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Research Article

Comparative Assessment of the Antiplasmodial Activity and Acute Toxicity of *Schumanniophyton magnificum* Good & Halle (Rubiaceae) Leaves, Trunk and Roots Methanolic Extracts

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Abstract

Background: Despite the various medicinal applications of *Schumanniophyton magnificum*, no comparative data are available to determine the part with best antiplasmodial activity and to guarantee its safety. This work deals with the evaluation of the antiplasmodial activity of methanolic extracts of *S. magnificum* organs as well as the study of acute toxicity of the most active part.

Materials and methods: The methanolic extracts of the leaves, stem bark, trunk wood, root wood and root bark, were obtained by maceration with 96% methanol. Phytochemical screening, based on precipitation and coloring reactions, to highlight the chemical groups present in the plant. The antiplasmodial test was ran against chloroquine sensitive *Plasmodium falciparum* 3D7 strains while the study of the *in vivo* acute toxicity was conducted according to guideline 423 of the OECD protocol at a fixed dose on Wistar rats.

Results: Phytochemical screening of the methanol extracts of these plants organs revealed the presence of alkaloids, triterpenoids, saponins, anthraquinones, polyphenols, coumarins anthocyanins and flavonoids. The antiplasmodial activity showed that only the leaves exhibited a moderate antiplasmodial effect with IC_{50} of 30.77 µg/ml. An oral administration of the methanolic extract of the leaves did not induce an abnormal variation of the physiological parameters in female Wistar laboratory rats, at non-toxic doses of 50, 300 and 2000 mg/kg body weight for 14 days.

Conclusion: The leaves of *S. magnificum* exhibited the best antimalarial activity and were non toxic, thus justifying the use of the plant in the treatment of malaria in traditional pharmacopoeia.

Keywords: Phytochemical screening, Antiplasmodial activity, Acute toxicity, *Schumanniophyton magnificum*, Medicinal plant.

1. INTRODUCTION

The spread of chloroquine-resistant *Plasmodium falciparum* and the alarming emergence of multi-drug resistant strains have raised an urgent need to search for new, safe and effective antimalarial drugs. [1,2] So, screening of herbal potions used in traditional medicine was indicated. Medicinal plant extracts as well as their occurring products have been used in the treatment of fever or other symptoms associated with malaria throughout the tropics and subtropics, with a folklore encompassing centuries of practical experience. [2] Despite the covid 19 pandemic, WHO calls on countries and global health partners to intensify the fight against malaria, a preventable and treatable disease that still claims hundreds of thousands of live search year. Better-targeted interventions, new tools and increased funding are needed to make a global difference and achieve internationally agreed targets. [3] In 1998, the WHO recommended the use of plants in the search for new therapeutic opportunities. In developing countries, 80% of the population uses herbal preparations for their health needs at least once a year. [4] These preparations, used without prior scientific evaluation of their toxicological or therapeutic properties, are at risk of intoxication or inefficient use for therapeutic purposes.

In our environment, *Schumanniophyton magnificum* Good & Halle, a plant of the Rubiaceae family, is known to traditionally treat malaria, anaemia and many urogenital infections. [5,6] Numerous researchers have evaluated on stem bark such as antimalarial, antimicrobial, anti-venomous and anticonvulsant activities. [7-12] Root bark was evaluated on antiviral activity against Acquired Immunodeficiency Virus and Herpes Simplex Virus infection. [13] The choice of this plant was made on the basis of an ethnobotanical survey and previous *in vitro* and *in vivo* studies conducted. Therefore in order to determine which part of *S. magnificum* would have the best antiplasmodial activity, we conducted a comparative experimental study to evaluate the antiplamodial activity of the methanolic extracts

of the leaves, trunk wood, stem bark, root wood, root bark of *S. magnificum*, and the acute toxicity of the most active part.

2. MATERIALS AND METHODS

2.1. Plant material

The plant material used consisted of leaves, trunk (bark and wood) and roots (bark and wood) of *Schumanniophyton magnificum*; they were collected on October 2019 in Okon, a village located in centre region of Cameroon. The botanical identification was carried out at the National Herbarium of Cameroon by Mr. Nana Albert by comparison with samples number 52128/HNC.

