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UPLC-ESI-QTOF-MS/MS PREDICTION AND IN VITRO ANTIPLASMODIAL ACTIVITY OF ETHANOLIC EXTRACTS OF DIFFERENTS PARTS OF SPATHODEA CAMPANULATA P. BEAUV. (BIGNONIACEAE)

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ABSTRACT

Malaria remains the first medical concern and a public health problem in many countries of the world, including Cameroon, where almost the entire population is exposed to the risk of infection. Given the presence of *Plasmodium* strains resistant to artemisinin derivatives reported in Southeast Asia and its emergence in East Africa, new strategies oriented towards the discovery of new antimalarial molecules are necessary and particularly urgent in an unfavorable economic and social context. Apart from conventional molecules, pharmacopoeia and traditional Cameroonian medicine constitute frequent recourses in the management of malaria by families. The objective of this work was to study the antiplasmodial activity of different organs of the plant *Spathodea campanulata* P. Beauv., also called tulip tree of Gabon, a tree used in traditional medicine in the management of malaria. Phytochemical screening was carried out on the various ethanolic

extracts obtained from the plant (flowers, leaves, branches, stem bark, roots) with the aim of identifying the various groups of secondary metabolites. Analysis by Ultra Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS) was carried out in order to identify the secondary metabolites present. The determination of the antiplasmodial activity of the extracts of *Spathodea campanulata* was carried out *in vitro* on the multi-resistant strain *Pf* Dd2 by measuring the growth of the parasites in the microtiter plates based on the reading of DNA fluorescence at the SYBER Green 1. Artemisinin and chloroquine were chosen as the reference antiplasmodial. The phytochemical profile of the extracts of the different organs (flowers, leaves, branches, stem bark, roots) showed the presence of sterols, polyphenols, saponins, polyterpenes and flavonoids. UPLC-MS analysis enabled us to identify in the different parts of *Spathodea campanulata*, 10 compounds: cinnamic acid, β-sistosterol, quercetin, naringinin, catechin, loganic acid, β-sitosterol 3-*O*-β-D-glucopyranoside, syringic acid and *n*-hexadecanoic acid (palmitic acid). The study of the antiplasmodial activity showed that the ethanolic extract of the leaves and stem bark at 10 µg/ml reduced the viability of the *Pf* Dd2 strains with values of IC_{50} at 93.68 µg/ml and 79.68 µg/ml respectively. Chloroquine and artemisinin gave IC_{50}s of 0.2953 and 0.02867 µg/ml respectively. It emerges from this study that only the leaves and stem bark of *S. campanulata* would present a weak activity on the *Pf*Dd2 strain, with the stem bark as the most active part. The abundance of polyphenolic compounds and the antiplasmodial activity of ethanolic extracts of leaves and stem bark of *S. campanulata* could confirm their good potential therapeutic properties attributed in the traditional pharmacopoeia to cure malaria.

KEYWORDS: *Spathodea campanulata,* phytochemical screening, UPLC-MS/MS, antiplasmodial activity.

1. INTRODUCTION

In 2020, there were an estimated 241 million cases of malaria worldwide, and 627,000 deaths attributable to malaria in the same year.[1] The WHO African Region bears a large and disproportionate share of the global malaria burden. In 2020, 95% of malaria cases and 96% of deaths due to the disease were recorded in this Region. Children under the age of 5 accounted for an estimated 80% of all malaria deaths in the Region.[1] In Cameroon, health facilities reported 1,722,188 cases of malaria, including 11,233 deaths in 2019.[2] In Rwanda, studies revealed a slower onset of action of CTAs in over 10% of patients, with prevalences of artemisinin resistance mutations also higher than those detected in previous studies.[3,4]

The work of Balikagala et al. in Uganda also highlighted mutations that appeared independently of those found in Rwanda and associated with artemisinin resistance.[5] The spread of such CTA-resistant strains in Africa would therefore be disastrous in the face of alternative molecules not currently available. Research into new anti-malarial molecules could eventually help to offer new drugs for combination therapy, and current malaria control strategies are geared towards the discovery of new, effective antimalarial molecules at lower cost.[6] Indeed, plants have the particularity of being an immense reservoir of structurally and pharmacologically diverse compounds known as metabolites. The search for new pharmacologically active agents through the screening of natural sources has led to the discovery of a large number of useful drugs which play a major role in the treatment of many human diseases, including malaria.[7,8,9,10] Furthermore, the World Health Organization (WHO) estimates that in Africa and Asia, 80% of the population use traditional medicines rather than modern ones for healthcare.[8] In Cameroon, several plant species are used to treat infectious diseases, often without any scientific proof of efficacy. Recourse to this practice is even more common today, due to the ever-increasing cost of specialties and services in health centers (public or private), as well as undesirable side effects, the unavailability of medicines in rural areas and the sale of counterfeit drugs.[9,10] This is how we chose to focus our work on evaluating the anti-plasmodial activity of the various organs (flowers, leaves, branches, stem bark, roots) of *Spathodea campanulata* P. Beauv. also known as the Gabon tulip tree, a member of the Bignoniaceae family and native to West and Central Africa. This plant is generally used as an ornamental and shade tree, but has numerous medicinal applications in both its native and introduced regions. In traditional medicine, extracts of its leaves are used to treat malaria.[11] The aim of the present work is to evaluate the potential of different parts (flowers, leaves, branches, stem bark and roots) of *S. campanulata*, and to determine the most active part of the plant in order to move towards possible new molecules that can be used in combinations.

2. MATERIALS AND METHODS

2.1. Plant materials collection

The flowers, leaves, branches, stem bark and roots of *S. campanulata* were collected in the early hours of the morning in Yaoundé in the Centre region, Nyong-et-Mfoumou Department, more precisely in Ayos, and identified by Mr NANA at the Herbier National du Cameroun by comparison with the specimen bearing number 50085/HNC.

2.2. Extraction

After harvesting, the various plant organs (flowers, leaves, branches, stem bark and roots) of *S. campanulata* were dried at room temperature in the shade for four (04) weeks, protected from humidity. Then, using an electric grinder, the various parts were ground separately to a fine powder. The powder from each part was macerated in a 70/30 (v/v) ethanol/water solvent system for 72 hours under magnetic stirring at room temperature. The macerates obtained were filtered twice on hydrophilic cotton with a mesh width of 1.5 cm, then once on Whatman chromatographic paper n°1 (25 mm in diameter). The filtrates obtained were concentrated using a rotary evaporator at 40°C, then freeze-dried to remove any residual water present in the extracts.

2.3. Phytochemical analysis

2.3.1. Preliminary hytochemical screening

Detailed phytochemical screening was performed on the ethanolic extract of *A. affinis* organs using standard methods, as reported in the literature.[12,15] Other specific phytochemical tests were also realized, all based on a precipitation reaction via the generation of insoluble complexes called precipitates, and on colorimetry reaction through the formation of colored soluble chemical species. The colored reactions were carried out in test tubes in the presence of the positive controls. The following tests were carried out: Drangendorff test (alkaloids), Tannins test (gallic tannins), Libermann Buchard test (steroids and triterpenoids), Shinoda test (flavonoids), Cardiotonic glycosides test (cardiotonic glycosides), Borntrager test (anthraquinones), Foam Index test (saponins), $FeCl_3$ test (polyphenols), Potash test (coumarin) and Reducing Sugars test. All observations were recorded.

