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Ethnopharmacological survey of antimalarial plants and the biological activities of *Conyza aegyptiaca* (Asteraceae)

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Abstract
Malaria is the most widespread disease in Cameroon and remains the major endemic and the first cause of morbidity and mortality in children under five and pregnant women. The increasing spread of drug-resistant *Plasmodium falciparum* strains has worsen the situation. Consequently, the medical treatment of malaria necessitates access to a good health care system. Unfortunately, most people resort to medicinal plants to treat themselves due to poverty. It is therefore crucial to promote and orientate research towards ethnobotanical studies in order to validate the therapeutic virtues of medicinal plant preparations. The objective of the present research work was to carry out an ethnopharmacological survey using in-depth interview of antimalarial plants in the Western Highlands of Cameroon with special focus on the biological activities of the leaves of *Conyza aegyptiaca* (Asteraceae). The interview of 76 tradi-practitioners reveals that, the knowledge of traditional medicine is a family secret and lies mostly in the hands of the older generation. The present study showed a good diversity of plants (37 plant species belonging to 25 families) used in the treatment of malaria by in the Haut-Plateaux Division with Asteraceae being the most represented plant family. The diagnosis of malaria in traditional medicine is based primarily on the symptoms while its management is dominated by monospecific recipes prepared mostly via decoction or maceration but administered mainly through oral route. Barks are the most preferred plant parts used in the preparations of recipes in traditional medicine. Though the antioxidant and antihemolytic activities of *C. aegyptiaca* (one of the plant species cited during the survey), further research is warranted to identify and isolates active compounds of all the antimalarial plants with higher performance indices in the Haut-Plateaux division.

Keywords: malaria, ethnopharmacological survey, *Conyza aegyptiaca*; antioxidant activity; anti-hemolytic activity

Introduction
Ethnopharmacology, which is the interdisciplinary scientific exploration of the biologically active agents (of plant, animal and mineral origin) traditionally used for therapeutic, curative, preventive or diagnostic purposes has played a vial in drug development using plants [1, 2]. The use of medicinal plants by man in general, and by Africans in particular, to treat themselves dates back to thousands of years [1, 3]. Medicinal plants represent a very important aspect in the history of medicine and have contributed enormously to the development of modern medicine [4, 5]. As a result, thanks to ethnopharmacological studies, 25% of conventional drugs used in modern medicine currently are of plant origin [6]. For instance, many drugs used derived solely from plants, produced as secondary metabolites to treat various diseases [7, 8]. Nevertheless, only 15% of the 70,000 plant species used for the treatment of diseases including malaria in the world have been investigated for their medical use [2].
Malaria is the most widespread disease in Cameroon; it remains the major endemic and the first cause of morbidity and mortality in the most vulnerable groups, namely children under five and pregnant women [9, 10]. Health statistics reveal that it is responsible for 35-40% of all deaths in health facilities [10]. It accounts for 50% of morbidity in children under five, 40-45% of medical consultations and 30% of hospitalisations [11]. Malaria is also the cause of 26% of absences from work and 40% of household health expenditure [12].
The protocol for the management of cases of uncomplicated malaria has undergone changes in recent years following the emergence of chloroquino-resistant strains of *Plasmodium* [13]. The use of chloroquine and monotherapies has been abandoned in favour of artemisinin-based combination therapy (ACT) or its derivatives. Unfortunately, due to their relatively high cost, ACTs remain inaccessible to poor populations who are most exposed to malaria. As a result, the control strategy of early and effective management of malaria cases remains unsatisfactory.

The increasing spread of drug-resistant *P. falciparum* strains has worsen the situation [14]. This leads to referrals to traditional medicines [15]. Additionally, population growth and poverty limit access to a good health care system for the medical treatment of malaria. In these conditions, people often resort to medicinal plants to treat themselves. It is therefore crucial to promote and orientate research towards ethnobotanical, phytochemical and pharmacological studies in order to validate the therapeutic virtues of these preparations. In this regard, traditional medicines would be improved and made accessible and available to the populations [16]. So far, in the Hauts-Plateaux Division and elsewhere in Cameroon, ethnopharmacological studies have led to the discovery of many new antimalarial drugs such as quinine and artemisinin obtained from *Cinchona officinalis* and *Artemisia annua*, respectively [16, 17].