2.2. Biological materials

The chloroquine-sensitive *P. falciparum* parasite line 3D7 purchased from Sigma-Merck.

2.3. Animal material

The experiments were carried out on 12 non-pregnant female rats aged from 8 to 12 weeks weighing 120 to 167 g. The rats were acclimatized for 2 weeks. The experimental animals were housed in standard plastic cages and provided access to food and water ad-libitum. The animals were fed a daily diet consisting of maize meal (43%), roasted soybean meal (26%), bone meal (1%), palm kernel cake (6%), wheat meal (10%), table salt (0.8%), table oil (0.1%), fish meal (13%), vitamins (0.1%) and drinking water.

2.4. Preparation of extracts

The different parts of *S. magnificum* (leaves, trunk wood, stem bark, root wood, root bark) were then dried at room temperature and powdered. The powder obtained for each part underwent maceration in 96% methanol at room temperature for 72 hours according to the protocol described by Mugiraneza et al in 2009. [14]

The extraction yields were calculated according to the following formula

$$yield = \frac{\text{weight of the dried crude extract}}{\text{weight of the powder}} \times 100$$

2.5. Qualitative phytochemical screening

The detection of secondary metabolites was carried out by specific phytochemical tests based on staining and precipitation reactions according to the methods described by Ronchetti and Russo (1971), Hegnauer (1973), Wagner (1983), Békro et *al.*(2007). [15-18] These tests included Liebermann Burchard test to identify sterols and triterpenoids; Dragendorff test to identify alkaloids; Bornstrager test for identification of anthraquinones, Saponins identification test; Coumarins identification test; Anthocyanins identification test; Shinoda test for identification of flavonoids and Ferric chloride test for identification of phenolic compounds

2.6. In vivo antiplasmodial tests

The antiplamodial tests were performed on chloroquine-sensitive 3D7 *Plasmodium falciparum* strains following the procedure previously describe by Desjardin et al., [19] with the modification as described by Mbosso et al. (2018), Fouokeng et al. (2019), Mbosso et al. (2020). [20-22] Briefly, the *P. falciparum* maintained in sealed T75 culture flasks containing RPMI 1640 supplemented with L-glutamine (2 mM), HEPES (25 mM) albumax II (5%), glucose (20 mM), hypoxanthine (650 µM), gentamycin (60 µg/ml), and human red blood cells (2-4% haematocrit) in at 37ºC under an atmosphere of 5% CO_2, 5% O_2, 90% N_2 in sealed T75 culture flasks. 2.5 µl of the mother extract solution were added to a 96-microplate filled with 97.5 µl of the infested culture medium. The final concentrations of the extracts were 100 µg/ml, DMSO were then 0.25%. After 48 h in a 37ºC CO_2 incubation, the cell viability was assessed using the quantification of the parasite Lactate Dehydrogenase (pLDH). To that end, 20 µl of each well were collected and add in corresponding well of a new 96-wells plates filled with 125 µl of Malstat/NBT/PES solution. After homogenisation, the plate was read at 620 nm against blank containing the parasite culture medium and the Malstat/NBT/PES prepared in the same condition. The control positive (chloroquine at 10 µM final concentration) and negative (antiparasitic drug replace with culture medium) were prepared in the same condition as the tests.

2.7. Single concentration screening and dose response assays

The initial evaluation of the antiplasmodial testing, the cell culture were incubated with tests sample at a fixed concentration of 50 µg/ml in triplicate. The percentage of growth inhibition was calculated relative to untreated control cultures using the upstated formulas. The validation of the tests was done using a positive control at 10 µM of chloroquine. The inhibitory parameter was the concentration that inhibits 50% of cell growth (IC_{50}) and was obtained through dose-response assays. The extracts were tested in a range of concentration ranging from 50 to 0.046 µg/ml using a 3-fold serial dilution. The dose-response curves of % cell viability vs. Log[extract] were plotted and the IC_{50} were determined by non-linear regression using GraphPad Prism (Version 5.02). IC_{50} values for some extract were not determined due to the low inhibition observed in the single concentration screening. All measurements were performed using the Biotek Synergy MX microplate reader.

