

***In vitro* Antiplasmodial Activity and Cytotoxicity of Extracts and Fractions of *Bidens pilosa***

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**Abstract**

Malaria is one of the most important health problems in Cameroon. The aim of this study was to evaluate the *in vitro* activity of the extracts and fractions of *Bidens pilosa* on *Plasmodium falciparum* strains. *Plasmodium falciparum* were cultivated by the method of Trager and Jensen (1976) and maintained in fresh O<sup>+</sup> human erythrocytes at 4 % haematocrit in complete medium (RPMI 1640 with Albumax II). The ring stage synchronized *P. falciparum* strains Pf3D7 (Chloroquine sensitive) and Pf INDO (Chloroquine resistant), 1% parasitemia, 2% hematocrit were incubated in a 96-well microplate for 48 h with different concentrations of plant extracts and fractions of the most active extract ranging from 1.56 to 100 µg/ml. RPMI and 0.4% DMSO were used as negative controls; while Chloroquine (1 µM) was used as positive control. The results were obtained by the microtiter plate using SYBR Green I fluorescence assay. Cytotoxicity was determined against HEPG2 and L929 cells using MTT assay. The most potent extract was chromatographed on reverse phase HPLC towards antiplasmodial activity guided purification of metabolites. Of the 3 plant extracts tested, the highest antiplasmodial (IC<sub>50</sub> of 8.18 ± 2.15 µg/ml) activity was observed with Ethyl Acetate extract of the leaves of *Bidens pilosa*. This extract was then selected for antiplasmodial activity guided fractionation and isolation of active fractions. Neighbouring fractions were combined judiciously to ensure maximum purity to obtain 15 pools. Among the 15 pools evaluated, the most potent was number 12 with an IC<sub>50</sub> of 0.73 ± 0.07 µg/ml (Pf 3D7) and 3.53 ± 1.85 µg/ml (Pf INDO). Pool 12 exhibited a CC50 of 88.34 ± 1.56 µg/ml (HEPG2 cells) and 81.31 ± 2.42 µg/ml (L929 cells). Therefore, pool 12 can be considered as highly active and non-toxic. Our findings, therefore, corroborate the use of *B. pilosa* as antimalarial in Cameroonian folk medicine.

**Keywords:** *Bidens pilosa*; Antiplasmodial activity; *Plasmodium falciparum*; Cytotoxicity; Cameroon.

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**Introduction**

Malaria is one of the most prevalent diseases in the world. It affects about 300–500 million people each year; mostly from sub-Saharan Africa and causes about 2.3 million deaths every year [1]. Malaria represents the world's greatest public health problem in terms of number of people affected, levels of morbidity and mortality (800,000 deaths among the 3 billion people at risk in 2009). About 91% of the total deaths occurred in Africa with pregnant women and children under 5 years being the most affected groups of the population [2]. Cameroon is among the 18 countries bearing 90% of deaths caused by malaria in Africa, with 71% of its population living in high-transmission areas [1]. The problems of the resistance of the vector mosquitoes to insecticides and that of parasites to most of the commercially available antimalarials seriously weaken the control approaches [3]. The success of quinine in the treatment of malaria for many decades, and later of

artemisinin and its derivatives for treatment of cerebral malaria, has turned attention to plants as potential sources of antimalarial drugs [4]. Most of the people in rural areas rely on traditional medicine for the treatment of many infectious diseases. They commonly treat the recurrent fever typical of malaria with plant extracts. It is not clear whether those plants contain ingredients with antimalarial activity or that they exert their actions through other mechanisms such as immunomodulation [5].

The emergence of multi-drug resistant strains of *Plasmodium* exacerbates the situation further; posing a major obstacle to successful chemoprophylaxis and chemotherapy of the disease [6]. The resistance of *P. falciparum* to the commonly used antimalarial drugs including the newly introduced Artemisinins has resulted in resurgence in treatment failures [7]. This rapid spread of parasite resistance has spurred a renewed interest in the search for new alternative antiplasmodial agents [8]. *Bidens*

*pilosa* is a medicinal plant used in Cameroon by traditional practitioners for the treatment of some diseases like helminthiasis, typhoids and malaria [9]. It is in this light that the present study wishes to assess the efficacy of this plant *in vitro* in order to justify its usage by traditional healers.

## Materials and Methods

### Plant material

Fresh leaves of plant were collected from Dschang-Cameroon in March 2015 and brought to the National Herbarium of Cameroon where a specimen was kept under number 18572/SRF-CAM. After identification as *B. pilosa* the leaves of the plant were collected for the second time, air dried and reduced to powder, before extractions were undertaken. Three types of extracts (methanolic, Ethyl Acetate and aqueous extracts) were prepared and tested on both Chloroquino-Sensitive 3D7 and Chloroquino-Resistant INDO strains of *P. falciparum*.

