	1.70±0.15 ^b	81.03±6.11 ^b
200	2.32±0.31 ^b	72.88±11.9 ^b	2.29±0.17 ^b	78.35±6.78 ^b
400	1.98±0.10 ^b	76.83±3.82 ^b	1.95±0.11 ^b	78.41±4.46 ^b
800	2.14±0.06 ^b	70.00±2.33 ^b	2.09±0.12 ^b	76.67±4.56 ^b
CQ	1.25±0.17 ^a	85.35±6.52 ^b	1.25±0.17 ^a	85.35±6.52 ^b
ED ₅₀ (mg/kg)		250.94±3.38 ^a		244.57±3.94 ^a
ED ₉₀ (mg/kg)		453.09±9.57 ^b		430.09±5.64 ^a

Keys: Data show mean ± SEM, n=5. 0 mg/kg: NC (Negative control); Normal saline, PC (Positive control); 10 mg/kg body weight Chloroquine. % P: percentage parasitaemia; % CS: percentage chemosuppression; ED₅₀: Effective dose in mg/kg that produced 50% chemosuppression; ED₉₀: Effective dose in mg/kg that produced 90% chemosuppression. Only values with different superscripts (a or b) within columns are significantly different (p < 0.05, one –way analysis of variance followed by the Student Newman Keuls' *post hoc* test, Superscripts a or b are statistical notations to depict significantly different activities in the values).

Table 4. Average survival time and percentage survivor of mice in the chemo-suppressive model

Doses (mg/kg) body weight	<i>Parquetina nigrescens</i>		<i>Spathodea campanulata</i>	
	Average survival time	Percentage survivor	Average survival time	Percentage survivor
0	17.2±2.18 ^a	20	17.2±2.18 ^a	20
100	15.4±2.96 ^a	80	21.8±1.56 ^a	40
200	16.4±2.94 ^a	80	17.6±2.52 ^a	60
400	17.4±1.50 ^{a, b}	40	17.6±5.05 ^a	60
800	27.0±0.63 ^b	60	18.0±1.82 ^a	60
CQ	28.0±0.00 ^b	100	28.0±0.00 ^b	100

Keys: Data show mean ± SEM, n=5. 0 mg/kg: NC (Negative control); Normal saline, CQ (Positive control) Chloroquine (10 mg/kg). AST: Average survival time. PS: Percentage survivor. Only values with different superscripts (a or b) within columns are significantly different (p < 0.05, one –way analysis of variance followed by the Student Newman Keuls' *post hoc* test, Superscripts a or b are statistical notations to depict significantly different activities in the values).

depicting a relatively high chemo suppressive effect of both extracts. The statistically different (p<0.05) ED₉₀ of the extracts shows a better activity of *P. nigrescens* over *Spathodea campanulata* in the chemosuppressive model, though ED₅₀ values were comparable (Table 3). The high chemosuppressive activities of both extracts depict the potential of these two plants to treat malarial especially in Africa.

P. nigrescens also presented a very high survival time (27.0±0.63) at the highest dose tested in the chemosuppressive model and was comparable (p>0.05) to that of the positive control, just like in the prophylactic model. For *S. campanulata*, the survival times were comparable (p>0.05) to that of the negative control at all doses with similar survivor of 60 except at 200 mg/kg which gave a value of 40 while the highest percentage survivor value of 80% was recorded at 100 and 200 mg/kg for *P. nigrescens*. The extract of *P. nigrescens* thus gave a better survivor and

survival time profile than *S. campanulata* in the chemosuppressive model (Table 4). These data coupled with its activities on the parasite confirmed *P. nigrescens* as the more active of the two as a chemosuppressive agent. In a similar experiment for *S. campanulata* the average mean survival time was between 13-25 days [41], this is in line with the results obtained in this present study.

The curative model gives the degree to which the extract can clear the parasite from the system of the mouse rather than reduction or suppression of the same in the prophylactic or suppression models respectively. The potential of the extracts to clear parasites is exemplified by the significant difference between the parasitaemia values at the tested doses during the days of treatment and the negative control upon progressive daily monitoring of the level of parasitaemia. The comparable levels of parasitaemia observed for the extracts of the two plants to each

Table 5. Average survival time and percentage survivor of mice in the curative model

Doses (mg/kg) body weight	<i>Parquetina nigrescens</i>		<i>Spathodea campanulata</i>	
	AST	PS	AST	PS
0	17.4±0.51 ^a	20	17.4±0.51 ^a	20
100	17.2±0.73 ^a	40	20.6±1.17 ^{a, b}	80
200	14.6±1.91 ^a	60	15.4±2.10 ^a	40
400	13.8±2.63 ^a	40	13.2±3.12 ^a	60
800	15.4±1.89 ^a	80	12.6±1.72 ^a	60
CQ	28.0±0.00 ^b	100	28.0±0.00 ^b	100