2.8. In vivo acute toxicity assessment

The acute toxicity test was conducted according to the modified OECD 423 guidelines, [23] in a sequential process using 3 animals per step. Thus, the absence or manifestation of mortality in a substance-related group of a given dose determines the next step, i.e. stopping the test, administering the next higher or lower dose to 3 more animals. A total number of 12 female Wistar rats were randomly selected and divided into four (4) different treatments groups, each group consisting of 3 healthy animals: a negative control group which only received distilled water and the others received the leaves extract at different doses, i.e. 50, 300 and 2000 mg/kg of body weight. Once treated, the animals were observed for 2 H and 4 H after the administration of the extract. They were then fed and observed after the first 30 minutes, 1 h, 2 h, 4 h, 8 h, and then every 48 h until 14 days during which the symptoms of intoxication (modification of the coat, body mass, stool appearance, motility, tremors, grooming, breathing, sensitivity to noise, as well as deaths) were noted. The dead rats in each batch were counted for the determination of the LD_{50}. This observation was made by following the parameters shown in Table 3. The animals were killed on the fourteenth day.

We weighed the animals daily for 14 days. At the end, the rats were fasted for 18 hours followed by a gentled earth with ether after administration of ketamine. Blood sampling was performed, followed by dissection. The organs removed were the liver, kidneys, lungs and heart. These were rinsed with 0.9% saline and weighed. The relative weight of each organ was calculated using the formula:

$$Pr = \frac{Po}{Pa} \times 100$$

Pr: relatif weight of the organ (g); Po : weight of the organ (g); Pa : rat body weight (g)

Several biochemical parameters were measured, in particular: transaminases (ALT, AST), creatinine, urea.

All assays were performed on blood collected before the animals were killed, following the protocol proposed in the SGM- Italia kit.

3. RESULTS

3.1. Extraction

The extraction of the different parts of *S. magnificum* allowed us to have the concentrations of the crude extracts (Table 1).

Table 1: Extraction yield of the methanolic extracts of the different parts of *Schumanniophyton magnificum.*

Vegetal material	Powder (g)	Crude extract (g)	Yield (%)
Root wood	1200	22	1.83
Root bark	1200	25.1	2.05
Trunk wood	1200	30	2.5
Stem bark	1500	12	0.8
Leaves	1300	60	4.6

The leaves extract of *S. magnificum* has the highest yield (4.6%) followed by trunk wood extract (2.5%), root bark extract (2.05%), root wood extract (1.83%) and trunk bark extract (0.8%).

3.2. Phytochemical screening of extracts

The results of the qualitative tests carried out on the various extracts have enabled us to highlight several families of chemical compounds represented in the following table

Table 2: Secondary metabolites tested.

Tests	Chemical compounds family	SMMrw	SMMrb	SMMtw	SMMsb	SMMle
Dragendorff	Alcaloids	+	+	+	+	+
Shinoda	Flavonoids	-	-	-	-	-
Potash test	Coumarins	-	-	-	-	-
$FeCl_3$	Polyphenols	+	+	+	+	+
Foam index	Saponins	-	-	+	-	+
Libermann Buchard	Triterpenoids	+	+	+	+	+
Anthocyanins	Anthocyanins	-	-	-	-	-
Borntrager	Anthraquinones	+	+	-	-	+

Legend: + = presence; - = absence; SMMrw: *S. magnificum* methanolic extract of root wood, SMMrb: *Schumanniophyton magnificum* methanolic extract of root bark, SMMtw: *S. magnificum* methanolic extract of trunk wood, SMMsb: *S. magnificum* methanolic extract of stem bark, SMMle: *S. magnificum* methanolic extract of leaves

Table 2 shows the different metabolites present in the extracts of the separated organs of *S. magnificum*

3.3. Antiplasmodial activity

Extracts from different parts of *S. magnificum* were obtained with different viability percentage (PV) (Figure 1) at a concentration of 50 µg/ml, on the 3D7 line of *P. falciparum* evaluation; 103.9% for trunk wood, 98.8% for root wood and 100% for root bark; The stem bark showed low activity with a viability percentage of 61.8%. The most important yield was obtained with leaves (17,1%).

