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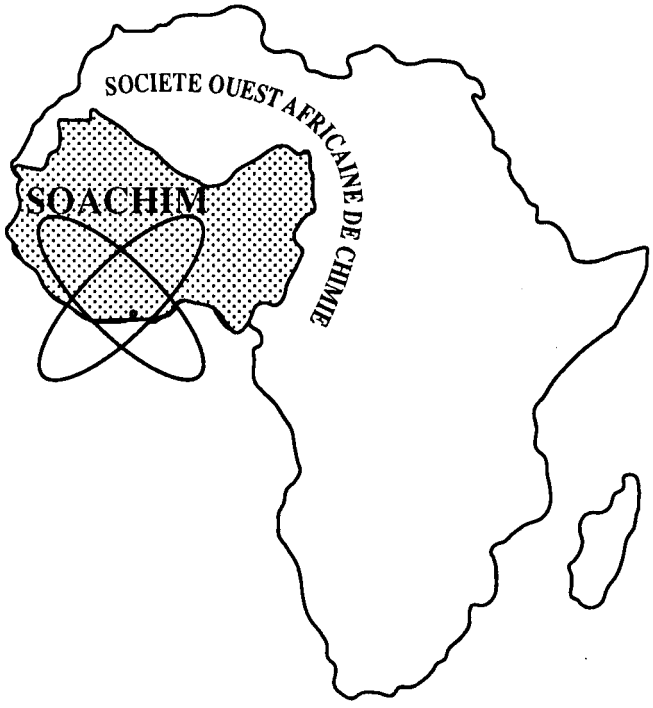

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In vitro Evaluation of the antiplasmodial activity of *Cymbopogon giganteus* extracts from Burkina Faso

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Abstract: Dicholoromethane, ethyl acetate, and methanolic extracts from leaves, flowers, stems, and roots of *Cymbopogon giganteus* have been evaluated for their total antioxidant contents using spectrophotometric methods. It has been revealed that, methanol is better for extracting antioxidants (36.271 µg of TE/mg); followed by the ethyl acetate (22.330 µg of TE/mg) and dichloromethane (16.029 µg of TE/mg). Against chloroquino-resistant K1 strain of *Plasmodium falciparum*, extracts from flowers, roots, stems and leaves had respectively average IC_{50} of 2.56; 1.32; 6.79 and 4.603 µg/Ml. Relating to 3D7 strain, extracts from flowers had good antiplasmodial activity (IC_{50} = 2.33 µg/mL) compared to those from roots (IC_{50} = 8.215 µg/mL), leaves (IC_{50} = 12.93 µg/mL) and stems (IC_{50} = 13.84 µg/mL). Moreover, antiplasmodial activity depended on the extraction solvent. Independently of the considered part, it was found that according to Wilcox scale, four dichloromethane extracts were very active on K1 strain ($0.17 \leq IC_{50} \leq 1.36$ µg/mL) and one from the same solvent was very active on the 3D7 strain (IC_{50} = 1.05 µg/mL) of *Plasmodium falciparum*. Ethyl acetate extracts presented a good antiplasmodial activity on the chloroquino-sensitive 3D7 strain and a mean activity on K1. Finally, methanolic extracts had moderate activity on both strains.

Key words : antioxidants, *antiplasmodial activity*, phytochemicals, Phenolic compounds

Evaluation *in vitro* de l'activité antiplasmodiale d'extraits de *cymbopogon giganteus* du Burkina Faso