2.3.2. UPLC-MS/MS analysis for determination of individual compound groups

Samples were analyzed on a Bruker Daltonics Compact QTOF mass spectrometer using a positive-mode electrospray ionization probe. The mass spectrometer was coupled to a Thermo Scientific Ultimate 3000 Dionex UHPLC system consisting of an RS WPS-3000 autosampler, RS HPG-3400 pump and RS DAD-3000 detector, using an Acclaim RSLC 120 C18, 2.2 µm, 2.1 x 100 mm column (P/N 068982) at 40ºC, flow rate 0.3 ml/min, solvents: Water (H_2O) and acetonitrile (MeCN), each solvent containing 0.1% formic acid (FA). A stepwise gradient condition method was performed with a constant ramp between segments and defined as follows: 0-5 min (H_2O-MeCN-AF, 95:5:0.1, v/v/v), 10-15 min (H_2O-MeCN-AF, 85:15:0.1, v/v/v), 20-25 min (H_2O-MeCN-AF, 75:25:0.1, v/v/v), 30-35 min (H_2O-

MeCN-AF, 65:35:0.1, v/v/v), 40-45 min (H_2O-MeCN-FA, 45:55:0.1, v/v/v). Final sample concentration was 1 mg/ml and injection volumes were 10 µl. MS source parameters in positive mode were defined as follows: End plate offset 500 V, capillary voltage 4500 V, nebulizer pressure 3.0 bar, dry gas flow 9.0 l/min, dry temperature 220°C.

2.4. Evaluation of antiplasmodial activity

The *Plasmodium falciparum* Dd2 strain (multidrug resistant) was obtained from the Biodefense and Emerging Infections (BEI) Research Resources (Manassas, VA) and maintained using a modified method from Trager and Jensen in 1976.[16] Parasites were grown in fresh human O^+ red blood cells from healthy volunteer donors after PCR confirmation at 3% (v/v) hematocrit in RPMI 1640 culture medium containing glutamax and $NaHCO_3$ (Gibco, UK) supplemented with 25 mM HEPES (Gibco, UK), hypoxanthine1X (Gibco, USA), 20 µg/ml gentamicin (Gibco, China), and 0.5% Albumax II (Gibco, USA). Stock solutions of the extracts were diluted with unsupplemented RPMI 1640 culture medium. Assays were carried out in 96-well plates, each containing 100 µg/ml of extract from different parts of the test plant, 0.2% dimethyl sulfoxide (DMSO) and parasite inoculum (1% *Pf*Dd2 parasitemia, 2% hematocrit) in a final volume per culture of 100 µl. Artemisinin and Chloroquine at 1 µM were used as references. After 72 h incubation at 37°C, the plate was stored at -20°C until further processing. After thawing, hemolyzed parasite suspensions were collected from each well and transferred to a new plate containing 3X concentrated SYBR Green I lysis buffer. The plate was kept for 30 min in the dark, after which fluorescence was measured using a spectrophotometer (Fluoroskan Ascent multiwell) with excitation and emission wavelengths at 485 and 538 nm respectively.[17]

2.5. Data processing methods

Results were expressed as percentage reduction in parasitaemia compared with artemisinin and chloroquine, chosen as reference, and inhibitory concentration 50 (IC_{50}) values were determined from drug dose response curves. Each sample was tested in triplicate. GraphPadPrism 8.0 and Microsoft office Excel 2010 were used to process the data, plot the curves and calculate the IC_{50}s of the extracts tested.

3. RESULTS AND DISCUSSION

3.1. Results

After filtration and evaporation of the ethanol using a rotary evaporator, we obtained brown crude extracts of the various parts with some residual water. Freeze-drying enabled us to

eliminate the quantities of water contained in the extracts in order to obtain a leg of each part, and the yields were calculated. The ethanolic extract of *S. campanulata* flowers showed the highest yield at 3.25%, while the ethanolic extract of the roots showed the lowest yield at 2.25%. The other plant parts showed a yield of 2.27%.

3.1.1. Phytochemical analysis

3.1.1.1. Preliminary phytochemical screening

The results in Table 1 show the different groups of compounds tested: in the ethanolic extract of flowers (EESCFl), sterols, polyphenols and saponins were present, while polyterpenes, flavonoids and anthraquinones were absent. The ethanolic extract of leaves (EESCLe) contains 4 groups of compounds including polyterpenes, flavonoids, saponins and polyphenols; and the absence of sterols and anthraquinones. The ethanolic extract of branches (EESCb) and of Stem bark (EESCSb) contains 4 groups of compounds including sterols, flavonoids, saponins and polyphenols, and an absence of polyterpenes and anthraquinones. The ethanolic extract (EESCr) of roots contains 2 groups of compounds, including saponins and polyphenols, and an absence of sterols, polyterpenes, flavonoids and anthraquinones.

Table 1: Phytochemical constituents.

Types of tests	Phytochemicals	Extracts				
		EESCFl	EESCLe	EESCb	EESCSb	EESCr
Libermann Buchard	Sterols Polyterpènes	+ -	- +	+ -	+ -	- -
Schinoda	Flavonoïds	-	+	+	+	-
Anthraquinones	Anthraquinones	-	-	-	-	-
Foam index	Saponins	+	+	+	+	+
Ferric Chloride ($FeCl_3$)	Polyphenols	+	+	+	+	+

Legend: EESCFl = ethanolic extract of S. campanulata flowers; EESCLe = ethanolic extract of S. campanulata leaves; EESCb = ethanolic extract of S. campanulata branches; EESCSb = ethanolic extract of S. campanulata stem bark; EESCr = ethanolic extract of S. campanulata roots.

3.1.1.2. UPLC-MS/MS analysis for determination of individual compound groups

3.1.1.2.1. Flowers

Figure 1 shows the UPLC-MS results for the ethanolic extract of *S. campanulata* flowers.

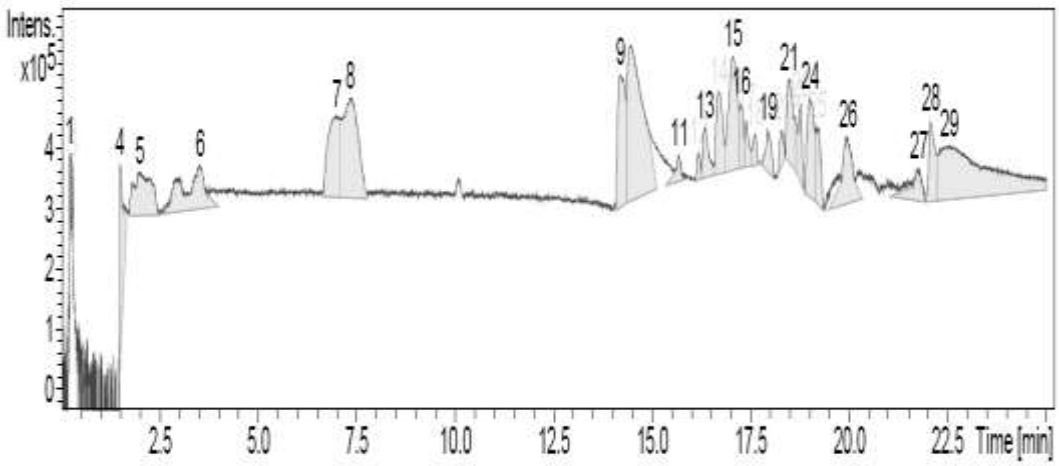

Figure 1: Chromatogram of the ethanolic extract of *Spathodea campanulata* flowers at a concentration of 1 mg/ml.