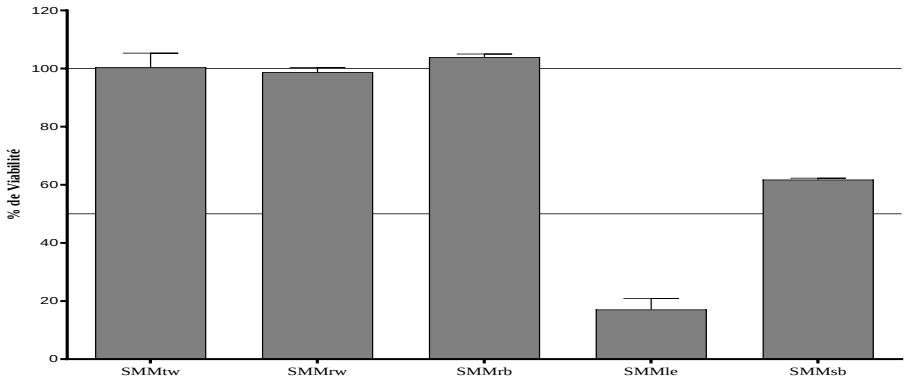

Figure 1: Viability percentage of *Plasmodium falciparum* 3D7 cells

SMMrw: *S. magnificum* methanolic extract of root wood, SMMrb: *Schumanniophyton magnificum* methanolic extract of root bark, SMMtw: *S. magnificum* methanolic extract of trunk wood, SMMsb: *S. magnificum* methanolic extract of stem bark, SMMle: *S. magnificum* methanolic extract of leaves

Due to the fact that the viability percentage of leaves extracts was less than 30%, the IC_{50} was determined. The curves of evolution of crude extract of the leaves and chloroquine, the reference drug were plotteds. GraphPad Prism program version 5.02 was used for data analysis.

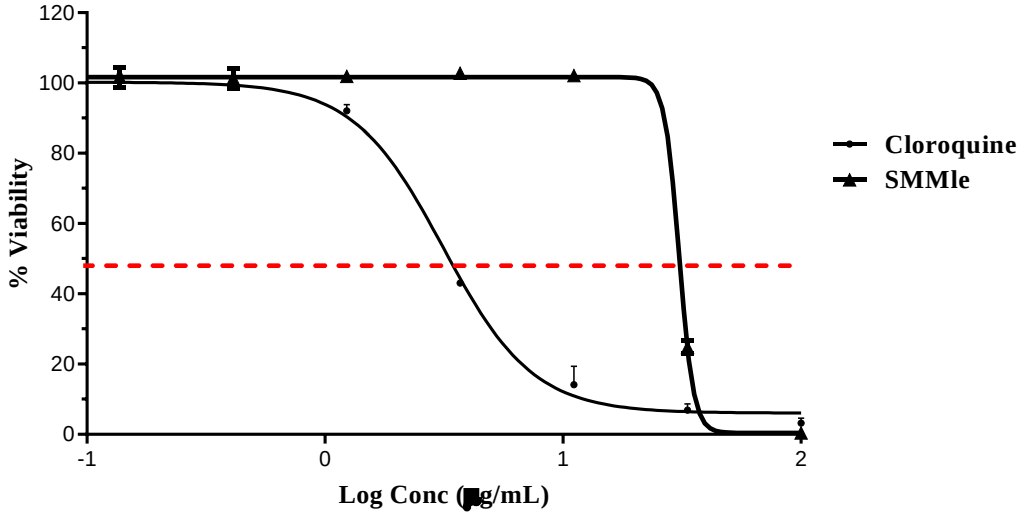

Figure 2: Dose-response curves of the antiplasmodial assay of *Schumanniophyton magnificum* methanolic extract of leaves

SMMle: *S. magnificum* methanolic extract of leaves; Chloroquine: reference drug.