Résumé : Les teneurs en antioxydants totaux des extraits au dichlorométhane, acétate d'éthyle et au méthanol des feuilles, fleurs, tiges et racines de *Cymbopogon giganteus* ont été évaluées par des méthodes spectrophotométriques. Le méthanol est le meilleur solvant d'extraction des antioxydants totaux (36,271 µg d'ET/mg) ; suivi de l'acétate d'éthyle (22,33 µg d'ET/mg) et du dichlorométhane (16,029 µg d'ET/mg). L'évaluation de l'activité antiplasmodiale sur la souche chloroquino-résistante K1 a montré que les extraits des fleurs, racines, tiges et des feuilles possèdent respectivement des CI_{50} moyennes de 2,56 ; 1,32 ; 6,79 et 4,603 µg/mL. Sur la souche 3D7, les extraits des fleurs possèdent une bonne activité antiplasmodiale (CI_{50}=2,33 µg/mL) comparativement à ceux des racines (CI_{50}=8,215 µg/mL), des feuilles (CI_{50}=12,93 µg/mL) et des tiges (CI_{50}=13, 84 µg/mL). Par ailleurs, l'activité antiplasmodiale de ces extraits dépendait du solvant d'extraction. Indépendamment de l'organe de la plante et selon l'échelle de Wilcox, quatre extraits au dichlorométhane sont très actifs sur la souche K1 ($0,17 \leq IC_{50} \leq 1,36$ µg/mL) et un autre du même solvant est très actif sur la souche 3D7 (CI_{50}=1,05 µg/mL) du *Plasmodium falciparum.* Les extraits à l'acétate d'éthyle possèdent plutôt une bonne activité antiplasmodiale sur la souche chloroquino-sensible 3D7 et moyenne sur K1. Enfin, les extraits au méthanol ont une activité modérée sur les deux souches.

Mots clés: antioxydants, *activité antiplasmodiale*, produits phytochimiques, composés phénoliques

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1. Introduction

Malaria is a fairly old pathology that appeared in 1550 before Jesus-Christ. World Health Organization (WHO) enumerates between 250 and 400 million cases of malaria and one millions of children die every year, meaning one child every 30 seconds [1]. Today about 40% of the world's population, particularly those in poor countries, are exposed to malaria, mainly in sub-saharan Africa, where 90% of deaths are recorded [2]. Malaria is the leading cause of mortality of children under 5 years of age and covers about 40% of public health expenditure. In the 1930 years, the first drugs aroused much hope in the management of this disease. This hope was short because in 1960, there was an increase in the prevalence of the disease showing the resistance of Plasmodium to standard treatments [3, 4].

Thus, the research for new antimalarial compounds, both chemical and natural, would be an alternative. Nowadays, traditional medicine covers the primary care needs of about 80% of the world's population. Indeed, research is being carried out on local plants that could be an alternative to the endogenous management of this real public health problem [5-7]. This study of the different parts of a plant from Burkina Faso is therefore in line with this dynamic and aims to identify the parts of the plant that contains the active ingredients and the best extraction solvent.

2. Material and methods

2.1. Plant material

The present work has focused on various parts of *Cymbopogon giganteus*. Indeed, leaves, flowers, stems and roots from this plant were collected from the Botanical Garden of the Institute of Applied Sciences and Technology Research (IRSAT) between December 2012 and January 2013. The plant material was dried in the open air, away from the light, for 2 weeks. The different parts of the plant were crushed and the powders obtained were stored in the refrigerator at 4 ° C until extraction.

2.2. Extraction

50 g of powder of each part were macerated successively with 300 mL of hexane, dichloromethane, ethyl acetate and methanol under magnetic stirring for 24 h. After filtration, on Wathmann papers N°3, extractions were repeated twice with the same volume of solvent for 24 h. Extracts obtained were concentrated under vacuum until almost dry. Dried extracts were stored in the refrigerator for the various tests.

2.3. Chemical reagents

The reagents used are: RPMI-1640 (a solution from Roswell Park Memorial Institute), Gentamycin, Albumax, hypoxanthine, sodiumhydroxide solution (1 M), Hepes buffer solution (200 mM), L-Glutamine.6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2_-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Quercetin, rutin, potassium persulphate ($K_2S_2O_8$), iron chloride (III) hexa-hydrate, acetate trihydrate sodium gallic were purchased from Sigma-Aldrich (St. Louis, MO) and used.

2.4. Phytochemical screening

Qualitative testing of the chemical families was carried out in tubes and by thin-layer chromatography (TLC) according to characterization methods described by Wagner et al. (1996).

2.5. Determination of total antioxidant Contents (TAC)

2.5.1. DPPH method

The antioxidant capacity of each extract to reduce the DPPH radical was determined by using the method of Brand-Williams et al [8, 9]. DPPH radical reduced by the antioxidant compounds has a maximum absorption wavelength at 515 nm. 50 µL of each extract are added to 200 µL of DPPH methanol solution (2 mg/50 mL). After 10 min of incubation at 37°C, absorbance was read at 515 nm in contrast with a blank not containing the dosed sample. TACs of the extracts were determined by reporting the absorbance read on the calibration curve established using the Trolox ($y = -27.63x + 0.873$; $R^2 = 0.984$) as standard. The results are expressed in micrograms of Trolox equivalents per milligram of extract (µg of TE/mg).