### Preparation of extract

The methanolic and Ethyl Acetate extracts were obtained using the procedure described by Wabo Poné et al. [10]. Briefly, 100 g of stored powder were macerated in 1.5 l of 95% methanol which removed the polar ingredient of the plants. The mixture was placed on a shaker. 72 h later, the suspension was sieved and filtered using filter paper of pore size 2.5 µm. The filtrate was aliquoted in a portion of 250 ml, introduced in a vial and concentrated for about 5 minutes using a rotavapor Buchi-R-210 model heated at 65°C. The concentration of all the filtrate took about 8 h.

For the ethyl acetate extract, the same quantity of plant powder was macerated in 1.5 l of ethyl acetate for 72 h. After filtration, the same procedure was followed for methanolic extract.

For the aqueous extract, a similar procedure was carried out; except for the fact that maceration in distilled water took 48 h. The filtrate was evaporated in a vacuum pump heated at 42°C and the residues were lyophilized. The methanolic, Ethyl Acetate and aqueous extract obtained were kept in a refrigerator at 4°C for further usages.

### Reference drugs

The reference drugs, Chloroquine (CQ) and Artemisinin (ATR), were obtained from SIGMA and were used as positive controls for *Pf3D7* and *PfINDO* respectively. Zero point four (0.4%) DMSO was used as negative controls.

### Dilution of extracts and reference drugs

6.5 mg of plant extracts and ATR were dissolved in 250 µl of DMSO while the same quantity of CQ was diluted using water (Milli-Q grade) making stock solutions of 25 mg/ml. Complete culture medium (RPMI 1640 with 0.2% sodium bicarbonate, 0.5% Albumax, 45 mg/l hypoxanthine and 20 mg/l gentamicin) was then added to solutions to prepare the desired concentrations ranging from 100 to 1.56 µg/ml.

### Maintenance of culture

*Plasmodium falciparum* was cultivated *in vitro* by the method of Trager and Jensen [11] with minor modifications. Cultures were maintained in fresh O<sup>+</sup> human erythrocytes at 4% haematocrit in complete medium at 37°C under reduced O<sub>2</sub> (gas mixture 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>).

### Synchronization of *Plasmodium falciparum* parasite development using sorbitol

The cell culture was centrifuged at 1800 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the pellet retained. Then, 10 pellet volume of 5% sorbitol solution was added. This solution was mixed and kept at 37°C for 7 minutes. The sorbitol solution containing the cells was taken out of the incubator and centrifuged at 1800 rpm for 5 minutes. The supernatant (sorbitol) was discarded and the synchronized culture was suspended in fresh cRPMI and transferred to the Petri dishes. The parasite culture was incubated at normal cultures conditions (37°C under reduced O<sub>2</sub>). After one hour, a thin blood smear was prepared and stained slides were examined under a microscope at 100 X magnification for the parasites stages identification and parasitemia [12].

### Antiplasmodial assay of *Bidens pilosa* extracts

The *in vitro* antiplasmodial activity of *Bidens pilosa* extracts was determined by fluorescence against CQ sensitive *Pf3D7* and resistant *PfINDO* strains of *Plasmodium falciparum*. Synchronized culture at 2% hematocrit and 1% parasitemia was aliquoted with test drugs [plant extracts (100, 50, 25, 12.5, 6.25, 3.125 and 1.56), 1Nm of CQ and ART] to 96-well flat-bottom tissue culture to a final volume of 100 µl. After 48 h of incubation, wells tested were supplemented with equal volumes of Lysis buffer (Tris-20 mM, EDTA-5 mM, Saponin-0.008%, Triton-X 100 – 0.08%) containing 1X SYBR Green I dye. After the addition of lysis buffer, the plates were incubated for another one hour. After this period, the plates were read using 96-well fluorescence plate reader (Victor, Perkin-Elmer), with excitation and emission wavelengths of 497 and 520 nm respectively. The fluorescence readings were plotted against drug concentration, and IC<sub>50</sub> values were determined. In order to validate the SYBR green data, thin blood smears of treated and untreated wells were prepared and stained [13].