Figure 2 show that crude extract of the leaves displayed antiplasmodial activity with an IC_{50} of 33.77 µg/ml while positive control, chloroquine exhibited an IC_{50} of 2.9 µg/ml.

3.4. Study of the acute toxicity of methanolic extract of Schumanniophyton magnificum leaves

3.4.1. Behaviour of rats

The main behavioural reactions of the rats 2 hours after gavage with single doses of the SMMle extract were noted in the table 3. The results show that: 2 hours after gavage, the animals showed some behaviour disturbances including increased aggression, salivation, locomotion, sleep and grooming. These changes were accompanied by the emission of pastys tools with the colour of the extract. All these changes disappeared after 4 hours of observation. All rats recovered their faculties.

Table 3: Observation of physiological parameters of rats.

Period	2H	4H	D0	D2	D4	D6	D8	D10	D12	D14
Mobility	E	E	N	N	N	N	N	N	N	N
Grooming	E	E	N	N	N	N	N	N	N	N
Agression	E	E	N	N	N	N	N	N	N	N
Faeces	E	E	N	N	N	N	N	N	N	N
Salivation	N	N	N	N	N	N	N	N	N	N
Feeding	N	N	N	N	N	N	N	N	N	N
Tremor	E	E	N	N	N	N	N	N	N	N
Drowsiness	N	N	N	N	N	N	N	N	N	N

H: Hour; D: Day; N: Normal; E: Exaggerated.

3.4.2. Determination of mortality rate and lethal dose 50 (LD_{50})

At the end of the fourteen days of observation, no anomalies were found in the parameters studied. In addition, no deaths were recorded among the treated animals. The calculated mortality rate was therefore 0%.

Table 4: Variation in mortality in 14 days after administration of a single dose of methanolic extract of *Schumanniophyton magnificum* leaves.

Groups	Number of rats per batch at the start	Mortality
Batch 1 (control)	3	0
Batch 2 (50 mg/kg)	3	0
Batch 3 (300 mg/kg)	3	0
Batch 4 (2000 mg/kg)	3	0
Total	12	0

Table 4 gives the values of the mortality proportions of the different batches. No deaths were recorded. The LD_{50} is therefore greater than 2000 mg/kg.