UPLC-MS analysis of the ethanolic extract of the flowers revealed the presence of 29 peaks, 10 of which were identified and corresponded to 9 compounds (Table 2, Figure 2).

Table 2: Annotation by UPLC-HR-ESI-MS/MS of the main compounds in ethanolic extract from *Spathodea campanulata* flowers.

Peak N°	t_R (min)	Structure N°	*m/z* $[M]^+$	*m/z* $[M + H]^+$	*m/z* $[M + 2H]^+$	*m/z* $[M + K]^+$	*m/z* $[M + Na]^+$	*m/z* $[M + COOH]^+$	Annotation	Molecular formula
4	1.5	1				186.9909			Cinnamic acid	$C_9H_8O_2K$
5	2.0	2	197.0868						Syringic acid	$C_9H_{10}O_5$
6	4.0	3		415.2077		453.3390			β-sistosterol	$C_{29}H_{51}O$
12	16.5									
7	7.0	4				340.2759 and 340.2757			Quercetin	$C_{15}H_{10}O_7K$
8	7.5									
9	14.5	5			274.2718				Naringinin	$C_{15}H_{13}O_5$
10	15.0	6	290.2666						Catechin	$C_{15}H_{14}O_6$
11	16.0	7					387.1765		Longanic acid	$C_{16}H_{24}O_{10}Na$
24	19.0	8						621.3015 and 621.3009	β-sitosterol 3-*O*-β-D-glucopyranoside	$C_{36}H_{61}O_8$
25	19.5									

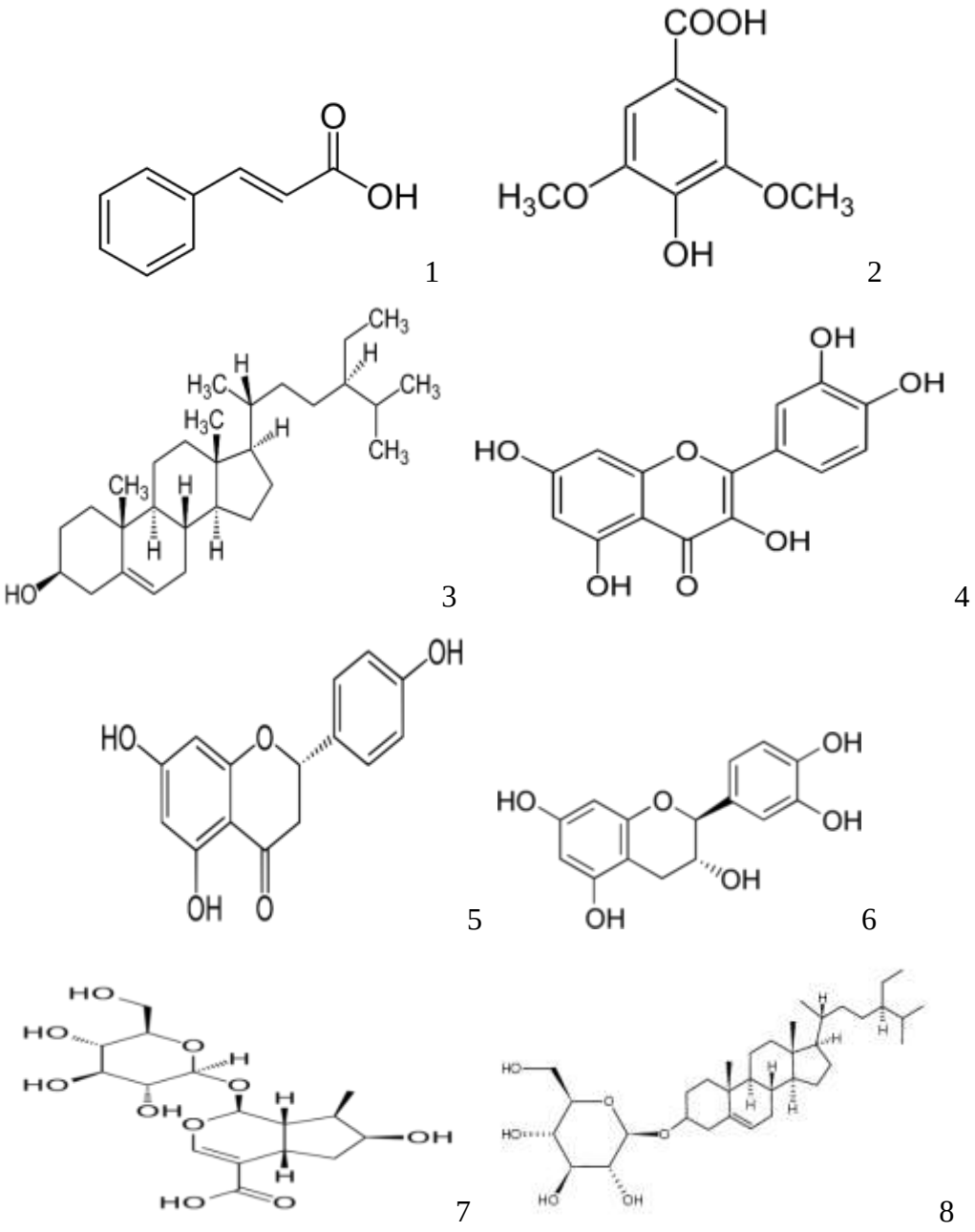

Figure 2: Chemical structures of annotated compounds in ethanolic extract from *Spathodea campanulata* flowers.

3.1.1.2.2. Leaves

Figure 3 shows the UPLC-MS results for the ethanolic extract of *S. campanulata* leaves.