Unfortunately, more than 63% of the cameroonian plants used by traditional healers remain uninvestigated for their presumed antimalarial properties [14].

To improve this low rate of exploitation of plants species with potential antimalarial properties, we intended to carry out an ethnopharmacological survey of antimalarial plants in the Western Highlands of Cameroon with special focus on the biological (antihemolytic and cytotoxic) activities of the leaves of *C. aegyptiaca* (Asteraceae).

Material and Methods

Collection of plant material, identification and working sites

The plant material is made up of all the plants listed in the ethnobotanical surveys carried out among traditional practitioners in the Haut-Plateaux Division of Western Region of Cameroon. The plants were harvested fresh from their environment. A herbarium plate per species was made and deposited at the National Herbarium of Cameroon for identification and confirmation of names.

The ethnobotanical survey led to the selection of namely *C. aeryptiaca* (leaves), identified by Dr. Tchiengue Barthelemy at the National Herbarium of Cameroon by comparison with the botanical collection of Geerling number 4732 registered at the National Herbarium referenced under the following number 36311/ HNC. The same plant was evaluated for its anti-malarial, antioxidant and anti-haemolytic properties.

The equipment used in the field included survey sheets to collect information and instructions on the plants by traditional practitioners, a digital camera for photographing the plants, pruning shears for collecting samples, newspaper and wood-slat paper presses to facilitate the drying of samples, alcohol at 95°for preserving plant organs during drying, and a notebook for field observations of the plants.

In order to carry out this study, the research protocol was submitted to the Institutional Ethical Review Committee of the Université des Montagnes for a research authorisation. The local councils of the towns of Baham, Bâtie, Bangou and Bamendjou eased the identification and contacting of the recognised traditional practitioners.

Type of study and sample collection

This study was conducted over a period of five months (January 2019 to May 2019) including three months of ethnopharmacological survey and 2 months of experimental studies. To successfully complete this study, a list of traditional practitioners was obtained at each district medical centre in order to schedule meetings with them. Several mutually agreed visits were made to workplaces (business premises), some of them following a pre-arranged schedule. The interview was exclusively oral and often ended with a prescription which was later used to fill in the questionnaire. Volunteers were interviewed individually, using a survey form (Appendix-make it available). Two standard ethnobotany techniques described by Foutse Yimta [18, 19] were used: (i) the field evolution, which consisted in collecting information from traditional practitioners during the field visit; (ii) and the comparative ethnobotany, which consisted in collecting comparative information on plants already known and used by both parties for the management of malaria. The plants mentioned in the questionnaire were either local and spontaneous plants or cultivated or imported plants. While in the field, picture of each plant in its natural environment was taken with a digital camera and samples were collected and tightly bound with rubber after spraying with 95 °C alcohol. The identification of plants was done at the National Herbarium based on observation of photos, plant samples and documentation.

Extraction and preparation of hydro-ethanolic extracts

The plant material (leaves) was harvested and was dried for 14 days at room temperature away from light to preserve the integrity of its properties. The plant leaves were crushed using a Royalty Line® brand crusher until a fine powder was obtained.

Extraction was done by maceration and decoction. The fresh leaves (500 g) of *Conyza aeryptiaca*, were macerated in 2 L of distilled water for 24 and 48 hours respectively. The resulting powder (100 g) was decanted in 700 mL of water distilled at 180 °C on a hot plate. The solutions obtained were sieved and then filtered using Whattman N° 2 paper. The filtrate was dried at 45 °C in the oven and yielded 10 and 29.53 g of crude extract respectively, representing 10.1 and 5.9 % extraction yield. The powder obtained was stored at room temperature in airtight boxes. The dry extracts were then stored in a refrigerator at +4 °C.

Phytochemical screening of hydro-ethanolic extract

Investigations into the targeted chemical groups in the plant extracts were conducted according to the protocols described by Harborne (1976), Odebeyi and Sofowara (1978), Trease and Evans (1989), Sofowora, (1993) 20, [21, 22, 23].