### Evaluation of plants extracts cytotoxicity

Plant extracts cytotoxicity were evaluated on animal cell lines fibroblast L929 and Hella cells using MTT assay as described by Mosmann [14]. Briefly, cells (104 cells/200 ml/well) were seeded into 96-well flat-bottom tissue culture plates in complete medium (10% foetal bovine serum, 0.21% Sodium Bicarbonate (Sigma, USA) and 50 mg/ml gentamicin). After 24 h, plant extracts at different concentrations were added and plates incubated for 48 h in a humidified atmosphere at 37 °C and 5% CO<sub>2</sub>, 10% DMSO (v/v) was used as a positive inhibitor. Thereafter, 20 µl of a stock solution of MTT (5

mg/mL in 1X phosphate buffered saline) was added to each well, gently mixed and each plate was incubated for another 4 h. After spinning the plates at 1500 rpm for 5 min, supernatants were removed and 100 ml of 10% DMSO were added in each well to stop the reaction of extracts. The formation of formazon obtained after the transformation of tetrazolium was read on a microtiter plate reader at 570 nm. The 50% cytotoxic concentration (CC<sub>50</sub>) of plant extract was determined by analysis of dose–response curves, according to the cytotoxicity gradient of plant extracts established by Malebo et al. (Table 1) [15]. Also, the Selectivity index (SI) was calculated using the following formula:

**Table 1:** The IC<sub>50</sub>, the CC<sub>50</sub> and the selectivity index (SI) of the effect of the different extract on the development of *P. falciparum* strains and mammalian cells line (Legend: AE: Aqueous extract, ME: Methanolic extract, EAE: Ethyl Acetate extract, SI: Selectivity index).

Strains	<i>Bidens pilosa</i>		
	AE	ME	EAE
	IC <sub>50</sub> (µg/ml)		
3D7	14.31 ± 1.37	26.66 ± 2.56	8.18 ± 2.15
INDO	22.6 ± 1.99	14.07 ± 2.09	13.37 ± 0.04
	CC <sub>50</sub> (µg/ml)		
HEPG2	73.23 ± 1.22	29.36 ± 4.01	71.23 ± 2.11
L929	76.11 ± 3.21	29.02 ± 1.68	68.46 ± 3.17
	SI		
	5.86	4.83	12.54

**Bioassay guided fractionation and isolation of active fractions**

The ethyl acetate extract of *B. pilosa* which showed good antiplasmodial potency was chosen for antiplasmodial activity

guided reverse phase HPLC fractionation. DMSO supernatant of 200 mg/ml was injected into C18 Deltapak (19 × 300 mm, 15 m) column (Waters, USA) using water–methanol gradient (5–95%, 1%/min) at a flow rate of 9 ml/min on a Gilson prep HPLC system. Dual wavelength detections were made at 214 and 254 nm. Fractions were collected, dried, weighed and evaluated for antiplasmodial and cytotoxic activities

**Determination of molecules contents in the ethyl acetate extract and fractions by GCMS**

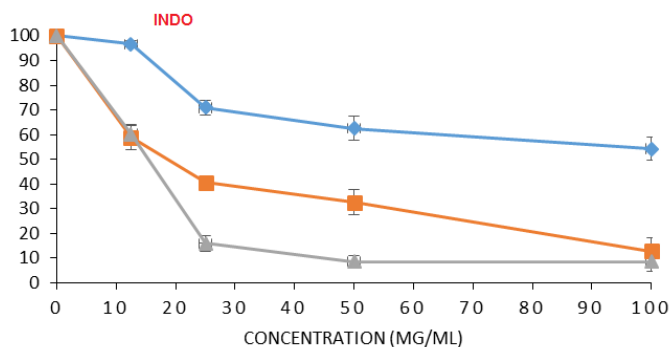
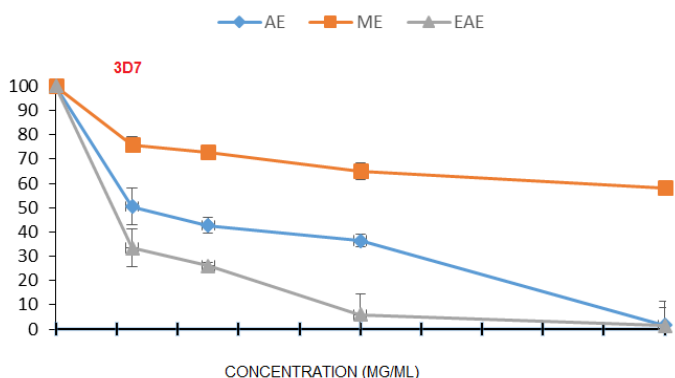
Molecules contained in the crude Ethyl Acetate extract and in most effective fractions were determined using Chromatogram D:\GCMS-QP2010Ultra\GCMS method.

**Statistical analysis**

Data obtained from this work were analyzed statistically using Student’s t-test and ANOVA (One-way) followed by a post test (Turkey–Kramer multiple comparison test) using SPSS (version 22.0). Differences between means was considered significant at 5% level of significance (P<0.05). The 50% inhibitory concentrations (IC<sub>50</sub>) were determined from linear regression curve obtained between the inhibition rate expressed in probit and the decimal logarithm of the concentrations (µg/ml).