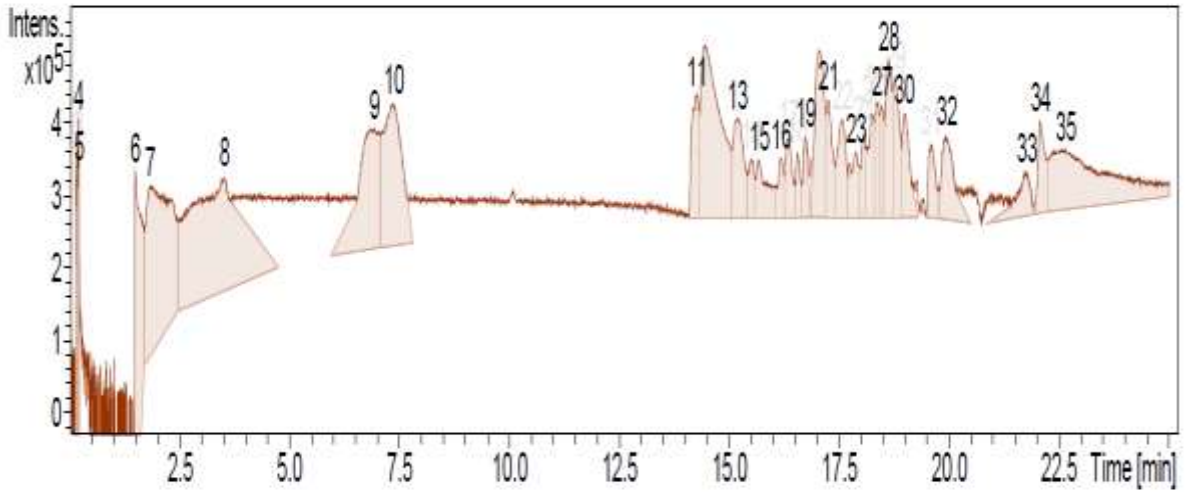

Figure 3: Chromatogram of the ethanolic extract of *Spathodea campanulata* leaves at a concentration of 1 mg/ml.

UPLC-MS analysis of the ethanolic extract of the leaves revealed the presence of 35 peaks, 5 of which were identified and corresponded to 4 compounds (Table 3, Figure 4).

Table 3: Annotation by UPLC-HR-ESI-MS/MS of the main compounds in ethanolic extract from *Spathodea campanulata* leaves.

Peak N°	t_R (min)	Structure N°	*m/z* $[M]^+$	*m/z* $[M + H]^+$	*m/z* $[M + K]^+$	Annotation	Molecular formula
7	2.0	1			186.9909	Cinnamic acid	$C_9H_8O_2K$
8	3.5	2		415.2089	453,3398	β-sistosterol	$C_{29}H_{51}O$
16	16.5						
10	7.5	3			340.2569	Quercetin	$C_{15}H_{10}O_7K$
12	14.5	4	290.2674			Catechin	$C_{15}H_{14}O_6$

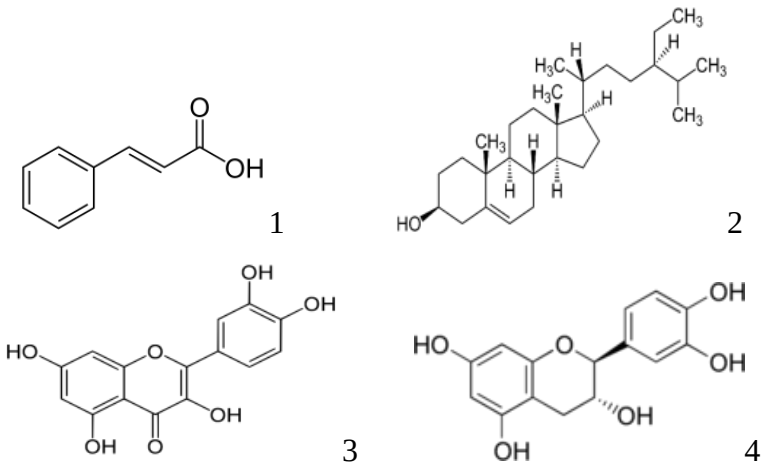

Figure 4: Chemical structures of annotated compounds in ethanolic extract from *Spathodea campanulata* leaves.

3.1.1.2.3. Branches

Figure 5 shows the UPLC-MS results for the ethanolic extract of *S. campanulata* branches.

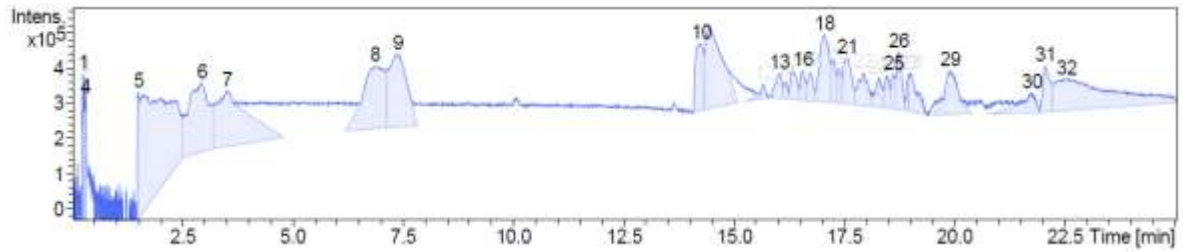

Figure 5: Chromatogram of the ethanolic extract of *Spathodea campanulata* Branches at a concentration of 1 mg/ml.

UPLC-MS analysis of the ethanolic extract of the Branches revealed the presence of 32 peaks, 7 of which were identified and corresponded to 6 compounds (Table 4, Figure 6).

Table 4: Annotation by UPLC-HR-ESI-MS/MS of the main compounds in ethanolic extract from *Spathodea campanulata* branches.

Peak N°	t_R (min)	Structure N°	*m/z* $[M]^+$	*m/z* $[M + H]^+$	*m/z* $[M + K]^+$	*m/z* $[M + Na]^+$	*m/z* $[M + COOH]^+$	Annotation	Molecular formula
4	0.5	1			186.9915			Cinnamic acid	$C_9H_8O_2K$
16	16.5	2	197.0868					Syringic acid	$C_9H_{10}O_5$
7	3.5	3		415.2096	453.3414			β-sistosterol	$C_{29}H_{51}O$
14	16.5								
11	15.0	4	290.2681					Quercetin	$C_{15}H_{10}O_7K$
12	15.5	5				387.1793		Longanic acid	$C_{16}H_{24}O_{10}Na$
28	19.0	6					621,3071	β-sitosterol 3-*O*-β-D-glucopyranoside	$C_{36}H_{61}O_8$