The flavonoid detection test

Five millilitres of a 25% diluted ammonia solution were added to 5 mL of a 2 mg/mL concentrated aqueous extract solution. Five millilitres of concentrated sulphuric acid were added to the mixture. A yellow colouring that disappeared over time characterised the presence of flavonoids [24].

The test for the detection of saponins

Twenty-five milligrams of extract were mixed with 15 mL of distilled water in a test tube and the whole was brought to the boiling water bath for 5 min. After cooling, 5 mL of this solution were introduced into a test tube and then vortexed vigorously for 10 seconds. The presence of a one-centimetre-thick foam that persisted for more than a minute after agitation indicated the presence of Saponins [25].

The tannin test

To five millilitres of an alcoholic or aqueous solution of extract concentrated at 2 mg/mL, 3 drops of 1 M ferric chloride were added. The presence of the tannins was manifested by a change in colour of the solution which turned

dark blue (gallic tannins) or blackish green marking the presence of the catechic tannins [22].

Testing for polyphenols
Fifty milligrams of extract were dissolved in 15 mL of methanol and the solution was heated in a boiling water bath for 15 min. To the mixture, 3 drops of a freshly prepared ferric cyanide solution (1 mL $FeCl_3$ 1% and 1 mL $K_3Fe(CN)_6$) were added. The formation of a green precipitate highlighted the presence of the phenols [24].

The triterpenes detection test
To 10 mL of a 10% (w/v) concentrated extract solution, 2 mL of chloroform were added and the whole was homogenised. A volume of 3 mL of concentrated sulphuric acid was added to form two phases. The formation of a reddish-brown interface indicated the presence of terpenoids [24].

The glucoside detection test
One gram of extract was dissolved in 5 mL of HCl and then neutralised with 5 mL of 5% sodium hydroxide solution. A Fehling solution (A + B) was added drop by drop to the mixture. The presence of glucosides was manifested by the appearance of a brick-red [24].

The anthocyanin detection test
Fifty milligrams of extract were mixed with 15 mL of 1% HCl and brought to the boil. The variation in colouring from red-orange to blue-orange was indicative of the presence of anthocyanins [24].

Testing for anthraquinones
Fifty milligrams of extract were diluted in 4 mL of a mixture of chloroform and petroleum ether (v/v), homogenised and then filtered. To 1 mL of filtrate, an equal volume of 10% NaOH was added. The development of a red colour highlighted the presence of anthraquinones [24].

The steroid detection test
Two hundred milligrams of extract were dissolved in 10 mL of chloroform. Two millilitres of acetic acid were then introduced into the solution and the whole was cooled in an ice bath. Concentrated sulphuric acid was added and the formation of a blue-grey ring showed the presence of the steroids [25].

Determination of the antioxidant property
The Ferric Reducing Antioxidant Power (FRAP) method and 2,2-diphenyl-1-picryl-hydrazyl free radical scavenging (DPPH) method were used to evaluate the antioxidant activity of our plant extracts.
2,2-diphenyl-1-picryl-hydrazyl free radical scavenging (DPPH) method
Briefly, in 3 mL of each diluted extract, 1 mL of methanol solution of DPPH (0.1 mmol/L) was added. The mixture was kept in the dark at room temperature for 30 min and the absorbance was measured at 517 nm against a blank. The following equation was used to determine the percentage of the radical scavenging activity of each extract. Percentage of radical scavenging activity = [(OD control - OD sample) / OD control] x 100. Where OD is the optical density. The IC value (µg/mL) which, is the effective concentration at which DPPH radicals were scavenged by 50% value was obtained by interpolation from linear regression analysis.

Total antioxidant activity by ferric reducing antioxidant power assay (FRAP)
The FRAP method was used to determine the total antioxidant activity which measures the reduction of ferric ion to the ferrous form in the presence of antioxidant compounds [26]. The fresh FRAP reagent consists of 500 mL of acetate buffer (300 mmol/L pH 3.6), 50 mL of 2,4,6-Tris (2-pyridyl)-s-triazin (10 mmol/L), and 50 mL of $FeCl_3 \cdot 6H_2O$ (50 mmol/L). For the assay, 75µL of each extract were mixed with 2 mL of FRAP reagent and the optical density was read after 2 min at 593 nm against the blank.