**Results**

The variation of the mean inhibition rate on the growth of *Pf3D7* and *PfINDO* strains of *P. falciparum* according to the different concentrations of extracts of *B. pilosa* is shown in Figure 1.



**Figure 1:** Percentage growth of *Plasmodium falciparum* 3d7 and INDO strains according to the different concentrations of *Bidens pilosa* extract. (Legend: AE: Aqueous Extract, ME: Methanolic extract, EAE: Ethyl Acetate Extract).

Figure 1 shows that RPMI 1640 and 0.4% DMSO did not affect (0% inhibition rate) the development of *P. falciparum*. In the treated wells, the inhibition rate increases with the

increasing concentration of the tested extracts. Considering the CQ sensitive 3D7 strain, the mean inhibition rates of the aqueous extract (49.55 ± 7.46, 57.35 ± 3.30 and 63.58 ± 2.68)

and methanolic extract ( $24.01 \pm 9.31$ ,  $27.10 \pm 8.77$  and  $35.00 \pm 4.36$ ) at the concentrations of 12.5, 25, and 50  $\mu\text{g/ml}$  respectively were quite similar ( $P > 0.05$ ). Meanwhile, these inhibition rates were less ( $P < 0.05$ ) than that of the Ethyl Acetate extract ( $66.54 \pm 7.62$ ,  $73.92 \pm 18.35$  and  $94.15 \pm 8.68$ ). However, at 100  $\mu\text{g/ml}$  concentration, Ethyl Acetate and aqueous extracts exhibited mean inhibition rates of  $98.59 \pm 9.94$  and  $98.44 \pm 7.28\%$  respectively which are higher as compared to the mean inhibition rate of methanolic extract ( $41.79 \pm 9.00\%$ ).

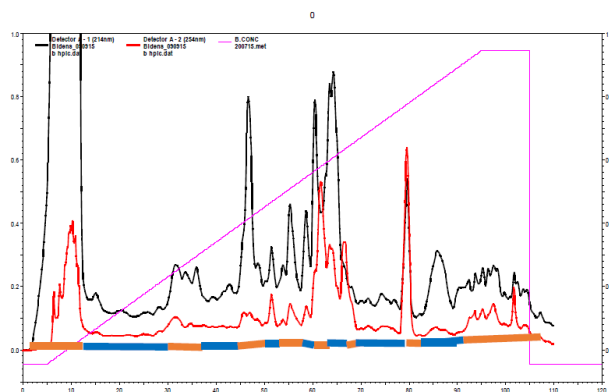
On the CQ resistant INDO strain, the aqueous extract exhibited a poor activity with a mean inhibition rate of  $3.35 \pm 1.3$ ,  $29.11 \pm 3.11$ ,  $37.51 \pm 4.97$ ,  $45.70 \pm 4.70$  at the concentrations of 12.5, 25, 50 and 100  $\mu\text{g/ml}$  respectively. For concentrations greater than 12.5  $\mu\text{g/ml}$ , the mean inhibition rates of methanolic extract ( $59.43 \pm 2.44$ ,  $67.44 \pm 5.20$ ,  $87.30 \pm 5.30$ ) were less ( $P < 0.05$ ) than the effect of Ethyl Acetate extract ( $84.09 \pm 3.33$ ,  $91.44 \pm 2.47$ ,  $91.51 \pm 3.81$ ). Below these concentrations, the antiplasmodial activity is similar for both extracts.

The  $\text{IC}_{50}$ , the  $\text{CC}_{50}$  and the selectivity index (SI) of the different extracts are shown on Table 1. The effect of the different plant extracts was more potent on CQ sensitive 3D7 strain compared to the CQ sensitive INDO strain. The Ethyl Acetate extract exhibited the highest antiplasmodial activity ( $\text{IC}_{50}$  of  $8.18 \pm 2.15$ ) with a ( $P < 0.05$ ) as compared to other extracts except for the  $\text{IC}_{50}$  of the methanolic extract. Aqueous extract showed the highest cytotoxic concentration ( $76.11 \pm 3.21 \mu\text{g/ml}$ ). However, the best selectivity index (121.01) was obtained in the Ethyl Acetate extract.

**Bioassay guided fractionation of Ethyl Acetate extracts of *Bidens pilosa***

Among the extracts of *B. pilosa* tested, the ethyl Acetate extract was the most potent and possessed the highest selectivity index. Therefore, it was selected for activity guided

fractionation by RPHPLC using water–methanol gradient (Figure 2). Orange and blue color bars indicate fractions (1–60) collected. The zoom of 60–120 min (marked by dotted box) has been shown just above the chromatogram tracing.