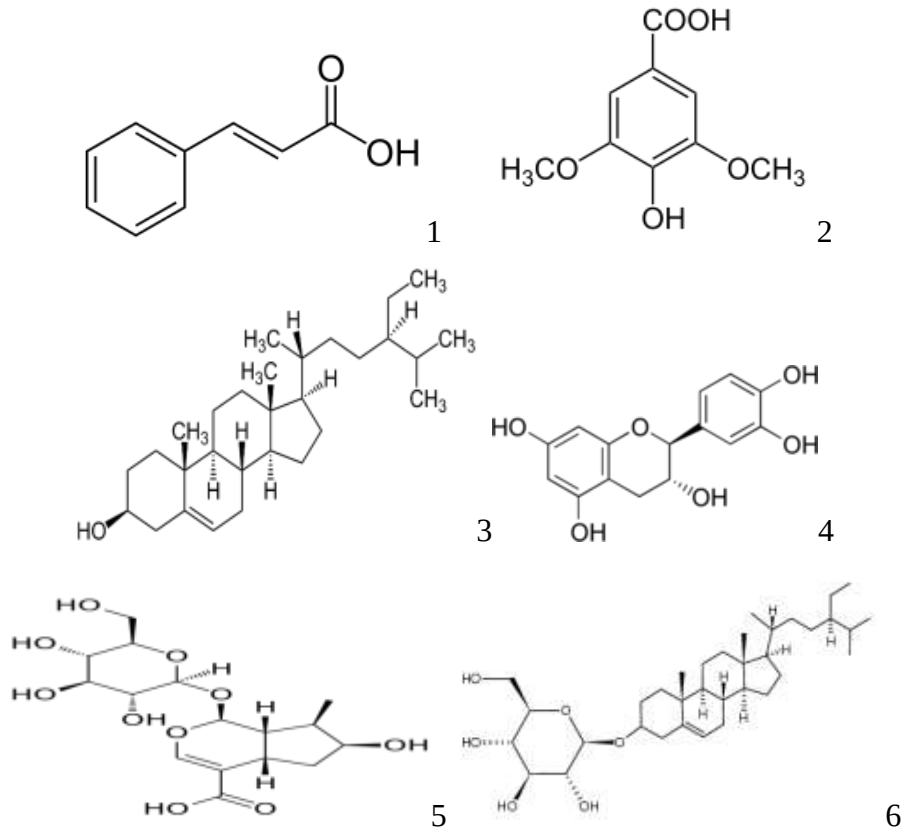

Figure 6: Chemical structures of annotated compounds in ethanolic extract from *Spathodea campanulata* branches.

3.1.1.2.4. Stem bark

Figure 7 shows the LC-MS results for the ethanolic extract of *S. campanulata* stem bark.

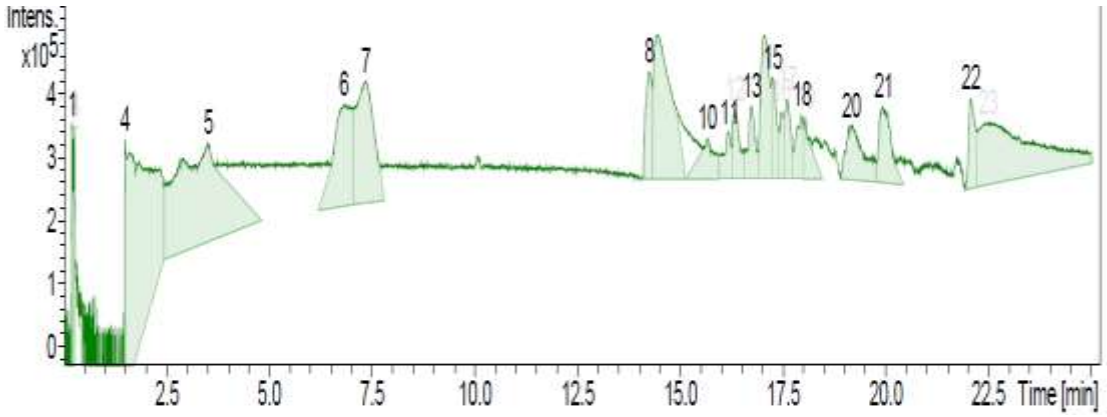

Figure 7: Chromatogram of the ethanolic extract of *Spathodea campanulata* stem bark at a concentration of 1 mg/ml.

UPLC-MS analysis of the ethanolic extract of the stem bark revealed the presence of 22 peaks, 5 of which were identified and corresponded to 5 compounds (Table 5, Figure 8).

Table 5: Annotation by UPLC-HR-ESI-MS/MS of the main compounds in ethanolic extract from *Spathodea campanulata* stem bark.

Peak N°	t_R (min)	Structure N°	*m/z* $[M]^+$	*m/z* $[M + H]^+$	*m/z* $[M + K]^+$	Annotation	Molecular formula
20	19.5	1			186.9920	Cinnamic acid	$C_9H_8O_2K$
7	7.5	2			340.2557	Quercetin	$C_{15}H_{10}O_7K$
9	14.5	3	290.2663			Catechin	$C_{15}H_{14}O_6$
11	16.5	4		415.2070		β-sistosterol	$C_{29}H_{51}O$
19	18.5	5	256.2615			Palmitic acid	$C_{16}H_{32}O_2$

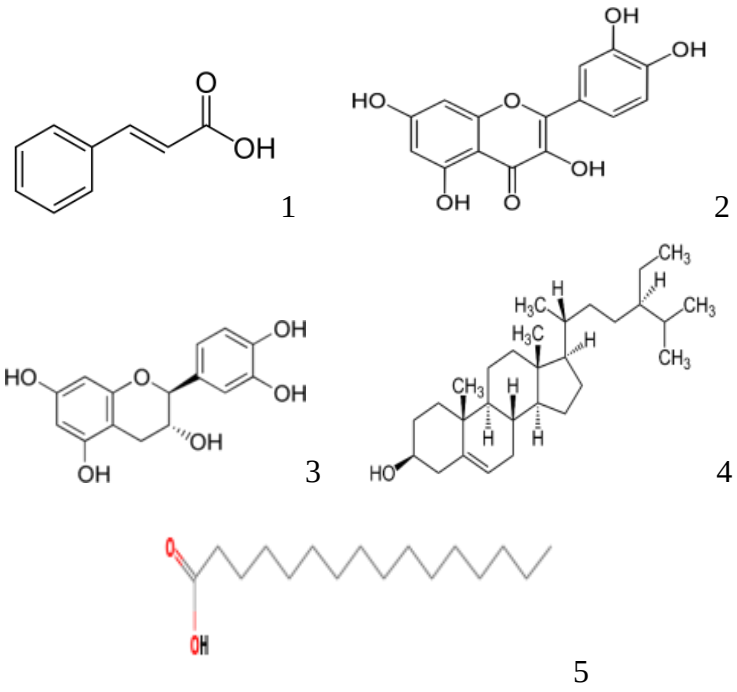

Figure 8: Chemical structures of annotated compounds in ethanolic extract from *Spathodea campanulata* stem bark.

3.1.1.2.5. Roots

Figure 9 shows the UPLC-MS results for the ethanolic extract of *S. campanulata* roots.

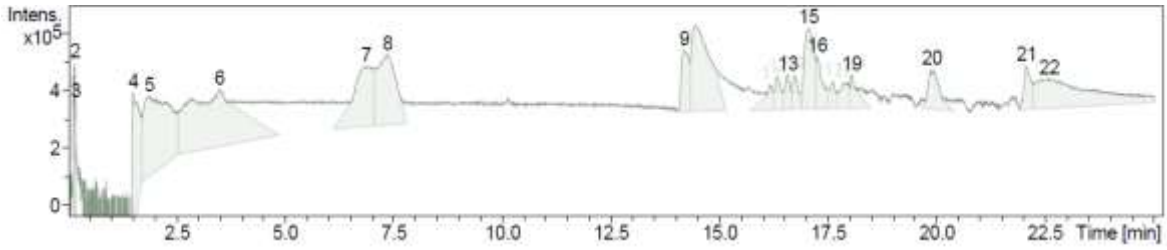

Figure 9: Chromatogram of the ethanolic extract of *Spathodea campanulata* roots at a concentration of 1 mg/ml.

UPLC-MS analysis of the ethanolic extract of the roots revealed the presence of 22 peaks, 8 of which were identified and corresponded to 5 compounds (Table 6, Figure 10).