2.5.2. ABTS method

The antioxidant capacity of extracts is also evaluated by studying the discoloration of $ABTS^{+\cdot}$ radical-cation. This method, reported for the first time by Miller and Rice-Evans [10], is based on the

reduction of $ABTS^{+}$ radical-cation by antioxidant compounds in plant extracts. $ABTS^{+}$ radical-cation was generated by mixing 10 mL of aqueous solution of $K_2S_2O_8$ potassium persulphate (39.2 mM) and 50 mL of aqueous solution of ABTS (7.01 mM). The mixture was kept in the dark at room temperature for 16 hours. For the dosage, the $ABTS^{+}$ solution is suitably diluted with distilled water to have an absorbance of about 0.7 ± 0.02 at 734 nm.120 µL of the $ABTS^{+}$ solution of was added to 50 µL of every plant extract and the absorbance was read at 734 nm after 10 min of incubation at room temperature. The absorbance is reported on the calibration curve established with the Trolox (y = -19,41x + 0.7197; R^2 = 0.9953). The TAC was expressed in µg/mg of extract.

2.5.3. FRAP method

In this method, the ferric salt Complex, Fe (III) $(TPTZ)_2Cl_3$ (TPTZ = 2, 4, 6-tripyridyl-*s*-triazine) was used as an oxidant [11, 12]. 30 µL of distilled water, 20 µL of extract were added to 200 µL of the FRAP solution. Absorbance of the intense blue coloration is read at 593 nm using a microplate reader (MP96 spectrophotometer, Safas) after 10 min of incubation. TAC values were determined from the equation of the calibration curve (y = 40.648x + 0.3419; R^2 = 0.9904) and were expressed in µg of Trolox equivalents (TE) per milligram of extract. All measurements were triplicated.

2.6. Biological material

The chloroquino-resistant K1 and chloroquine-sensitive 3D7 strains of *Plasmodium falciparum* were used throughout this study. They were maintained in continuous culture by the method of Jensen and Trager (1977) in fresh human O^+ erythrocytes at 2.5% hematocrit in the complete culture Medium (CCM) (RPMI 1640 with 25 MM buffer HEPES, 25 mM $NaHCO_3$, 10% Human O^+ Serum) at 37°C under O_2 vacuum atmosphere (gas mixture 6% CO_2, 14% O_2, 80% N_2). The parasitaemia was maintained between 1% and 6% daily.

2.7. Preparation of plant extracts

The initial stock solutions of the extracts were prepared at the concentration of 10 mg/mL. The used solvent was DMSO for all organic extracts. From these initial solutions dilutions were then carried out in the medium RPMI-II containing 1% DMSO in order to obtain concentrations of 100 µg/mL.
From an initial solution of a given extract (100 µg/mL) a cascade dilution was carried out (from 50 µg/mL to 0.78 µg/mL) with 100 µL in a 96-well microplate having a flat bottom.

2.8. Evaluation of the antiplasmodial activity

. The method of pLDH was used. In the 96-well microplate containing the plant extracts, 100 µL of 1% parasitized blood and 4% Hematocrit were transferred according to the same scheme of the microplate used to dilute the products to be tested. The final volume of parasitized blood in the wells was 200 µL with Parasitemie of 1% and Hematocrit rate of 2%. Every drug was tested in duplicate.
Infected and uninfected erythrocytes O+ were used as positive and negative controls, respectively.
. The microplate was covered and placed in the CO_2 incubator at 37 ° C for 72 hours.
The antiplasmodial activity of the molecules was determined by the immuno-enzymatic ELISA method based on the quantification of pLDH (Plasmodium lactate dehydrogenase) present in the culture. This detected antigen is a Plasmodium-specific, intracellular metabolic enzyme found in parasitized red blood cells [13]. Its synthesis and accumulation can be used both *in vivo* and *in vitro* as indices of parasitic viability [14]. After 72 hours of incubation, 25 µL of the homogenized supernatant is withdrawn and mixed with 120 µL of a mixture consisting of 20 µL of a MALSAT solution. A new incubation of 10 minutes at room temperature is carried out in a light free room. Then, absorbance is read at 650 nm. The Table Curve 2D Jandel Scientific version 3.0 software is used to plot the concentration of product curve in relation with the percentage of inhibition (**Figure 1**). A nonlinear regression established between the percentage of parasitic inhibition relating to the control and the different concentrations of the extracts were used to determine this IC_{50}.