Keys: Data show mean ± SEM, n=5. 0 mg/kg: NC (Negative control); Normal saline, CQ (Positive control) Chloroquine (10 mg/kg). AST; Average survival time. PS: Percentage survivor. Only values with different superscripts (a or b) within columns are significantly different (p < 0.05, one –way analysis of variance followed by the Student Newman Keuls' *post hoc* test, Superscripts a or b are statistical notations to depict significantly different activities in the values).

other at all the doses tested in each of the days and the significant parasitaemia reduction on the last day with PN at doses 200mg/kg and 8000mg/kg on day 4 and with SC at 200, 400 and 800mg/kg showed there was a reasonable reduction of parasitaemia at each of the doses with progressive administration of both extracts (Figs. 1 and 2).

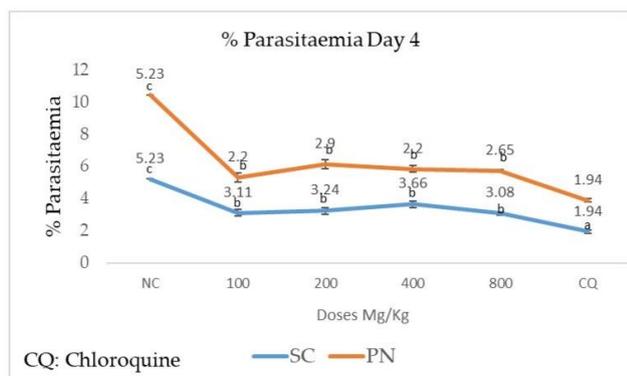


Figure 1. Comparative Percentage parasitaemia in the curative model of the extracts of *S. campanulata* and *P. nigrescens*

These seem to confirm the curative effectiveness of both extracts. However, at all doses, *P. nigrescens* seems to elicit a higher percentage clearance than *S. campanulata*. *P. nigrescens* elicited a clearance of 58% at 100 mg/kg, the lowest dose while *S. campanulata* elicited 49% at the highest dose of 800 mg/kg. The significantly different (p<0.05) clearance of 63% given by the positive control, chloroquine (Fig. 2) is an indication of its better curative activity than each of the extracts.

Also, the ED₅₀ elicited by both extracts were not significantly different (p>0.5) from each other, but the ED₉₀ of *S. campanulata* (827.76 ± 91.87) was significa-

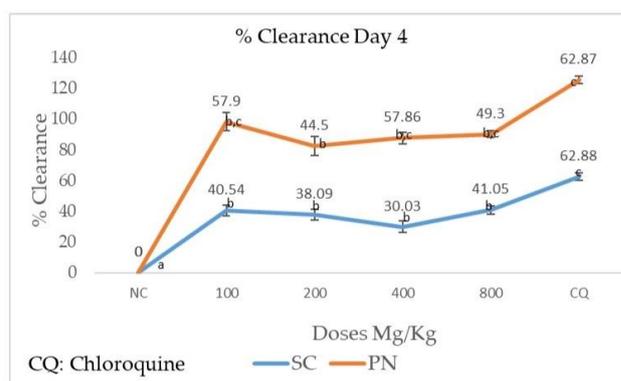


Figure 2. Comparative Percentage clearance in the curative model of the extracts of *S. campanulata* and *P. nigrescens*

ntly higher than that of *P. nigrescens* (582.38±63.99) indicating that the extract of *P. nigrescens* displayed a better curative activity than that of *S. campanulata* (Fig. 3). Effective doses elicited by antimalarial plants have been used to rank order their activities in a comparative study [42].

For the curative model, a doubled value of the survival times obtained for an extract is an express indication of curative antiplasmodial activities [43]. However, despite the moderately average percentage clearance, the extract could not give a doubled survival time. Truly, the survival time at all doses tested for both extracts gave values that were comparable to the negative control and so neither of them possessed intrinsic curative activities. However, the highest values of percentage survivor were elicited at the lowest dose tested for *S. campanulata* and the highest dose tested for *P. nigrescens* (Table 5). The survival time profile was thus better for *S. campanulata* than *P. nigrescens* in the curative experiment.