Table 6: Annotation by UPLC-HR-ESI-MS/MS of the main compounds in ethanolic extract from *Spathodea campanulata* roots.

Peak N°	t_R (min)	Structure N°	*m/z* $[M]^+$	*m/z* $[M + H]^+$	*m/z* $[M + K]^+$	Annotation	Molecular formula
22	23.0	1			186.9924	Cinnamic acid	$C_9H_8O_2K$
7	7.0	2			340.2573	Quercetin	$C_{15}H_{10}O_7K$
9	14.5	3	290.2730			Catechin	$C_{15}H_{14}O_6$
11	16.5	4		415.2128		β-sistosterol	$C_{29}H_{51}O$
19	18.5	5	256.2626			Palmitic acid	$C_{16}H_{32}O_2$

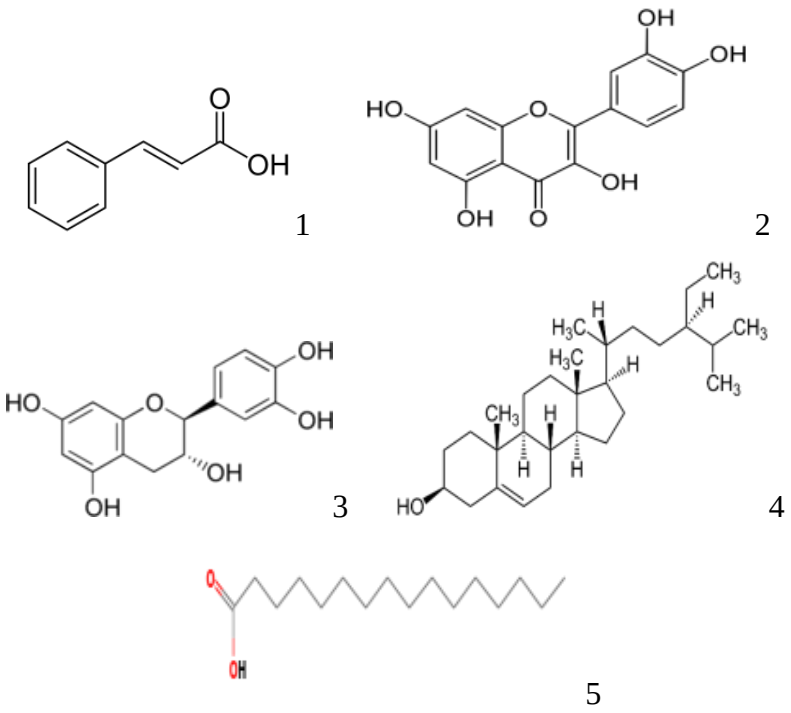

Figure 10: Chemical structures of annotated compounds in ethanolic extract from *Spathodea campanulata* roots.

3.1.2. Study of antiplasmodial activity

Figure 11 shows the dose-response curve as a function of the logarithm of the concentration of artemisinin and chloroquine extracts. These results confirm the significant inhibitory activity of artemisinin, with an IC_{50} of 28.67 nM or 0.02867 µg/ml (Figure 11A and C). Our results also confirm the chloroquine-resistant property of the *Pf*Dd2 strain, with an IC_{50} of 295.3 nM or 0.2953 µg/ml, 10 times higher than that of artemisinin (Figure 11B and C).

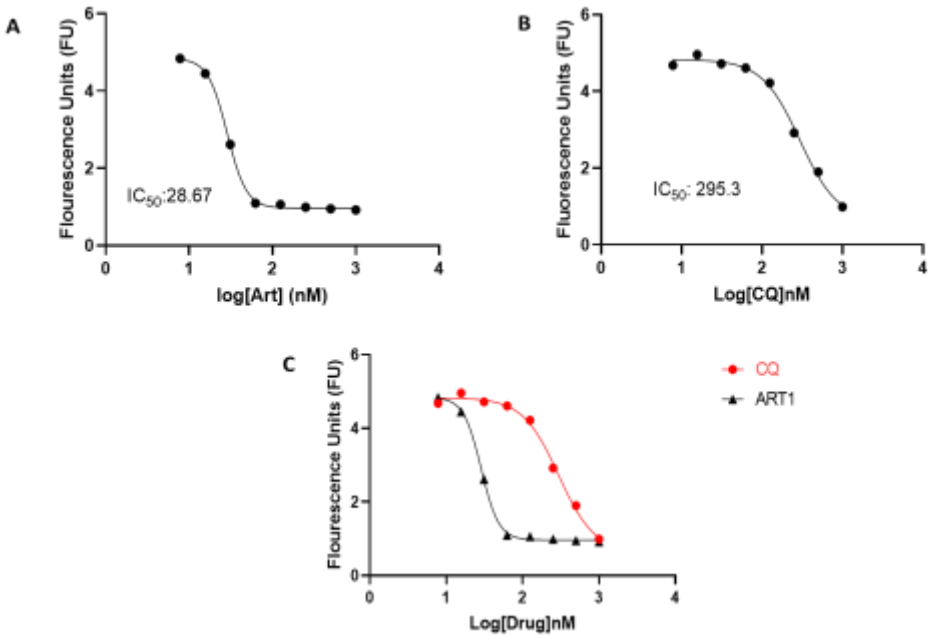

Figure 11: Individual antiplasmodial activities of drugs taken as reference for Artemisinin (A) and Chloroquine (B) and compared (C).

Figure 12 shows the dose-response curve for extracts from the various plant parts tested. Branch and root extracts show IC_{50} values in excess of 100 µg/ml. Leaf extract showed less activity than stem bark extract (93.6 µg/ml vs. 79.68 µg/ml).

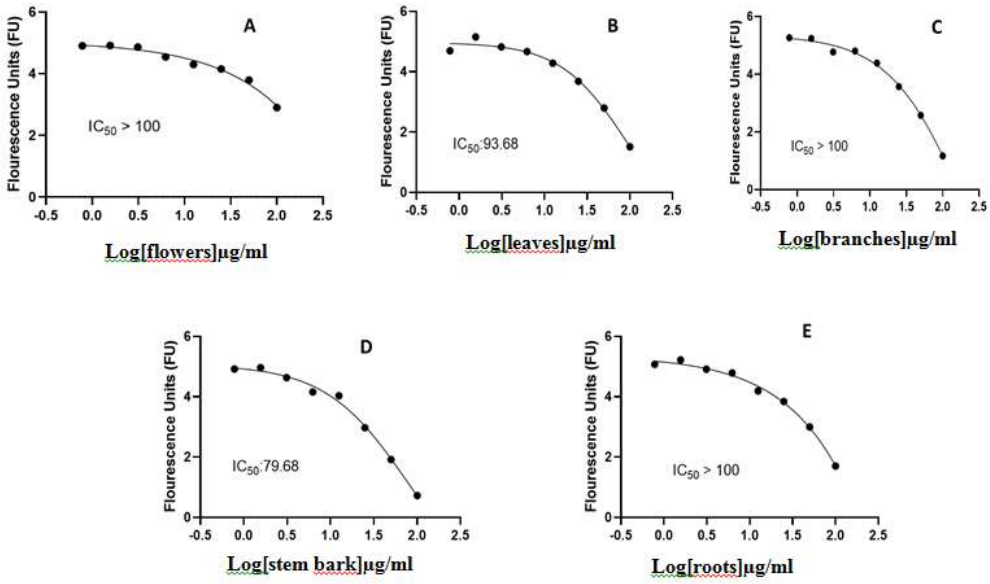

Figure 12: Dose-response curve as a function of the logarithm of extract concentration for the different parts of the *Spathodea campanulata* plant: flowers (A), leaves (B), branches (C), stem bark (D) and roots (E).