**Figure 2:** Semiprep Reverse phase HPLC Chromatogram of ethyl acetate extract of *Bidens pilosa*.

Table 2 shows the  $\text{IC}_{50}$  and the  $\text{CC}_{50}$  fractions of Ethyl Acetate extract of *B. pilosa* on the development of *P. falciparum* strains and mammalian cells line.

The fractions showed a wide range of antiplasmodial potencies with  $\text{IC}_{50}$  values ranging from 2.38 to 71.46  $\mu\text{g/ml}$  against a CQ resistant strain (INDO) and 0.73 to 67.36  $\text{mg/ml}$  for CQ sensitive strain (3D7) of *P. falciparum* (Table 2).

Just like crude extracts of *B. pilosa*, most chromatographic fractions derived from these products were also found to be more effective against a CQ sensitive 3D7 strain than CQ resistant INDO strain. More interestingly, fractions led to a significant increase on the inhibition of the development of *P. falciparum* with the lowest  $\text{IC}_{50}$  of  $0.73 \pm 0.07$  against *Pf3D7* compared to crude extract which exhibited a lowest  $\text{IC}_{50}$  of  $8.18 \pm 2.15$  ( $P < 0.05$ ).

**Table 2:** The  $\text{IC}_{50}$ , the  $\text{CC}_{50}$  and the selectivity index (SI) of the effect of the different fractions on the development of *Plasmodium falciparum* strains and mammalian cells line (Legend: SI Selectivity index).

Fractions	weight (mg)	PfINDO	Pf3D7	HEPG2	L929	SI
		$\text{IC}_{50}$ ( $\mu\text{g/ml}$ )	$\text{CC}_{50}$ ( $\mu\text{g/ml}$ )			
1 (F1-F5)		$59.26 \pm 1.21$	$58.48 \pm 3.29$	$17.42 \pm 9.92$	$23.28 \pm 4.87$	0,30
2 (F6-F14)	5.99	$65.38 \pm 0.88$	$61.22 \pm 1.81$	$4.62 \pm 3.82$	$2.46 \pm 9136$	0,08
3 (F15-F20)	5.26	$50.15 \pm 2.2$	$53.18 \pm 1.52$	$14.30 \pm 1.22$	$12.02 \pm 3.98$	0,27
4 (F21-F23)	2.6	$61.75 \pm 1.55$	$59.41 \pm 2.30$	$2.21 \pm 1.81$	$0.87 \pm 0.23$	0,04
5 (F24-F27)	3.64	$71.46 \pm 1.48$	$68.73 \pm 2.33$	$3.75 \pm 1.58$	$1.45 \pm 0.64$	0,05
6 (F28)	0.65	$8.29 \pm 1.35$	$6.95 \pm 1.89$	$17.72 \pm 5.65$	$29.31 \pm 2.42$	2,55
7 (F29-31)	2.65	$31.76 \pm 1.73$	$67.36 \pm 11.04$	$9.49 \pm 1.01$	$11.38 \pm 2.87$	0,14
8 (F32)	0.75	$16.8 \pm 8.33$	$14.65 \pm 7.51$	$21.6 \pm 0.9$	$17.46 \pm 1.36$	1,47

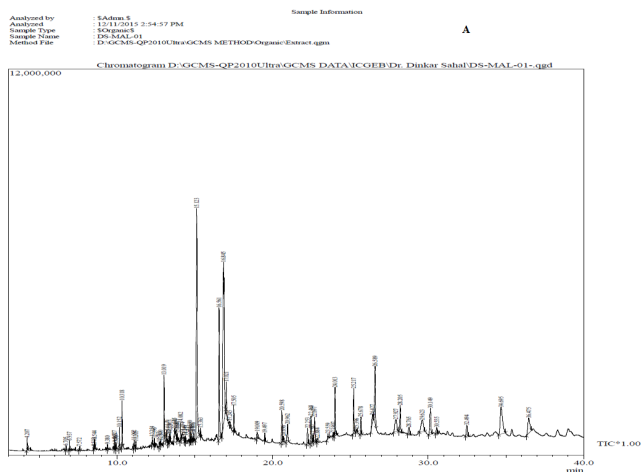
9 (F33-34)	2.17	5.33 ± 0.01	4.87 ± 0.20	36.6 ± 5.16	25.02 ± 3.98	0,08
10 (F35-36)	2.45	6.64 ± 0.87	4.39 ± 0.30	40.3 ± 7.01	36.47 ± 5.23	9,18
11 (F37-38)	3.96	13.55 ± 0.66	12.41 ± 1.24	22.2 ± 2.35	30.45 ± 6.64	1,79
12 (F39-43)	4.53	3.53 ± 1.85	0.73 ± 0.07	88.34 ± 1.56	81.31 ± 2.42	121,01
13 (F44-45)	0.69	2.38 ± 0.09	1.86 ± 0.10	62.2 ± 6.40	33.40 ± 0.62	33,44
14 (F46-51)	1.61	3.49 ± 0.04	3.11 ± 0.12	77.51 ± 616	41.93 ± 6.67	24,92
15 (F52-60)	11.12	3.91 ± 0.11	3.24 ± 1.27	73.70 ± 1.69	62.80 ± 7.02	22,75

