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Research Article
In vitro Antiplasmodial Properties of Five Antimalarial Plants Used in Burkina Faso

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Abstract

Background and Objective: Malaria remains one of the most deadly diseases in developing countries. Most populations in these countries use fold medicine without any scientific evidence. In the present study, the *in vitro* antiplasmodial activity has been assessed from five medicinal plants commonly used traditionally in Burkina Faso to treat malaria. **Materials and Methods:** Thus, *Spermacoce verticillata*, *Gardenia erubescens*, *Mitracarpus villosus*, *Fadogia agrestis* and *Mitragyna inermis* belonging to the Rubiaceae family were extracted with water, ethanol, methanol, dichloromethane and hexane. Total phenolics and total flavonoids were determined by colorimetric methods. The *Plasmodium* lactate dehydrogenase assay was used to determine the drug susceptibility. The cytotoxicity was determined with the red blood cells by the calculation of the percentage of hemolysis. **Results:** The results indicated that most of the extracts had total phenolics and total flavonoids. All aqueous and hexanic extracts of the five plants failed to inhibit parasite growth. However, ethanolic macerate of *Fadogia agrestis* and ethanolic extract to the soxhlet of *Spermacoce verticillata* significantly inhibited the growth of 3D7 parasites with IC_{50}s of 9.56±0.63 and 6.86±0.39 μg mL^{-1}, respectively. The percentage of hemolysis in all extracts was less than 5% indicating the hemolysis effects of the extracts. **Conclusion:** Current study showed that *Spermacoce verticillata* and *Fadogia agrestis* exhibited good antiplasmodial activity *in vitro*. Bio-guided phytochemical studies could lead to interesting natural molecules with antiplasmodial activity.

Key words: Rubiaceae, *Plasmodium falciparum*, antiplasmodial activity, hemolytic activity, total phenolics, total flavonoids

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Malaria is a parasitic endemic caused by *Plasmodium* and transmitted by mosquitoes[1]. Of all types of *Plasmodium*, *P. falciparum* is the most dangerous species and is responsible for 98% of cases worldwide[1]. While 229 million cases and 409,000 deaths in 2019, compared to 241 million cases and 627,000 deaths in 2020 that this disease inflicts globally[1,2]. The increase in both the number of cases and deaths requires attention so that this disease does not continue to bereave families[1].

Burkina Faso is one of the 10 most affected countries in the world by this parasitic disease, with more than 11 million people infected and 4,000 deaths, making it the leading cause of death in community health centers[1].

Despite ongoing efforts to develop a malaria vaccine, bed net distribution, home sprays and prophylaxis remain the main prevention methods and antimalarial drugs remain the only treatment option[3]. To reduce the risk of developing chemotherapy resistance to most antimalarial drugs, the World Health Organization (WHO) recommended artemisinin-based combination therapy (ACT) for the treatment of uncomplicated *Plasmodium falciparum* cases.

The emergence and spread of resistance and the inaccessibility of drugs to poor populations due to their high cost are leading people to resort to medicinal plants[4,5].

Quinine and artemisinin, along with several other secondary metabolites with antimalarial properties, were isolated from *Cinchona ledgeriana* and *Artemisia annua* species, respectively, confirming medicinal plants as a potential drug source[6-10]. Today, 30% of drugs in the pharmaceutical market come from nature[11] and medicinal plants are a popular source of potential antimalarials.

Several families of plants are recognised as having quite interesting properties such as Rubiaceae in the African pharmacopoeia have a great reputation. Plants of this family are used by traditional practitioners in the fight against some pathologies such as oedemas, jaundice, coughs, fevers and malaria[12,13]. The Rubiaceae family with more than 630 genera and about 13,000 species consist of herbaceous plants, annuals or perennials, shrubs, bushes, trees, lianas and epiphytes widely distributed in cold, temperate, subtropical and tropical regions[14].

Spermacoce verticillata L., *Gardenia erubescens* Stapf and Hutch, *Mitracarpus villosus* (Sw.) Cham and Schltdl. E×DC, *Fadogia agrestis* Schweinf. ex Hiern and *Mitragyna inermis* (Willd) O. Ktze are plants from the Rubiaceae family used by traditional practitioners to control this parasite[15]. In this study, the *in vitro* antiplasmodial activity of the extracts of the different plants was evaluated to have a way for potential pharmaceutical development.