Evaluation of the antihemolytic activity of *Conyza aegyptiaca* leaves
Erythrocytes were obtained from healthy donors and processed as described by Hebbani *et al*., 2014 [26]. After the removal of plasma and buffy coat, the erythrocytes were washed thrice with phosphate buffer saline (PBS) and resuspended in the same buffer. For hemolysis, modified protocol as described by Okoko *et al*. [27], was used. The reaction mixture contained 200 µL of erythrocyte suspension and 10 µL of the tested plant fraction. The mixture was incubated for 30 min at 37 °C. Hemolysis was induced by addition of 100 µL of 100 µM of H_2O_2 followed by incubation at 37 °C for 3 hours. The supernatant (200 µL) was diluted with 1.4 mL of PBS and the samples were centrifuged at 5000 rpm for 5 min to separate the red blood cells from the other blood components and absorbance of the content was measured at 540 nm. For this experiment, the absorbance obtained from H_2O_2 alone without the plant extract was taken as 100% hemolysis. Hence, the absorbance values obtained at 540 nm were expressed as % hemolysis inhibition.

Cytotoxicity Test
The Vero ATCC CRL 1586 cell line was maintained in 25 cm^2 culture bottles (T-Flask) containing DMEM medium supplemented with 10% FBS, 0.2% sodium bicarbonate (w/v) (Sigma), 50 µg/mL, under standard conditions with 5% CO_2 at 37 °C. The medium was renewed every 3 days after culture. For the preparation of the inoculum, the cells were detached by trypsinisation, then centrifuged at 1800 rpm for 5 min and the pellet obtained was suspended in 1mL of medium. The suspension (10µL) was added to 10 µL of blue trypan and after 10 min of incubation, the cells were counted microscopically using the Neubauer hematimeter to calibrate the load.

Data analysis
The determination of the IC_{50}, CC_{50}, EC_{50} was done graphically using Statgraphics 5.0 software. The plotting of the curves and the calculation of the inhibition percentages were carried out using Microsoft Excel 2013 for Windows.

Results
Ethnopharmacological survey
Socio-demographic profile of traditional practitioners (TP) in the Haut-Plateaux Division
In this study, 76 traditional healers (TH) were interviewed, 52 of whom were males and 24 females (Table I). Their average age was 54 ± 4 years with a minimum of 20 and a maximum of 70 years. Results show that most of THs are found in two age groups, i.e. 40 to 50 years old and 50 to 60 years old. Most of them (45%) received primary education, while 35% reached secondary level and only 7% university level. Based on the origin of their knowledge of traditional medicine, 45%

of THs declared to have acquired it over time through revelation, while 16% had it from father to son; on the other hand, 35% were initiated into traditional medicine against 4% who got it otherwise. Results also show that only 17% of the participants were full-time traditional healers while the majority (61%) were farmers; moreover, 22% of the THs were involved in other activities.

Table 1: Characteristics of traditional practitioners

Sex	**Female**			**Male**		
Number	24			52		
Percentage (%)	32			68		
Age range	Under 30 years old	[30 - 40]	[40 – 50]	[50 – 60]	[60 – 70]	>70
Number	7	8	9	18	27	7
Percentage (%)	9	10	12	24	36	9
Education profile	Illiterate	Primary	Secondary	University		
Number	10	34	27	5	0	0
Percentage (%)	13	45	35	7	0	0
Origin of Knowledge	Heritage	Initiation	Revelation	Others		
Number	27	12	34	3		
Percentage (%)	35	16	45	4		
Status of TP	Full time		Farmer		Others	
Number	13		46		17	
Percentage (%)	17		61		22	

Etiology of Malaria in Traditional Medicine in the Haut-Plateaux Division

Traditional healers are completely unaware of the real cause of the malaria they claimed to treat.