**Characterization of molecules in the most effective extract and the most effective fractions by GCMS**

Figure 3 presents the number of molecules contained in a crude (A) and fraction 12 (B) of Ethyl Acetate extract of *B. pilosa* (F39-43).

Figure 3A has shown 68 pics of molecules contained in the crude Ethyl Acetate extract. Each pic corresponds to a specific molecule. The different names have been given according to the record time of pics, the area and the percentage of area (Table 3).

Peak#	R. Time	Area	Area%	Name
1	4.207	787779	0.52	ETHANONE, 1-PHENYL-
2	6.705	231503	0.15	OCTADECANE
3	6.937	443551	0.29	Dodecane, 4,6-dimethyl-
4	7.572	170061	0.11	Dodecane, 4,6-dimethyl-
5	8.503	65185	0.04	METHYL [(1R)-(1-ALPHA,4-ALPHA,5-BETA)-4-(BENZYLIDENE)-2-PHENYLETHYL]OXIRANEMETHANOL
6	8.544	490644	0.32	alpha-Methyl-alpha-[4-methyl-3-pentenyl]oxiranemethanol
7	9.380	219972	0.14	Heptadecane, 2,6,10,15-tetramethyl-
8	9.827	321064	0.21	Hexadecane, 1-iodo-
9	9.863	182967	0.12	Heptadecane
10	9.936	240535	0.16	Benzene, 1,4-bis(1-formylethyl)-
11	10.152	1077719	0.71	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-
12	10.318	2486719	1.63	Benzoic acid, 4-ethoxy-, ethyl ester
13	11.092	270201	0.18	HEXADECANE
14	11.205	346611	0.23	(-)-5-OXATRICYCLO[8.2.0.0(4.6)]DODECANE,12-TRIMETHYLBIS(1-ETHOXY)METHYLENE-1,1-DIIMINE
15	12.254	384625	0.25	IRON, TRICARBONYL[N-(PHENYL-2-PYRIDINYLMETHYL)AMINE]
16	12.396	443095	0.29	Eicosane
17	12.713	342184	0.22	2-Hexenal, 6-phenyl-, (E)-
18	12.800	231030	0.15	1-(9H-FLUOREN-9-YLIDENE)-1LAMBDA-5--DIAZYLENE
19	13.019	3659517	2.40	Tetradecanoic acid
20	13.176	720200	0.47	1-PHENYL-PENTA-2,4-DIYNE
21	13.295	155562	0.10	1-OCTADECENE
22	13.359	439939	0.29	EICOSANE
23	13.413	631585	0.41	3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl)-
24	13.685	654034	0.43	2,6,10-TRIMETHYL,14-ETHYLENE-14-PENTADECENE
25	13.809	386380	0.25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
26	13.884	367323	0.24	2-PENTADECANONE, 6,10,14-TRIMETHYL-
27	14.082	958391	0.63	2-Butenal, 2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
28	14.204	661914	0.43	Phthalic acid, isobutyl trans-dec-3-enyl ester
29	14.346	323251	0.21	PENTALENE, OCTAHYDRO-1-(2-OCTYLDECYL)-
30	14.411	279791	0.18	Heneicosane
31	14.690	493271	0.32	TETRATRIACONTANE
32	14.739	166949	0.11	01297107001 TETRANEURIN - A - DIOL
33	14.800	583339	0.38	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
34	14.939	530383	0.35	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-[R]]
35	15.123	23525862	15.40	PENTADECANOIC ACID
36	15.363	424661	0.28	HEXADECANOIC ACID, ETHYL ESTER
37	16.561	9498634	6.22	Phytol
38	16.845	31052991	20.33	cis-Vaccenic acid
39	17.021	2343140	1.53	Octadecanoic acid
40	17.245	613046	0.40	9-Octadecenal, (Z)-
41	17.505	1335161	0.87	Phytol, acetate
42	19.009	810860	0.53	(E)-Dodec-5-en-4-olide
43	19.497	500137	0.33	PENTACOSANE



**Figure 3A:** Chromatogram of pictures of the molecules contained in the crude extract of ethyl acetate (A) and in the most effective fraction (12: F39-43).