MATERIALS AND METHODS

Study area: The acquisition of plant species (leaves, stem barks and whole plants), preparation of extracts, plasmodial strains and evaluation and analysis of the results took a year from October, 2021 to September, 2022. The studies were done at the Nazi Boni University and the National Research and Education Center on Malaria.

Plant material and extraction: As 400 g of leaves and stem barks of *Mitragyna inermis* (Willd) O. Ktze and *Gardenia erubescens* Stapf and Hutch, whole plant of *Spermacoce verticillata* L., *Mitracarpus villosus* (Sw.) Cham and Schltdl. While E×DC and *Fadogia agrestis* Schweinf. ex Hiern were collected in Diaradougou, a village located 15 km Northwest of Bobo-Dioulasso in Burkina Faso. Collected plants have been identified by Dr. Hermann Ouoba, a botanist and cytoecologist at the Nazi Boni University (UNB e×UPB).

The different parts of the plants were pulverised with a blender (Retsch GM 200) and an aluminium mortar and extracted in ethanol, methanol, dichloromethane and hexane by maceration for 24 hrs. Also, a 4 hrs soxhlet extraction with petroleum ether followed by ethanol in 6 hrs was performed. The aqueous extracts obtained by decoction for 15 min were freeze-dried.

Determination of total phenols and total flavonoids

Total phenols: Total phenols were estimated according to the method described by Meda and *et al.*[16]. The colorimetric method described was evaluated[16]. Read the absorbance at 760 nm according to the standard curve for gallic acid. Results were expressed in mg gallic acid equivalent per 100 mg extract (mg EAG/100 mg).

Total flavonoids: Meda *et al.*[16] for the quantification of total flavonoid extracts. Absorbance was read at 415 nm against a quercetin standard curve. Results were expressed as milligram quercetin equivalents per 100 mg of extract (mg EQ 100 mg^{-1}).

In vitro antiplasmodial assays

Parasite culture: Asexual stage *P. falciparum* cultures were established using the method of Trager and Jensen[17] with a slight modification. The used *P. falciparum* strains provided by MR4 were the chloroquine (CQ) sensitive 3D7 and multidrug

resistant Dd2 strains. Parasites were cultured at 2% hematocrit (human A-positive red blood cells O-positive red blood cells) in Roswell Park Memorial Institute (RPMI 1640) medium containing 24 mM sodium bicarbonate (Sigma, Life Science, St. Louis), with the addition of 0.01% hypoxanthine (Sigma Chemical Co., USA), 20 Mm Hepes (Sigma Chemical Co., USA) and 2 mM L-glutamine (Sigma Aldrich, USA) 10 mg mL^{-1} gentamicin (Biosynt carbo synth, AG29671) at 37°C in a standard gas mixture consisting of 1% O_2, 5% CO_2 and 94% N_2.

As 3D7 and Dd2 strains were cultured in the presence of 5% Albumax II (lipid-rich bovine serum albumin, Gibco®, Life Technologie, USA).

Evaluation of the antiplasmodial effect of extracts: The extracts were dissolved either in their extraction solvent or in DMSO (Dimethylsulfoxide). The reference products chloroquine was used as a reference control for the asexual stage of 3D7 and dihydroartemisinin was used for Dd2.

The prepared extracts were put in 96-well plates and the serial dilutions were done with the complete culture medium and the final volume was 100 µL/well. As 100 µL of the parasite culture of parasitemia between 0.5% and 1% were distributed. The final volume and hematocrit were 200 µL/well and 1%, respectively.

The plate was placed in the jar containing water then gasified with mixed gas and incubated for 72 hrs at 37°C. The modified method of Makler and *et al.*[18] was used to assess the parasite Lactate Dehydrogenase production using MALSTAT (mixture of reagents such as Triton×100 (Sigma-Aldrich, 069K049), Lithium L-Lactate (Sigma Code L2250), Trizma-base (Sigma Code T6066) and 3-Acetylpyridine Adenine Dinucleotide (Sigma Code A5251)) and Nitro Blue Tetrazolium (Sigma Code N6876)/ Phenazine EthoSulfate (Sigma Code P4544) (NTB/PES) reagents. The absorbance readings were entered into Excel to calculate the percentages of parasite growth inhibition that were obtained by deducting the values of the negative control. The average growth percentages were transferred to Curve version 5.0, to plot the inhibition curve for determining the IC_{50}. The tests were performed three times.