It needs to be mentioned that antimalarial drugs may

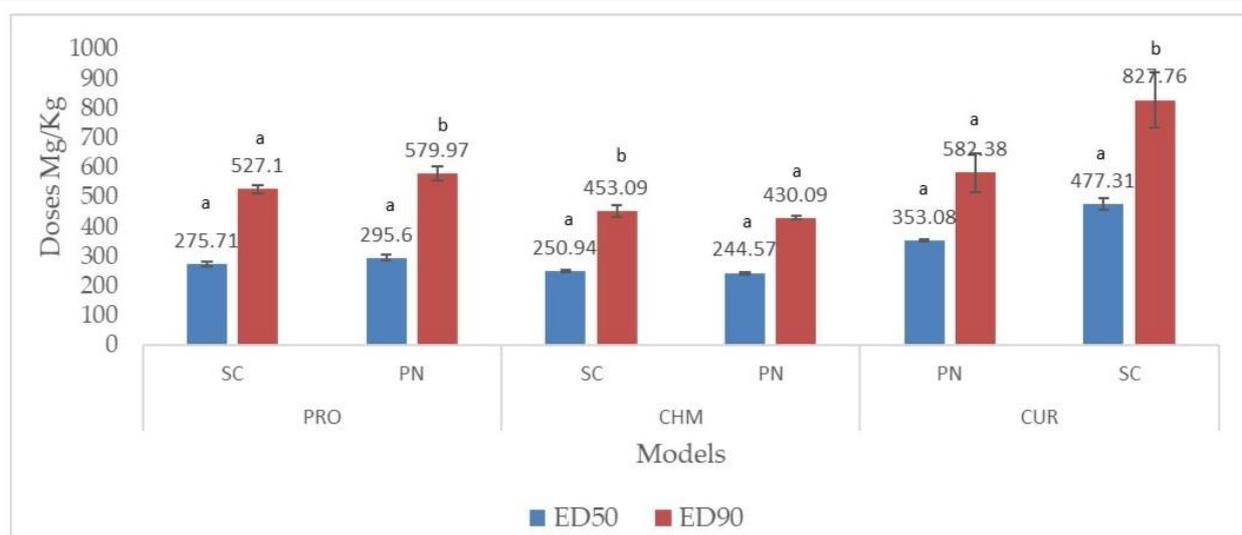


Figure 3. Effective dose values of the leaf extracts of *S. campanulata* and *P. nigrescens* for all the models

elicit their antimalarial activities either like chloroquine, by inhibiting the formation of haemozoin by the parasite as a result of detoxifying, by polymerization of haemoglobin which is toxic to the parasites [44], or also like mefloquine, by associating with the serum polypeptide apoA1 to which malaria-infected erythrocytes bind in order to obtain supply of exogenous molecules for membrane trafficking events which the drug eventually disrupts [45]. It may also like pyrimethamine, cause failure of nuclear division at the time of schizont formation in the erythrocytes by inhibiting the dihydrofolate reductase of plasmodia and thereby blocking the biosynthesis of purines and pyrimidines, which are essential for DNA synthesis and cell multiplication [46]. It does not seem unlikely that the extracts of *S. campanulata* and *P. nigrescens* in the different models of antimalarial activities act in either of those mechanisms based on some comparable activities they both elicited at some tested doses. The elucidation of the exact mechanism of action of the plant extracts and their compounds, after the isolation of the active constituents, should be the subject of a further work to be carried out on the extracts.

In summary, *S. campanulata* with significantly lower effective doses and better survival time profile in the prophylactic model was a better prophylactic drug than *P. nigrescens* with better survivor. On the other hand, though *P. nigrescens* elicited lower ED₉₀ in the

chemosuppressive and curative models, its additional high survival times and high survivor in both models made it a better chemosuppressive and curative drug.

4. Conclusions

In conclusion, the study shows that the leaf of *S. campanulata* is a better prophylactic drug because of its significantly lower effective dose and better survival time profile while *P. nigrescens* leaf gave better chemosuppressive and curative profile than *S. campanulata* because of its higher survival times and percentage survivor in both models. However, further studies will be needed to investigate the potential of both plants being combined together in an antimalarial remedy that will elicit activities of the three models of test and in addition identify the constituents responsible for these activities. The individual extracts and the combinations may also be tested against chloroquine resistant Plasmodium species to further highlight their potentials as antimalarial agents.

Institutional review board statement (Ethical statement)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Animal

Research Ethics Committee is Health Research Ethic Committee [HREC] Institute of Public Health, Obafemi Awolowo University, Ile Ife with Ethical Approval Number is IPH/OAU/12/2209.

Authors' contributions

Designed and coordinated the study, S.A.O, Carried out the extraction and the antimalaria assay, T.B.B., Data collection and analysis, T.B.B. and S.A.O. Writing–S.A.O. Original Draft Preparation, S.A.O.; Writing–Review and Editing, S.A.O. and T.B.B.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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