**Table 3:** Names and percentage area of different molecules illustrate in Chromatogram A: (crude extract of ethyl acetate).

Figure 3B has shown only 14 molecules in the most effective fraction as compared to the Ethyl Acetate extract (68 molecules). The different names have been given as earlier stated (Table 4).

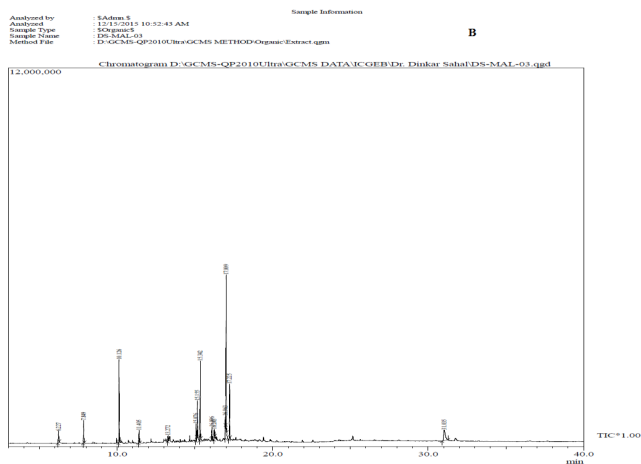


Figure 3B: Chromatogram of pictures of the molecules contained in the most effective fraction (12: F39-43)

Peak#	R. Time	Area	Area%	Name
44	20.598	2803075	1.84	Benzonitrile, m-phenethyl-
45	20.696	434505	0.28	4-Cyanobenzoic acid, hexadecyl ester
46	20.962	2278184	1.49	Tetratetracontane
47	22.253	1695359	1.11	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
48	22.468	2595722	1.70	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
49	22.582	588995	0.39	(2,3-Diphenylcyclopropyl)methyl phenyl sulfoxide, trans-
50	22.697	2945935	1.93	PENTACOSANE
51	22.884	321199	0.21	Octacosyl acetate
52	23.559	556830	0.36	5,5-Dimethyl-1,3-dioxane-2-ethanol, tert-butyl dimethylsilyl
53	23.907	285943	0.19	1,3,5,7,9,11,13,15,17,19,21,23-Cyclotetradecacosadodecane
54	24.013	3703986	2.43	TETRACONTANE
55	25.217	3842484	2.52	PENTACOSANE
56	25.396	296079	0.19	Octacosyl acetate
57	25.678	1516338	0.99	Squalene
58	26.427	1310175	0.86	
59	26.589	6279450	4.11	3-(3,5-DI-TERT-BUTYL-4-HYDROXY-PHENYL)-N-[2-(4-
60	27.927	3298920	2.16	
61	28.205	3081576	2.02	TETRACONTANE
62	28.765	1126422	0.74	Ergost-5-en-3-ol, (3.beta.)-
63	29.623	3964751	2.60	4,4-DIMETHYLCHOLESTA-5,7-DIEN-3-OL #
64	30.149	3899899	2.55	TETRACONTANE
65	30.555	855317	0.56	STIGMAST-5-EN-3-OL, OLEAT
66	32.494	1799636	1.18	TETRACONTANE
67	34.695	9095440	5.95	Stigmasterol
68	36.475	4310789	2.82	STIGMAST-5-EN-3-OL, (3.BETA.)-
		152738775	100.00	

Table 3: Names and percentage area of different molecules illustrate in Chromatogram A: (crude extract of ethyl acetate).

Peak#	R. Time	Area	Area%	Name
1	6.227	847179	2.94	Benzaldehyde, 2,4-dimethyl-
2	7.849	1254525	4.36	4-(DIMETHOXYMETHYL)-1,2-DIMETHYLBENZENE
3	10.126	4377850	15.20	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-
4	11.405	691927	2.40	1,2,4-TRIMETHOXY-5-[(1E)-1-PROPENYL]BENZENE #
5	13.272	269924	0.94	1-HEXADECENE
6	15.076	886027	3.08	HEXADECANOIC ACID
7	15.155	1792697	6.23	ETHYL 9-HEXADECENOATE
8	15.342	3991067	13.86	HEXADECANOIC ACID, ETHYL ESTER
9	16.096	553641	1.92	ETHYL 9-HEXADECENOATE
10	16.243	554279	1.92	n-Nonadecanol-1
11	16.963	567568	1.97	n-Propyl 9,12-octadecadienoate
12	17.009	7484432	25.99	ETHYL 9-OCTADECENOATE
13	17.225	2860086	9.93	OCTADECANOIC ACID, ETHYL ESTER
14	31.035	2666726	9.26	Cholesterol
		28797928	100.00	

Table 4: Names and percentage area of different molecules illustrate in Chromatogram B: [most effective fraction (12: F39-43)].