Hemolytic activity: The hemolytic activity of extracts was performed as described by Jansen *et al.*[19], Laurencin *et al.*[20] and Robert *et al.*[21], with minor modifications. This test was used to detect false positives.

As 5 mL of blood were collected and centrifuged at 2200 rpm for 15 min at 4°C. The blood collection was carried out by adding three times the volume of the pellet of saline phosphate-buffered (PBS) solution. The red blood cells were then diluted to 10% in PBS. The extracts were prepared in two concentrations C1 = 100 and C2 = 50 µg mL^{-1}.

In a 96-well plate, 190 µL of red blood cells were added to 10 µL of the samples in each concentration. Control solutions were also prepared. The extracts and controls were tested in triplicate and incubated under slow shaking for 1 hr.

The solutions were centrifuged at 2200 rpm min^{-1} over 5 min and 150 µL of the supernatant from each tube is placed in a well of a 96 plate. The absorbance's (A) were read with a spectrophotometer (Biotek EL×808) at 630 nm.

The percentage of hemolysis in each extract was determined using the following formula:

$$\text{Hemolysis (\%)} = \frac{\text{A sample tested} - \text{A negative control}}{\text{A positive tested} - \text{A negative control}} \times 100$$

An extract induces hemolysis if the percentage of hemolysis is higher than 5%[19-21].

Statistical analysis: The statistical analysis (calculation of means, standard deviations and p-values) was done with the IBM SPSS Statistics 25.0. software. The IC_{50} is the dose that 50% inhibition of parasite viability, calculated from sigmoidal dose-response curves using Gen5 1.10 software available on a plate reader [Biotek].

RESULTS

Total phenolics: The contents of total phenolics were recorded in Table I. For *M. inermis*, values varied from 3.07±0.09 to 48.95±0.11 mg GAE/100 mg extract and the aqueous extract and the ethanolic macerated leaves were the most enriched with 47.56±3.64 and 48.95±0.11 mg EAG/100 mg extract, respectively. All extracts from the leaves of *G. erubescens* had these but were more concentrated in the ethanolic macerate leaves with 42.95±0.75 mg EAG/100 mg extract and the methanolic with 49.49±4.38 mg EAG/100 mg extract. For whole plants, total phenolics are found in all extracts except hexanic ones. The ethanolic macerate of *S. verticillata* had 39.95±1.82 mg GAE/100 mg, *F. agrestis* with 20.71±1.18 mg GAE/100 mg and *M. villosus* with 34.49±2.78 mg GAE/100 mg for the methanolic extract had the high contents. The dichloromethane extract of *S. verticillata* had the highest amount of 10.14±0.65 mg GAE/100 mg. Among the hexane extracts, the whole plant extract of *F. Agrestis* had the highest concentration of 7.43±0.45 mg GAE/100 mg.

Table 1: Total phenolics content of extracts

	Total phenolics (mg GAE/100 mg extract)					
Extracts	H_2O	Macerated EtOH	Soxhlet EtOH	MeOH	DCM	Hexane
BMi	9.75±0.32^{bc}	25.21±0.89^{jkl}	24.42±0.30^{ijkl}	18.64±0.57^{gh}	ND	ND
LMi	47.56±3.64^{p}	48.95±0.11^{pq}	14.03±1.18^{de}	16.35±0.87^{efg}	8.43±0.69^{b}	3.07±0.09^{a}
BGe	21.86±2.66^{ghij}	41.24±1.61^{o}	13.82±0.11^{de}	32.78±3.02^{m}	ND	ND
LGe	26.28±1.94^{kl}	42.95±0.75^{o}	32.9±0.5^{m}	49.49±4.38^{q}	7.36±0.54^{b}	6.7±0.12^{ab}
S.v	20.14±0.21^{gh}	39.95±1.82^{no}	27.85±0.4^{l}	34.42±3.09^{m}	10.14±0.65^{bcd}	ND
F.a	13.21±0.62^{cde}	18.14±1.31^{fg}	12.43± 1^{cde}	20.71±1.18^{ghi}	7.07±0.37^{b}	7.43±0.45^{b}
M.v	14.43±0.21^{ef}	23.14±0.93^{hij}	25.5±1.3^{jkl}	34.49±2.78^{m}	7.21±0.45^{b}	ND