3. Results and Discussion

Phytochemical Screening: Except alkaloids, ethyl acetate extracts did not contain any chemical family investigated in this work. Like dichloromethane extracts from leaves, those from flowers, stems and roots contained alkaloids, saponins. Polar compounds such as tannins and flavonoids were encountered in methanol extracts (**table I**).

Total Antioxidant Contents (TAC): values of obtained TAC by the three methods (DPPH, ABTS, and FRAP methods) (**table II**) showed a significant differences between the extracts of the different parts of the species.

Regardless of the part of the studied plant material and the extraction solvent, it is appeared that FRAP method, based on the reduction of ferric ions by antioxidants had the highest TAC in extracts with an average value of 43.126 ± 3.749 µg of TE/mg followed by the DPPH method (23.61 ± 2.793 µg of TE/mg) and finally by ABTS method (2.946 ± 0.746 µg of TE/mg).

Depending on the extraction solvent, it was clear that methanol better extracted the antioxidant compounds with a mean antioxidant content of 36.271 ± 3.55 µg of TE/mg. Ethyl acetate and dichloromethane contained TAC of 22.355±2.074 and 16.029±2.001 µg of TE/mg, respectively.

Regarding the studied different parts, flowers contained the highest TAC with an average value of 29.681 ± 2.264 µg of TE/mg. The other parts: roots, leaves and stems followed, with contents of 25.702 ± 2.724; 20.796 ± 2.701 and 20.574 ± 2.028 µg/mg, respectively.

Antiplasmodial activity: antiplasmodial activity was evaluated by calculating the concentrations that inhibit 50% (IC_{50}) of the growth of parasites determined by the half-logarithmic method. The variation in the inhibition of parasitic growth based on the concentrations of the various extracts during 72 hours, which allowed the determination of IC_{50}, was represented by the half-logarithmic curves plotted by usingTable Curve 2D Jandel Scientific version 3,0 software (Figure 1). IC_{50} values of extracts from the different parts of the plant are summarized in **table III**.

Table I: Summary of the results of phytochemical screening

Parts of plant	Extraction Solvents	chemical groups tested				
		Tannins	Flavonoids	Alkaloids	Saponins	Phenolic Acids and Coumarins
Leaves	DCM	-	-	+	-	+
	AcEt	-	-	+	-	-
	MeOH	+	+	-	-	-
Flowers	DCM	-	-	+	+	-
	AcEt	-	-	-	-	-
	MeOH	+	+	+	-	-
Stems	DCM	-	-	+	+	-
	AcEt	-	-	-	-	-
	MeOH	+	+	+	-	-
Roots	DCM	-	+	+	+	-
	AcEt	-	-	-	-	-
	MeOH	+	+	+	-	-

DCM :dichloromethane ; AcEt : Ethyl acetate ; MeOH : methanol

Table II: TAC obtained by the methods at DPPH, ABTS and FRAP

Parts of plant	Extraction Solvents	TAC in µg TE /mg of extract		
		DPPH	FRAP	ABTS
Flowers	DCM	2.514±0.257	14.891±1.43	ND
	AcEt	27.96±4.063	38.225±3.181	0.163±0.072
	MeOH	60.390±8.44	112.946±2.348	10.044±0.58
Leaves	DCM	0.145±0.00	20.305±1.51	ND
	AcEt	7.633±1.674	39.672±4.041	0.020±0.00
	MeOH	47.150±6.477	68.560±7.522	3.683±0.539
Stems	DCM	26.745±0.00	18.158±2.330	ND
	AcEt	27.474±2.550	36.732±0.952	0.448±0.00
	MeOH	19.215±2.342	31.459±6.490	4.363±0.117
Roots	DCM	19.336±1.797	47.606±8.454	1.268±0.212
	AcEt	16.1785±3.279	43.779±2.441	ND
	MeOH	28.688±2.636	45.1794±4.285	3.578±0.849