Discussion

In Cameroon, as in all developing countries, plants are regularly used by healers to treat recurrent fever and malaria. In this study, we evaluate the *in vitro* antiplasmodial activities of crude extracts and fractions from leaves of *B. pilosa* using SYBR Green assay. From the normal growth observed in the

negative control wells, the variation of parasitemia shown in the treated wells was due to the effect of tested products. The aqueous, methanolic and ethyl acetate extracts of *B. pilosa* were more active against CQ sensitive 3D7 strain than CQ resistant strain, with IC<sub>50</sub> values of 14.31 ± 1.37, 266.77 ± 0.49 and 8,18 ± 2.15 µg/ml respectively. These results are in contrast with those obtained by Niharika Singh et al. [13] which show that *P. hysterothorus* extracts and fractions were more effective against CQ resistant INDO strain than CQ sensitive 3D7 strain. However, our results are in conformity with those of Okokon et al. [17] which shows that, *Pf3D7* was more sensitive (IC<sub>50</sub>=35.35 ± 0.16) to butanol extract of *Zea mays* than *PfINDO* (IC<sub>50</sub>=44.81 ± 0.12). The Ethyl Acetate and methanolic extracts of *B. pilosa* demonstrated a higher antiplasmodial activity than the aqueous extract used as the solvent in the traditional preparation at all concentrations. Similar observations were reported by Sothearea et al. [18] and Lekana-Douki et al. [19] comparing the methanolic extracts of *Brucea javanica* and *Staudtia gabonensis* to aqueous extracts. This suggests that more active compounds were extracted with those solvents. The antiplasmodial activities of the extracts and fractions against plasmodium parasites were dose-dependent. According to [13], the plants were classified by their antiplasmodial potential as:

- (a) highly active (IC<sub>50</sub> ≤ 5 µg/ml), fractions number 9 (F33-34), 10 (F35-36), 12 (F39-43), 13 (F44-45), 14 (F46-51) and 15 (F52-60);
- (b) Promisingly active (IC<sub>50</sub>: 5.1–10 µg/ml), Ethyl Acetate extract of *B. pilosa* and fraction number 6 (F28);
- (c) Good activity (IC<sub>50</sub>: 10.1–20 µg/ml), aqueous extract of *B. pilosa*, fractions number 8 (F32) and 11 (F37-38);
- (d) moderate activity (IC<sub>50</sub>: 20.1–40 µg/ml);
- (e) Marginal potency (IC<sub>50</sub>: 40.1 –70 µg/ml), fractions number 1 (F1-F5), 2 (F6-F14), 3 (F15-F20), 4 (F21-F23), 5 (F24-F27), 7 (F29-31);
- (f) Poor or inactive (IC<sub>50</sub> 70.1 to 4100 µg/ml).

The reported antiplasmodial activity of *B. pilosa* may be attributed to the molecules contained in the plant extracts; especially the Phenol, 2, 4-Bis (1, 1-Dimethylethyl) which is a known antiplasmodial molecule. The percentage area of Phenol, 2, 4-Bis (1, 1-Dimethylethyl) was 0.71% in the crude ethyl acetate extract and up to 15.20% in the most effective fraction (F12). Several plants of the Asteraceae family have been revealed as good sources of antimalarials; the most famous one being *Artemisia annua* the Chinese herb from which artemisinin (qinghaosu) was isolated [15]. From gradient, the definition of the cytotoxicity concentrations used [16] was CC<sub>50</sub><1.0 µg/ml (high cytotoxicity), CC<sub>50</sub> 1.0–10.0 µg/ml (moderate), CC<sub>50</sub> 10.0–30.0 µg/ml (mild), and CC<sub>50</sub>>30 µg/ml (nontoxic). We realized that most of the tested extracts and fractions were found to be non-cytotoxic or with very low toxicity on HEPG2 and L929 mammalian cells line. This observation may be an indicator of their safety as drugs for mammalian organisms. Our findings, therefore, corroborate

with the use of *B. pilosa* as antimalarial in Cameroonian folk medicine.

## Conclusion

From this study, it was observed that RPMI 1640 and 0.4% DMSO did not influence the normal growth of *P. falciparum*. The three tested extracts inhibited the growth of both *Pf3D7* and *Pf1ND0* strains. The most effective fraction from the ethyl acetate extract showed high antiplasmodial activity with no cytotoxicity. The GCMS of crude extract and fraction 12 of Ethyl Acetate of *B. pilosa* show that this plant contained high number of molecules. Moreover, the bioassay guided of the fraction concentrate molecules to which the antiplasmodial activity can be attributed.

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