BMi: Barks of *Mitragyna inermis*, Lmi: Leaves of *Mitragyna inermis*, Bge: Barks of *Gardenia erubescens*, Lge: Leaves of *Gardenia erubescens*, S.v: Whole plants of *Spermacoce verticillata*, F.a: Whole plants of *Fadogia agrestis* and M.v: Whole plants of *Mitracarpus villosus,* ND: Not determined, Values are Mean±Standard Deviation (n = 3), Means in each column followed by a different letter are significantly different (p<0.05)

Table 2: Total flavonoids contents of extracts

	Total flavonoids (mg EQ/100 mg extract)					
Extracts	H_2O	Macerated EtOH	Soxhlet EtOH	MeOH	DCM	Hexane
BMi	0.41±0.01^{a}	1.51±0.08^{efg}	2.18±0.08^{hi}	1.32±0.17^{cdef}	ND	ND
LMi	4.32±0.05^{op}	4.02±0.38^{no}	5.53±0.05^{rs}	3.76±0.24^{mno}	1.19±0.14^{bcde}	0.78±0.04^{abc}
BGe	3.66±0.58^{mn}	1.91±0.35^{fgh}	2.62±0.08^{ijk}	2.12±0.09^{ghi}	ND	ND
LGe	4.05±0.24^{no}	5.45±0.12^{r}	3.36±0.09^{lm}	2.33±0.24^{hij}	0.31±0.01^{a}	1.4±0.05^{def}
S.v	5.11±0.13^{qr}	6.12±0.08st	6.80±0.28^{u}	5.40±0.08^{r}	1.26±0^{cde}	ND
F.a	4.95±0.12^{qr}	2.94±0.21^{jkl}	4.10± 0.12^{op}	4.69±0.21^{pq}	1.51±0.29^{efg}	0.60±0.04^{ab}
M.v	3.00±0.13^{kl}	6.28±0.08^{tu}	6.43±0.08^{tu}	6.30±0.12^{tu}	0.85±0.32^{abcd}	ND

BMi: Barks of *Mitragyna inermis*, Lmi: Leaves of *Mitragyna inermis*, Bge: Barks of *Gardenia erubescens*, Lge: Leaves of *Gardenia erubescens*, S.v: Whole plants of *Spermacoce verticillata*, F.a: Whole plants of *Fadogia agrestis* and M.v: Whole plants of *Mitracarpus villosus*, ND: Not determined, Values are Mean±Standard Deviation (n = 3), Means in each column followed by a different letter are significantly different (p<0.05)

Table 3: Antiplasmodial activity of extracts against the 3D7 asexual stages of *P. falciparum*

	IC_{50} (µg mL^{-1})					
Extracts	H_2O	Macerated EtOH	Soxhlet EtOH	MeOH	DCM	Hexane
BMi	>50	>50	>50	>50	32.97±0.48^{d}	>50
LMi	>50	27.13±2.29^{c}	>50	>50	10.05±0.27^{a}	>50
BGe	>50	>50	>50	>50	45.94±1.39^{f}	>50
LGe	>50	>50	>50	>50	>50	>50
S.v	>50	7.34±0.98^{a}	6.86±0.39^{a}	26.31±1.62^{c}	15.34±0.55^{b}	>50
F.a	>50	9.56±0.63^{a}	23.68±1.26^{c}	34.99±6.02^{d}	33.56±0.54^{d}	>50
M.v	>50	24.42±2.8^{c}	>50	>50	40.03±0.47^{e}	>50
Chloroquine			8.64±1.73*			

BMi: Barks of *Mitragyna inermis*, Lmi: Leaves of *Mitragyna inermis*, Bge: Barks of *Gardenia erubescens*, Lge: Leaves of *Gardenia erubescens*, S.v: Whole Plants of *Spermacoce verticillata*, F.a: Whole plants of *Fadogia agrestis* and M.v: Whole plants of *Mitracarpus villosus*. *Control, Values are Mean±Standard deviation (n = 3), Means in each column followed by a different letter are significantly different (p<0.05)

Total flavonoids: The total flavonoids contents were expressed in milligrams of quercetin equivalent per 100 mg of extract (mg EQ/100 mg extract) and were shown in Table 2. The contents varied from 0.31±0.01 to 6.80±0.28 mg QE/100 mg extract. All the extracts of *S. verticillata* were the richest with values of 5.11±0.13, 6.12±0.08, 6.80±0.28 and 5.4±0.08 mg EQ/100 mg extract for water, ethanolic macerate, ethanolic soxhlet and methanol, respectively. For dichloromethane and hexane extracts, the whole plant of *F. agrestis* and the leaves of *M. inermis* had high concentrations of 1.51±0.29 and 0.78±0.04 mg EQ/100 mg extract, respectively.