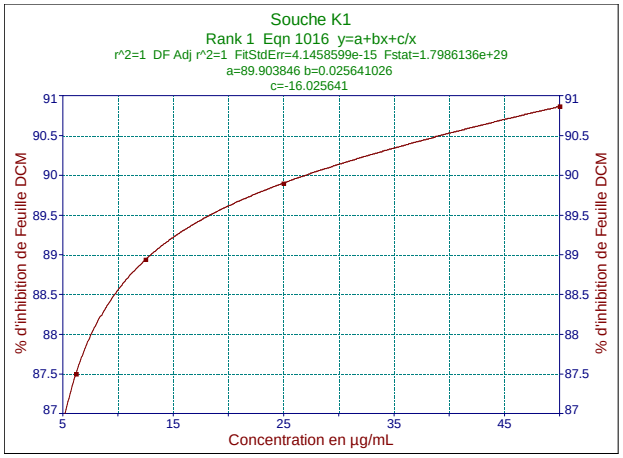

Figure 1: Inhibition of parasitic growth as a function of concentration

The analysis of the results (**table III**) shows that the antiplasmodial activity evaluated on the two strains chloroquino-resistant K1 and chloroquino-sensitive 3D7 of *Plasmodium falciparum* depended on the plant material part but also the extraction solvent.

The antiplasmodial activity show values related to the nature of the plant part that is used. Indeed, extracts of *C. giganteus* flowers have very good activity on the K1 strain (IC_{50}= 0.17 µg/mL); twice better than the leaves (IC_{50}= 0.37 µg/mL); thrice better than root extracts (IC_{50}= 0.45 µg/mL) by DCM. In contrast, on the chloroquino-sensitive 3D7 strain, the DCM extracts of the leaves were very active (IC_{50}= 1.05 µg/mL) approximately twice as much as those of the flowers (IC_{50}= 2.56 µg/mL) according to the scale proposed by Wilcox et al. [16].

Taking into account all four studied parts of *C. giganteus* and independently of the extraction solvent, it appeared that on the K1 strain, the flowers, roots, stems, and leaves respectively have average IC_{50} of 2.56; 1.32; 6.79 and 4.60 µg/mL. On the 3D7 strain, the inhibition of parasitic growth could be classified as followed: the best activity was found in flowers with an average IC_{50} of 2.33 µg/mL followed by roots (IC_{50}= 8.21 µg/mL), leaves (IC_{50}= 12.93 µg/mL), and finally by stems (IC_{50}= 13.84 µg/mL). The flowers of *C. giganteus* are the most appropriate part to fight against the two strains of *Plasmodium falciparum*.

In addition, the antiplasmodial activity depended on the extraction solvent. Among the plant extracts used for *in vitro* evaluation of antiplasmodial activity, dichloromethane extracts showed a very good antiplasmodial activity. Antiplasmodial activities of extracts from flowers using dichloromethane, ethyl acetate, and methanol, respectively, 0.17; 5.96 and

Table III: Concentrations that inhibit 50% (IC_{50}) of parasitic growth

	IC_{50} (µg/mL)					
	DCM extract		Ethyl acetate extract		Methanolic extract	
	K1	3D7	K1	3D7	K1	3D7
***C. giganteus* leaves**	0.37	1.05	2.54	ND	10.90	24.82
***C.giganteus* stems**	1.36	13.28	7.34	4.29	11.68	23.95
C. *giganteus* roots	0.45	9.59	0.22	ND	3.31	6.84
***C.giganteus* flowers**	0.17	2.56	5.96	3.6	17.45	0.82

Table IV: laboratory efficacy score for *in vitro* antiplasmodial activity of crude extracts

IC_{50} (µg/mL) (best)**	**Level of activity**
< 2.0	Very good
2.0 - 5.0	Good This is the concentration range that is generally considered as active in screening programmes for anti-malarial activity, warranting bioassay- guided fractionation.
5.1 - 10	Good to moderate This range may reasonably be considered for bioassay-guided fractionation.
11 à 25	Weak
26 - 50	Very weak
>100	Inactive

17.85 µg/mL revealed that DCM extracts had an excellent activity on the K1 strain. For the others extracts from leaves, roots and stems it was also noticed that DCM extracts were more active. So, it is the methanolic extract from flowers of *C.giganteus* which was more active (IC_{50}= 0.82 µg/mL) on the chloroquine-sensitive 3D7 strain in comparison with DCM (IC_{50}= 2.56 µg/mL) and ethyl acetate (IC_{50}= 3.6 µg/mL).