3.4.3 Evolution of the body masses

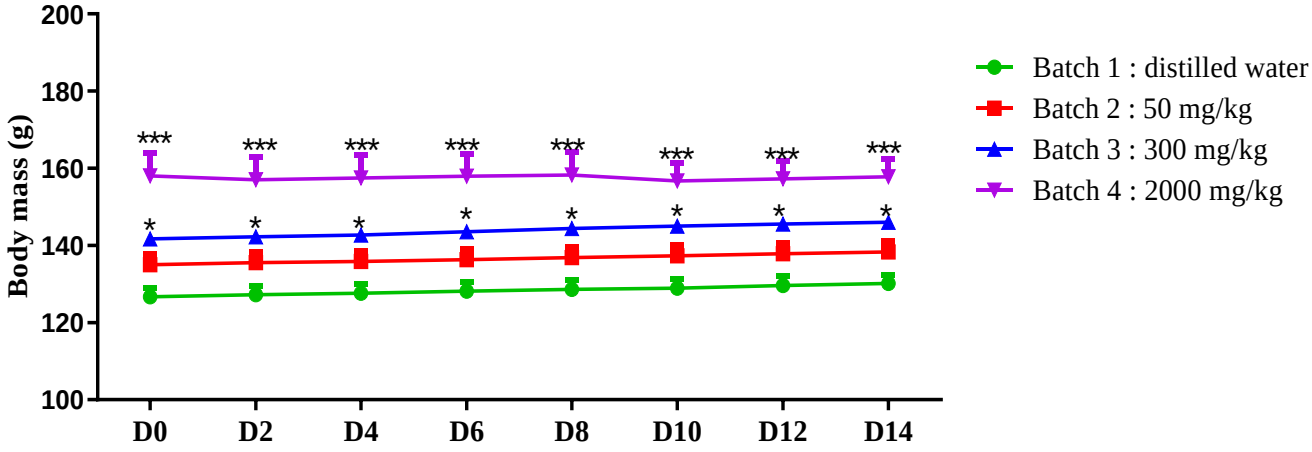

Figure 3: Evolution of the body masses of batches of rats

It can be seen from figure 3 that the administration of the extract leads to a non significant increase in body mass. However, a drop in body mass was observed on the 2nd day at a dose of 2000 mg/kg followed by a return to normal on the 4th day.

3.4.4. Effect of methanolic extract of Schumanniophyton magnificum leaves on the relative mass of the internal organs of batches of rats.

Table 5 summarises the relative mass of the kidney, heart, lung, liver and spleen of animals treated for 14 days. From this table, it can be seen that the administration of the extract does not lead to any variation in the mass of the detoxification organs compared to the control.

Table 5: Internal organs' mass within batches of rats.

	Weight (g)				
Groups	Kidney	Heart	Lung	Liver	Spleen
Batch 1 (control)	0.69 ± 0.01	0.48 ± 0.12	0.83 ± 0.0	4.25 ± 0.0	0.70 ± 0.0
Batch 2 (50 mg/kg)	0.96 ± 0.01	0.56 ± 0.0	0.85 ± 0.0	5.00 ± 0.0	0.64 ± 0.0
Batch 3 (300 mg/kg)	0.87 ± 0.01	0.57 ± 0.0	0.87 ± 0.0	5.06 ± 0.01	0.92 ± 0.01
Batch 4 (2000 mg/kg)	0.75 ± 0.12	0.59 ± 0.0	0.87 ± 0.0	5.09 ± 0.01	0.68 ± 0.01

Values represent means ± MSE. *** $p < 0.001$; ** $p < 0.01$ (significant difference from distilled water); control: distilled water.

3.4.5. The effects of methanolic extract of Schumanniophyton magnificum leaves on the blood biochemical parameters of the batches of rats studied

Four blood biochemical parameters of the groups of rats studied were assayed to understand the state of functioning of the kidneys and liver at the end of the study period. Table 6 gives us the values of our test rats in comparison with the control lot. The values show no signs of toxicity from a biochemical point of view, especially on the renal and hepatic functions.

Table 6: Biochemical parameters (ALT, AST, Creatinin and Urea of batches of rats).

Groups	ALT (UI/l)	AST (UI/l)	Creatinine (mg/dl)	Urea (mg/ml)
Batch 1 (distilled water 10 ml/kg)	40.00	21.41	0.76	6.6
Batch 2 (50 mg/kg)	47.02	20.80	0.80	7.09
Batch 3 (300 mg/kg)	50.00	19.32	0.86	9.06
Batch 4 (2000 mg/kg)	55.46	19.80	0.97	9.89
References values	20-90	15-40	0.5-1.5	6-23

4. DISCUSSION

The methanol extraction of the *S. magnificum* organs was carried out in a single step by maceration. The solvent was chosen on the one hand for its low boiling point of about 65°C and on the other hand for its capacity to dissolve a large proportion of polar and non-polar compounds. We obtained low yields ranging from 0.9 to 4.6% for the different extracts. These results do not corroborate those of Bend et al., in 2018 that made an aqueous decoction of *S. magnificum* leaves with a yield of 11.8%[24]. This difference could be explained on the one hand by the difference in the solvent and on the other hand by the extraction method, the place of harvesting and the temperature of the environment; Bend et al., in 2018 harvested during the month of March (dry season) in southern Cameroon and extract with water. [24]