Antiplasmodial activity: Five plants were extracted with different solvents (water, ethanol, methanol, dichloromethane and hexane). The antiplasmodial activity of 42 extracts was tested on the asexual stages of the chloroquine-sensitive *P. falciparum* strain 3D7 and the results were reported in Table 3. All aqueous and hexanic extracts of the five plants, ethanolic extracts of *M. inermis* and methanolic extracts of *G. erubescens* did not leaves of *M. inermis* which had a moderate effect with an inhibit parasite growth except inhibit parasite growth except for the ethanolic macerated IC_{50} of 27.13±2.29 µg mL^{-1}. Ethanolic extracts, whether macerated or soxhlet, considerably inhibited parasite growth with

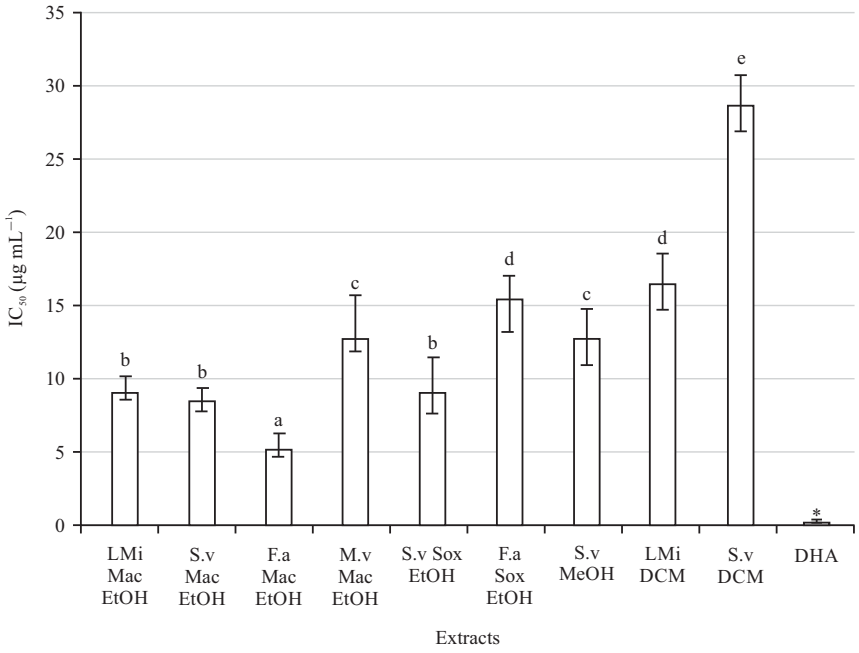

Fig. 1: Antiplasmodial activity of the extracts against the asexual Dd2 stages of *P. falciparum*

LMi: Leaves of *Mitragyna inermis*, S.v: Whole plants of *Spermacoce verticillata*, F.a: Whole plants of *Fadogia agrestis* and M.v: Whole plants of *Mitracarpus villosus*. DHA: Dihydroartemisinin, *Control. Values are Mean±Standard deviation (n = 3), Means in each stick followed by a different letter are significantly different (p<0.05)

Table 4: Hemolytic power of extracts

		Percentage of hemolysis (%)					
Extracts	Concentrations (µg mL^{-1})	H_2O	Macerated EtOH	Soxhlet EtOH	MeOH	DCM	Hexane
BMi	100	0.07	0	0	0	0.03	0
	50	0	0	0	0	0	0
LMi	100	0	0.11	0	0.09	0	0
	50	0	0	0	0	0	0
BGe	100	0.17	0.04	0	0	0	0
	50	0.09	0.03	0	0.13	0	0
LGe	100	0	0.08	0.12	0	0	0
	50	0	0	0	0	0	0
S.v	100	0	0	0	0	0	0
	50	0	0	0	0	0	0
F.a	100	0	0.1	0	0.04	0	0.05
	50	0	0	0	0.03	0	0
M.v	100	0	0.08	0	0.24	0	0
	50	0	0.17	0	0.17	0	0
NC				0			
PC				100			