3.2. DISCUSSION

Extraction by maceration of the various organs (flowers, leaves, branches, stem bark, roots) of *S. campanulata* was carried out in an ethanol/water mixture (70/30). Ethanol, a polar, protic, water-miscible solvent, was chosen in our study both because it is close to the conditions of use by traditional practitioners, and because it is low in toxicity. The results reveal the influence of the extraction solvent on yield. The solvent mixture used proved less effective, with yields ranging from 2.26% to 3.25%, compared with the results of preliminary studies carried out on flowers of the same plant, for which extraction by liquid-liquid fractionation had given higher yields of around 38.45% with water, 29.63% with Dichloromethane (DCM) and 27.92% with Hexane.[18] In all cases, the ethanolic extract of the flowers provided a yield of 3.25%, these results being identical to those found in a preliminary study carried out by Mbosso et al. in 2016 after maceration of flowers from the same plant with 95% methanol.[19] The groups of secondary metabolites such as alkaloids, coumarins, glycosides, polyphenols and saponosides found in the different parts of the plant are those already reported in another study conducted by Bayaga et al. in 2020 who showed the presence of secondary metabolites in aqueous and hydroacoholic extracts such as: alkaloids, coumarins, glycosides, polyphenols and saponosides.[20] Previous studies carried out on flowers of the same plant showed the presence of gallic tannins, steroids, terpenoids, cardiotonic glycosides and saponins in the methanol extract.[18] Talla et al. showed the presence of several families of compounds in the aqueous extract of *S. campanulata* stem bark, including alkaloids, saponins, phenolic compounds, flavonoids, tannins and reducing sugars.[21] The presence of alkaloids, tannins and reducing sugars observed in addition in his study would probably be linked to the polarity of the solvent used compared with the ethanol used in our study. Another study by Ngouela et al, in 1991 showed that leaves extracts from the same plant contained spathodol, caffeic acids, phenolic acids and flavonoids,[22] while Rangasamy et al.'s 2008 work on leaves found several families of compounds in *S. campanulata* leaves, including alkaloids in the petroleum ether extract, saponins in the aqueous and methanol extracts, flavonoids and steroids in the chloroform and methanol extracts, and terpenoids and tannins in the methanol extract.[23] Various studies have revealed the presence of secondary metabolites such as anthocyanins in *S. campanulata* flower extracts,[24] carbohydrates and glycosides in chloroform and petroleum ether extracts, and tannins and glycosides in methanol extracts.[25] Polyphenols, tannins, saponins and glycosides have also been found in fruit extracts from the same plant.[26] UPLC-MS analysis enabled us to identify 10 different compounds belonging to the sterol, flavonoid and polyphenol groups,

among others, in the ethanolic extract of the various parts of *S. campanulata*. This corroborates the results obtained during phytochemical screening, where these compound classes were highlighted. The unidentified peaks could be compounds not yet isolated from the plant, but which could belong to other classes of secondary metabolites highlighted during the phytochemical screening and which either needs to be identified with further analyses such as MZmine, or to be purified and characterized. Whatever the case, all these studies reveal differences in the composition of families and metabolic compounds contained in extracts from the same organs, which could be explained by a number of parameters, including harvesting location and period, extraction and drying methods, all of which can influence the chemical constituents of plants. The parameter linked to harvesting location is reinforced by the various results obtained from *S. campanulata* collected in different regions of Africa. Flower, branch and root extracts showed no anti-plasmodial activity on the chloroquino-resistant *Pf*Dd2 strain under our experimental conditions, with IC_{50} values > 100 µg/ml, yet their phytochemical profile indicates a wealth of secondary metabolites, although flower and root extracts showed no presence of flavonoids like other parts. However, according to the scale proposed by Wilcox et al. in 2011,[27] a very low antiplasmodial activity was found for ethanolic extracts of leaves and stem bark at a concentration of 100 µg/ml, with IC_{50} values of 93.68 µg/ml and 79.68 µg/ml respectively. These values are well below those of Chloroquine and Artemisinin, which showed IC_{50} values of 295.3 and 28.67 nM, i.e. 0.2953 and 0.02867 µg/ml. Our results corroborate those found by Rangasamy et al. in 2008 suggesting that ethanolic leaf extracts were not endowed with strong antiplasmodial activity; IC_{50s} ranged from 68.05 ± 10.60 to 108.2 ± 8.20 µg/ml on Chloroquine-sensitive and Chloroquine-resistant *Plasmodium falciparum* isolates isolated and adapted in the laboratory.[23] Whereas the butanol and chloroform fractions of the ethanolic leaf extract showed antiplasmodial activity with IC_{50s} of 18.7 ± 2.23 and 12.3 ± 1.32 µg/ml.[23] In addition, preliminary studies we carried out on *S. campanulata* flowers in 2018, showed that the hexane, dichloromethane and ethyl acetate fractions could significantly reduce *Pf*3D7 cell multiplication with respective IC_{50} values of 28.13; 29.69; 30.22 µg/ml at a concentration of 100 µg/ml.[18] In view of all this, the fractions would appear to be more active than the total extracts. All these studies reveal differences in the activity of the plant parts, which could be explained not only by the diversity of the metabolite composition, but also by the diversity of the parasite strains tested. In our study, the *Pf*Dd2 strain used is a Chloroquino-resistant strain which may also be resistant to ethanolic extracts of *S. campanulata*.

4. CONCLUSION

At the end of our study, phytochemical screening revealed numerous families of secondary metabolites whose nature varies according to the plant parts: sterols, polyphenols and saponins from flower extract; polyterpenes, flavonoids, saponins and polyphenols from leaf extract; sterols, flavonoids, saponins and polyphenols from branch and stem bark extract, which also contain sterols; saponins and polyphenols from root extract. UPLC-MS analysis revealed 10 compounds, notably Cinnamic acid, β-sistosterol, Quercetin, Naringinin, Catechin, Loganic acid, β-sitosterol 3-*O*-β-D-glucopyranoside, Syringic acid, *n*-hexadecanoic acid (palmitic acid). Evaluation of antiplasmodial activity on *Pf*Dd2 strains revealed very low activity of ethanolic extracts of *S. campanulata* leaves and trunk bark only, with the stem bark as the most active part. Finaly, these results indicate a potential antiplasmodial activity of *S. campanulata*, which could justify its traditional use in Cameroon. The presence of certain chemical molecules found in this plant with anti-inflammatory and antioxidant potential is also of interest in the treatment of numerous pathologies other than malaria.