The phytochemical screening carried out aimed at identifying secondary metabolites (alkaloids, triterpenoids, saponins, anthraquinones, polyphenols, coumarins, anthocyanins, and flavonoids). These metabolites were chosen on the basis of previous studies indicating their presence in *S. magnificum* harvested in the previous study by Feuya et al. in 2014. [7] These tests were based on the observation of changes in the colouring of the solutions indicative of the presence of the desired compound. The results obtained are not in agreement with those of Feuya et al. in 2014 who revealed the presence of flavonoids and the absence of triterpenes, saponosides in the stem bark. [7] These differences could be due to the concentration of these secondary metabolites in the extracts. Feuya et al.carried out a double maceration, whereas in our study we did just one maceration. Bend et al., in 2018 also obtained the presence of sugars from the aqueous decoction of leaves. [24] The difference could be due to the polarity of the solvent use for extraction, water in Bend et al. study and methanol in our.

The evaluation of the *in vitro* antiplasmodial activity of *S. magnificum* extracts at a concentration of 50 µg/ml, on the 3D7 cell line of *P. falciparum* showed that only the leaves extract (SMMle) significantly reduced the viability of the parasite with an IC_{50} of 30.77 µg/ml, comparatively to the other extracts which had no effect on the viability of the parasite with percentages of viability of 103.9% for trunk wood, 98.8% for root wood and 100% for root bark. The stem bark showed low activity with a viability percentage of 61.8%. According to Deharo et al. in 2001, we can affirm that the methanolic extracts of the leaves of *S. magnificum* have moderate activity on the 3D7 cells of *P. falciparum.* [25] According to the phytochemical screening, the activity of the leaves could be related to a higher level of anthraquinones and terpenes. These results are not in agreement with those of Bickii et al. in 2007 that showed mixed results with three extracts of *S. magnificum* stem bark on *P. falciparum* line W2. The hexanolic and dichloromethane/MeOH (1:1) extracts showed low activity with IC_{50} values of 78.1 and 47.8 µg/ml respectively while the methanolic and H_2O/EtOH (1:1) extracts showed moderate activity with IC_{50} values of 28.7 and 25.5 µg/ml respectively. [9] This difference observed for stem bark in the two studies could be justified since in the study of Bickii et al., the *Plasmodium falciparum* line used is the chloroquine resistant strain W2, while in ours its the *P. falciparum* strain 3D7.

Toxicity tests done on the methanolic extracts of *S. magnificum* leaves did not lead to any sign of toxicity at the limit doses of 50, 300 and 2000 mg/kg body weight; however, the leaves of *S. magnificum* have not yet been the subject of previous toxicological studies. Nevertheless, the aqueous extract of *Heinsia crinita*, a species of the Rubiaceae family, did not show acute toxicity from a behavioural point of view at the limit doses of 200, 400 and 2000 mg/kg. A study conducted by Boumba et al., in 2018, agrees with our results. [26] In addition, the work of Bend et al., in 2018 fates on stem bark and on rat fertility at 800 mg/kg did not show any significant influence on the elevation of sex hormones[24]. Thus, based on the globally harmonised classification system of OECD guideline number 423, the LD_{50} of the methanolic extract of *S. magnificum* leaves was greater than 2000 mg/kg (LD_{50} > 2000 mg/kg). As a result, the extract was classified in category 5 of the said system. [23]

CONCLUSION

The general objective of this research work was to evaluate the antiplasmodial activity of methanolic extracts of the leaves, trunk and roots of *S. magnificum* and study the acute toxicity of the most active part. Phytochemical screening of the different methanolic extracts of *S. magnificum* revealed the presence of alkaloids, terpenes, polyphenols and anthraquinones distributed in the different extracts. The extracts of stem bark, trunk wood, root wood, root bark, leaves had almost no effect at a concentration of 50 µg/ml on *Plasmodium falciparum* strain 3D7 with the exception of the methanolic leaves extract which significantly reduced the viability of the parasite with an IC_{50} of 30.77 µg/ml and was the most active part. The presence of antiplasmodial activity in the plant could thus justify its use for the treatment of malaria. It would be more judicious to suggest to local populations the use of the leaves instead of the stem bark. Leaves with a lethal dose ≥ 2000 mg/kg could be used without risk of toxicity in the management of malaria.

Conflicts of Interest: None.

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