PC100BMi: Barks of *Mitragyna inermis*, LMi: Leaves of *Mitragyna inermis* , BGe: Barks of *Gardenia erubescens,* LGe: Leaves of *Gardenia erubescens*, S.v: Whole plants of *Spermacoce verticillata*, F.a: Whole plants of *Fadogia agrestis*, M.v: Whole plants of *Mitracarpus villosus*, NC: Negative control and PC: Positive control

IC_{50} of 7.34±0.98 and 6.86±0.39 µg mL^{-1} for *S. verticillata*, 9.56±0.63 and 23.68±1.26 µg mL^{-1} for *F. agrestis*. Only the ethanolic macerate of *M. villosus* inhibited parasite growth with an IC_{50} of 24.42±2.8 µg mL^{-1}. For the dichloromethane extracts, *M. inermis* leaves and the whole *S. verticillata* plant was active with IC_{50} of 10.05±0.27 and 15.34±0.55 µg mL^{-1}.

All the extracts having an IC_{50} less than or equal to 26 µg mL^{-1} were tested on the Dd2 strain and the results were reported in Fig. 1. All extracts inhibit parasite growth with IC50s ranging from 5.38±1.83 (S.v Mac EtOH) to 28.63±3.39 µg mL^{-1} (S.v DCM). The ethanolic macerates of the whole plants of *S. verticillata*, *F. agrestis*, *M. villosus* and

leaves of *M. inermis* were active on *Plasmodium* with IC_{50} of 8.56±0.18, 5.38±1.83, 12.84±0.84 and 9.19±0.8 μg mL^{-1}, respectively. Ethanolic soxhlet extracts of whole plants of *S. verticillata*, *F. agrestis* inhibit parasite growth with IC_{50}s of 9.17±1.67 and 15.64±1.64 μg mL^{-1}. Methanolic extract of *S. verticillata* inhibited with an IC_{50} of 12.81± 3.54 μg mL^{-1} dichloromethane extracts, *S. verticillata* (28.63±3.39 μg mL^{-1}) inhibited less than *M. inermis* leaves (16.72±1.82 μg mL^{-1}).

Hemolytic activity: The results of the hemolytic power were presented in Table 4. Most of the aqueous extracts had a hemolysis percentage equal to 0 except for *M. inermis* (C = 100 μg mL^{-1}) and *G. erubescens* (C = 50 and 100 μg mL^{-1}) barks with 0.07, 0.17 and 0.09%. The ethanolic extracts with soxhlet, only the leaves of *G. erubescens* had a hemolysis percentage of 0.12%. All the dichloromethane extracts had a hemolysis percentage of 0 except for the barks of *M. inermis* with 0.03%. All the types extract, the methanolic extract of *M. villosus* which has the highest percentage of 0.24%. This percentage was strictly less than 5% which means that none of the extracts induced hemolysis.

DISCUSSION

Total phenolics and flavonoids were determined in this study. These compounds are known to possess very interesting biological properties[15]. Phytochemical screening helps revealed the chemical properties of plant extracts components. It can also be used to find bioactive agents that can be used to synthesize very useful pharmaceuticals[22]. All extracts were rich in these compounds but at different levels from one plant to another (Table 1, 2). This could be explained by the plant species, the type of solvent, the extraction method, the extraction time and temperature[23]. These compounds are known to possess antiplasmodial activities[24-26]. Gossipol, a polyphenol, isolated from cotton (*Gossypium* sp.) has also been found to be active *in vitro* on *Plasmodium falciparum*. Some flavonoids (artemetin, casticin) have antispasmodic activity but at high doses[27]. Like flavonoids extracted from *Artemisia indica*, exigua flavanone A and exigua flavanone B showed antispasmodic activities *in vitro* (IC_{50} = 4.6 and 7.0 μg mL^{-1}, respectively)[28].