IC_{50} values (**table IV**) showed that the inhibition of parasitic growth of apart on a strain (e.g., chloroquino-resistant) decreases when the polarity of the solvent increased. Dichloromethane extracts were generally more active than those of the corresponding methanol on 3D7 strains. Ethyl acetate extracts exhibited good activity on 3D7 (chloroquino-sensitive) strain and moderate activity on K1 (chloroquino-resistant) strain considered. methanolic extracts revealed moderate antiplasmodial activity. Thus, dichloromethane was the appropriate solvent for obtaining an extract possessing a strong parasitic control.

Considering DCM, extract from flowers was twice as active as the leaves extract; itself more active than the extract from roots which in turn was more active than that from stems.

The phytochemical screening of the whole extracts revealed that dichloromethane extracts contained quinones, triterpenes and steroids. This chemical composition of these extracts would therefore justify their highest antiplasmodial activity. Indeed, active principles responsible for the antiplasmodial activity of a plant such as sterols, triterpenes [17-19, 13], and alkaloids [14-16, 18-19] are soluble in apolar organic solvents. Thus, β-sitosterol showed an IC_{50} of the order of 2 µM on the chloroquin-resistant strain (FcB1). In 2002, Ziegler et al had demonstrated that lupeol, a triterpene pentacyclic, had an antiplasmodial activity [20]. The amino-4-quinoline known as amodiaquine is an alkaloid known for its efficacy against some chloroquino-resistant of *P.falciparum* strains, although cross-resistance exists [21]. Artemisinin, a lactose sesquiterpene extracted from leaves of *Artemisia annua* is also recognized, as a potent blood schizontocide active against all species of *plasmodium* [22-24].

Results of alcoholic extracts were in agreement with some previous studies. Garcia-Alvarez et al, 2013 [25] obtained an IC_{50} = 34.5 µg/mL on FcM29 for methanolic extracts from *Sebastiania Chamaelea* and anIC_{50}= 6.6 µg/mL on W2 from the aqueous extract of the whole plant. Shuaibu et al. 2008 obtained anIC_{50} of 15.28 µg/mL (K1) from the methanolic extract of *Prosopis africana* [26]. Benoît-Vical et al showed for *Chrozophoras enegalensis*, anIC_{50} of 13 ± 3 µg/mL (FcM29) for the ethanolic extract from leaves and 10 ± 1 µg/mL (FcM29) for the ethanolic extract from stems [15]. In 2013, Ouattara et al showed that the polar solvent extracts of some plants harvested in the Comoe forest region had a meanI $C_{50} \geq 20$ µg/mL. According to this work, active elements against Plasmodium were the ellagic and gallic acids [27]. The phytochemical screening of these alcoholic extracts revealed that they were rich in tannins, polyphenols and flavonoids. However, each of these secondary metabolite families can potentially contain molecules having antiplasmodial activity: tannins and flavonoids [6, 28-31]. Phenolic compounds (ellagic acid, quercetin) soluble in polar solvents are therefore responsible for the antiplasmodial activity of methanolic extracts of the plant material [32-35]. In contrast, Tona et al. 2004, obtained for the extracts from *Western Cassia* leaves an IC_{50} = 2.8 ± 0.5 µg/Ml. The activity was due to the presence of some molecules of the group of quinones, flavonoids, triterpenes and steroids [36, 38].

4. Conclusion

Extracts from the various parts were preferentially active on either K1 and 3D7 strains of *P.falciparum*. Thus, irrespective of the extraction solvent, extracts from the roots showed a good antiplasmodial activity on the K1 strain. But on 3D7 strain, extracts from flowers presented a good activity. In addition, DCM was the appropriate solvent for obtaining rich extracts in phytochemicals with a very good power of inhibition of parasitic growth.

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