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REFERENCES

1. OMS. Rapport sur le paludisme dans le monde, 2021. [Accessed on 20 August 2024]. Available online: https://www.who.int.
2. PNLP activity report. [Internet]. 2019 [Accessed on 20 August 2024]. Available online: http://onsp.minsante.cm. File in french.
3. Roux AT, Maharaj L, Oyegoke O, Akoniyon OP, Adeleke MA, Maharaj R, Okpeku M. Chloroquine and Sulfadoxine-Pyrimethamine Resistance in Sub-Saharan Africa-A Review. Front Genet, 2021; 12: 668574. DOI: 10.3389/fgene.2021.668574.
4. Bergmann C, van Loon W, Habarugira F, Tacoli C, Jäger JC, Savelsberg D, et al. Increase in Kelch 13 Polymorphisms in *Plasmodium falciparum*, Southern Rwanda. Emerg Infect Dis, 2021; 27(1): 294-6. DOI: 10.3201/eid2701.203527.
5. Balikagala B, Fukuda N, Ikeda M, Katuro OT, Tachibana SI, Yamauchi M, Opio W, Emoto S, Anywar DA, Kimura E, Palacpac NMQ, Odongo-Aginya EI, Ogwang M, Horii

T, Mita T. Evidence of Artemisinin-Resistant Malaria in Africa. N Engl J Med, 2021; 385(13): 1163-71. DOI: 10.1056/NEJMoa2101746.

6. WHO Strategy for Traditional Medicine 2014-2023. World Health Organization. [Internet]. 2013 [Accessed on 20 August 2024]. Available online: https://apps.who.int. File in French.
7. Gurib-Fakim A. Medicinal plants: Traditions of yesterday and drugs of tomorrow. Mol Aspects Med, 2006; 27(1): 1-93.
8. Place of traditional medicine in the health system: Facts and nombers [Internet]. Afrique Sub-Saharian Africa. [Accessed on 20 August 2024]. Available online: https://beta.scidev.net/afrique-sub-saharienne/features/place-de-la-m-decinetraditionnelle-dans-le-syst-me-de-sant-faits-et-chiffres. File in french.
9. Focus on traditional medicine in Cameroon [Internet]. Senegal Direct. [Accessed on 20 August 2024]. Available online: https://senegaldirect.com/zoom-sur-la-medecinetraditionnelle-au-cameroun. File in french.
10. Waffo Tchounga CA, Sacré PY, Ciza Hamuli P, Ngono Mballa R, Nnanga Nga E, Hubert P, et al. Poor-quality medicines in Cameroon: A critical review. Am J Trop Med Hyg, 2021; 105(2): 284-94.
11. Etame LG, Ngoule CC, Mbome B, Kidik PC, Ngene JP, Yinyang J et al. Contribution to the study of medicinal plants and their traditional uses in the Lom and Djerem department (East, Cameroon). J Ani Plant Sci, 2018; 35(1): 5560-78. File in french.
12. Bekro YA, Mamyrbekova JA, Boua BB, Bi FT, Ehile EE. Ethnobotanical study and phytochemical screening of *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). Sci Nat., 2007; 4(2): 217-25. File in french.
13. Kunyima P, Mbomba NB, Habari M, Lami N, Mpiana T, Muganza M. Chemical screening, antioxidant and antiplasmodial activities of stem bark extracts of *Enantia olivacea* Robyns & Ghesq (Annonaceae), a plant used by bonobos, *Pan paniscus* in Lui-Kotale in DR Congo. Rev Primatol, 2013; 5. DOI: 10.4000/primatologie.1314. File in french.
14. Gbadamosi AE. Genetic variation in *Enantia chlorantha* (Oliv.)–a medicinal plant. J Food Agri Environ, 2005; 3(1): 153–6.
15. Ronchetti F, Russo G, Bombardelli E, Bonati A. A new alkaloid from *Rauwolfia vomitoria*. Phytochemistry, 1971; 10(6): 1385-8.
16. Trager W, Jensen JB. Human malaria parasites in continuous culture. Science, 1976; 193(4254): 673-5. DOI: 10.1126/science.781840.

17. Vossen MG, Pferschy S, Chiba P, Noedl H. The SYBR Green I malaria drug sensitivity assay: performance in low parasitemia samples. Am J Trop Med Hyg, 2010; 82(3): 398-401. DOI: 10.4269/ajtmh.2010.09-0417.
18. Mbosso TJE, Hassan O, Siwe NX, Meyer F, Megalizzi V, Hoppe HC, Krause MRW, Wintjens R. Evaluation of *in vitro* antiplasmodial, antiproliferative activities, and *in vivo* oral acute toxicity of *Spathodea campanulata* flowers. Scientific African, 2023; 21: e01871. DOI :10.1016/j.sciaf.2023.e01871.
19. Mbosso TJE, Assob NJC, Meyer F, Donfack VE, Ndjakou LB, Ngouela S. *In vitro* antimicrobial and anti-proliferative activities of plant extracts from *Spathodea campanulata*, *Ficusbubu*, and *Carica papaya*. Pharm Biol, 2016; 56: 1086-95.
20. Bayaga HN, Guedje NM, Tabi OY, Pola YE, Njinkio NBL, Assong EDC et al. Antibacterial power of aqueous and hydro-ethanolic extracts of the mixture of stem barks of *Albizia gummifera* (J.F. Gmel.) C.A. Sm and *Spathodea campanulata* P. Beauv. J Appl Biosci, 2020; 154: 15881-7. File in french.
21. Talla C. Evaluation of the aphrodisiac activity of the aqueous extract of the stem bark of *Spathodea campanulata* P. Beauv. in rats; [Pharmacy Thesis], Douala: University of Douala, 2017. File in french.
22. Ngouela S, Tsamo E, Sondengam BL, Connolly JD. Spathodol, a new polyhydroxysterol from the leaves of *Spathodea campanulata*. J Nat Prod, 1991; 54: 873-6.
23. Rangasamy D, Asirvatham D, Muthusamy J, Rajamanickam K, Muthusamy P, Jeyaraman A. Preliminary phytochemical screening and antimalarial studies of *Spathodea campanulata* P. Beauv. leaf extracts. Ethnobot Leafl, 2008; 12: 811-9.
24. Banerjee A, Bratati D. Anthocyanins in some flowers of West Bengal. J Med Aro Plant Sci, 1993; 23: 600-4.
25. Zahid Z, Aniruddha P, Sagar D, Subur K, Rana ZA. Comparative phytochemical screening of flowers and bark of *Spathodea campanulata*. Int J Applied Biol Pharm, 2011; 2.
26. Amusan OOG, Adesogan E, Makinde JM. Antimalarial active principles of *Spathodea campanulata* stem bark. Phytother Res, 1996; 10: 692-3.
27. Somsak V, Srichairatanakool S, Yuthavong Y, Kamchonwongpaisan S, Uthaipibull C. Flow cytometric enumeration of *Plasmodium berghei*-infected red blood cells stained with SYBR Green I. Acta Trop, 2012; 122(1): 113-8. DOI: 10.1016/j.actatropica.2011.12.010.