Resistance of *P. falciparum* to chemical treatment remained important. New plant sources with anti-plasmodial activity and low toxicity could be a viable, low-cost and easily accessible alternative for the poor communities where these species are found[22]. Therefore, natural products isolated from plants used in traditional medicine possess potent antimalarial activity and are potential sources of novel antimalarial drugs[29-31].The antiplasmodial activity was evaluated spectrophotometrically by measuring the production of the enzyme lactate dehydrogenase of the parasite. All aqueous extracts of the five plants did not inhibit parasite growth. There are several reasons for the lack of activity: Traditional methods are not reproduced in the laboratory, possible degradation of the active ingredients during extraction, effectiveness is dependent on plant associations and no direct action on the parasite[32,33]. All extracts from the leaves and barks of *M. inermis* and *G. erubescens* were unable to inhibit parasite growth of strain 3D7 except of the ethanolic macerate of *M. inermis* leaves which had an IC_{50} of 24.52±5.06 μg mL^{-1} and dichloromethane extracts (Table 3). However, Soré *et al.*[26] had found for aqueous, ethanolic and methanolic extracts of *M. inermis* leaves had IC_{50}s of 21.6±6.1, 25.9±11.7 and 42.54±11.4 μg mL^{-1}. This difference could be explained by he nature of the soil, the type of microclimate and also the bioclimatic stages where these plants grow[23]. Ethanolic macerates of the plant of *S. verticillata* and *F. agrestis* showed very interesting activities with IC_{50}s of 7.34±0.98, 9.56±0.63 μg mL^{-1} for strain 3D7 and 8.56±0.18, 5.38±1.83 μg mL^{-1} for strain Dd2, respectively (Fig. 1). The different activities of these plants could be due to the presence of these chemical compounds in the extracts[34,35]. Originally from America, *S. verticillata* was found in the Cape Verde Islands, tropical Africa and Madagascar and is used in traditional medicine for the treatment of diseases in internal use such as malaria with gastrointestinal pain, dysmenorrhea, female sterility, infantile diarrhea, bilharzia, gonorrhea, paralysis, furunculosis, antivomiting and as a uterine and milk booster[15].

de sa Peixoto Neto *et al.*[36] revealed the antimicrobial activity of a methanol extract from the roots of *S. verticillata*. The extract showed high antimicrobial activity against six different strains of *Pseudomonas aeruginosa*, with an inhibition range of 10-18 mm, even against resistant strains. Molecules such as plant-derived isoborreverin and borreverine also showed significant and broad-spectrum antimicrobial activity against both Gram-positive and Gram-negative bacteria[37] and may have antiplasmodial properties. The *in vivo* oral administration of the ethanolic extracts of the whole plant of *S. verticillata* on mice at a dose of 250 mg kg^{-1} of body weight had a percentage reduction in parasitemia of 27.6%[38].

Fadogia agrestis ranges from Mali to Nigeria to Cameroon, the Central African Republic and Sudan and is used in the treatment of fever, chills, malaria, rickets, dysentery, nephritis, convulsions and anorexia[22]. Chloroformic, methanolic and ethyl acetate extracts of *F. agrestis* bark showed moderate inhibitory activity on Gram-negative and Gram-positive disease-causing bacteria, the most inhibited being *Proteus vulgaris*, *Bacillus subtilis* and *Escherichia coli*[22]. The ethanolic

extract tested *in vivo* on *Plasmodium berghei* had a percentage inhibition of parasitemia of 40.3%[39].

The anti-plasmodial activity of extracts could be influenced by extract-induced hemolysis. None of the extracts induced hemolysis as their hemolysis percentages were all below 5% (Table 4).

Studies on these plants used by traditional practitioners against malaria have shown very interesting activities in some parts, which is proof of their potentials. These plants could be an alternative in the fight against this parasitosis. The isolation of the molecules responsible for these activities are envisaged.

CONCLUSION

The total phenolics and flavonoids, the *in vitro* antiplasmodial activity and the cytotoxicity of the extracts of five plants have been investigated in this study. All the plant extracts had total phenolics, total flavonoids, condensed tannins. The methanolic macerate of *G. erubescens* leaves had a high content of total phenolics. For total flavonoids, the soxhlet ethanolic extract of the whole plant of *S. verticillata* was the most enriched. The ethanolic macerates of the wholeplant of *S. verticillata* and *F. agrestis* significantly inhibited parasite growth and had effects classified as moderate to promising. *Spermacoce verticillata* and *Fadogia agrestis* could contain very interesting molecules. However, a bioguided fractionation could have very good activity. None of the extracts had hemolytic activity. These results confirm the appropriateness of their use in traditional medicine.

SIGNIFICANCE STATEMENT

In plant extracts, secondary metabolites such as total phenolics and flavonoids were quantified. Also, their antiplasmodial activity was evaluated and the plants studied were able to inhibit the growth of parasites in the asexual stages. This study confirms that these plants are sources of antiplasmodial molecules.

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