Diagnosis of malaria by traditional healers in the highlands department. The diagnosis of malaria in traditional medicine is clinical but also biological. It is based primarily on the symptoms, but also on the diagnosis made by doctors. The most common symptoms are fever (39%), asthenia (29%), headache (17%), chills (7%), muscle pain (4%), vomiting (3%), loss of appetite (1%). Each of the TPs encountered cited at least four of the symptoms listed in Figure 1.

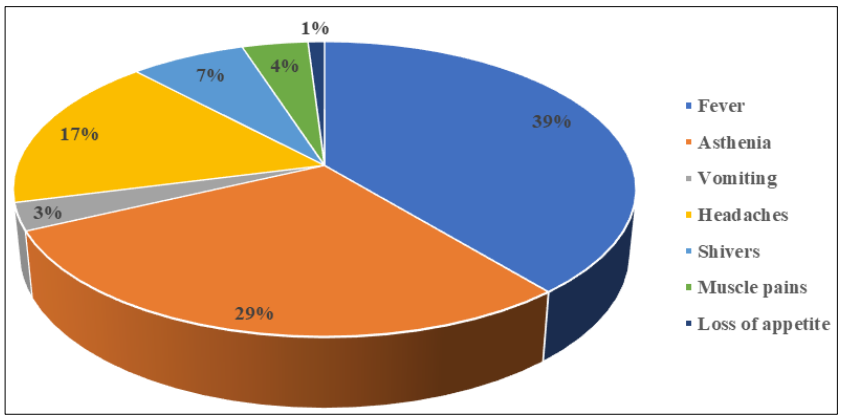


Fig 1: Malaria symptoms cited by traditional healers

The means of confirming the diagnosis of malaria by traditional healers were revelation, thick drop, present symptoms and Vidal as highlighted in Figure 2 below.

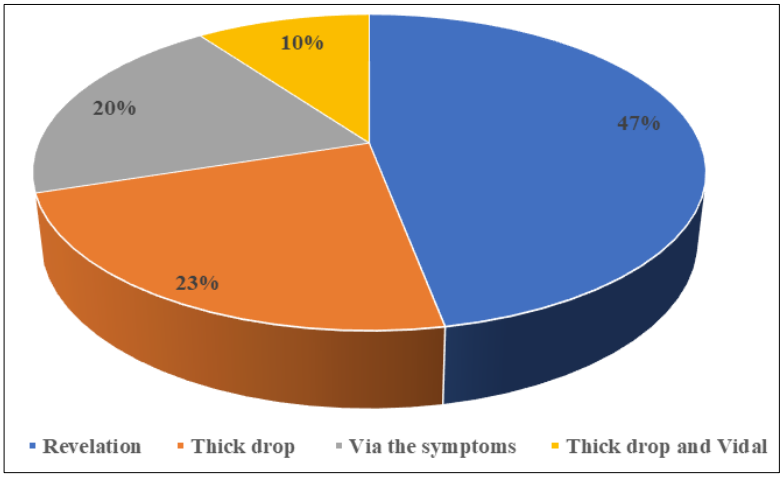


Fig 2: Means of confirming the diagnosis used by traditional practitioners

Malaria management in traditional medicine

Malaria management in traditional medicine in the Haut-Plateaux Division consisted of prescribing a few hygienic and dietary measures during treatment with medicinal plants over a period of 3 to 7 days, depending on the symptoms, the parasite load and the recipe used. After the duration of treatment prescribed by the healer, the patient had to carry out biological tests.

Treatment of malaria with medicinal plants in the Haut-Plateaux Division

The present study in Figure 3 identified 53 recipes prepared from 37 plant species, including 32 recipes with a single plant and 23 recipes obtained through plant associations, i.e. 65% and 35% respectively. The number of associated plants for these recipes varies between two and four. The methods of preparation observed were decoction (71%), infusion (26%) and maceration (33.33%), administered mainly orally at the majority dose of two glasses (500 mL) per day, i.e. 800 mg/kg/day for 3 to 7 days, kept on average for 1 week. The side effects cited were nausea, vomiting due to the bitterness of certain plants. However, for the majority of these treatments (90%), the traditional practitioners did not control the side effects associated with them.

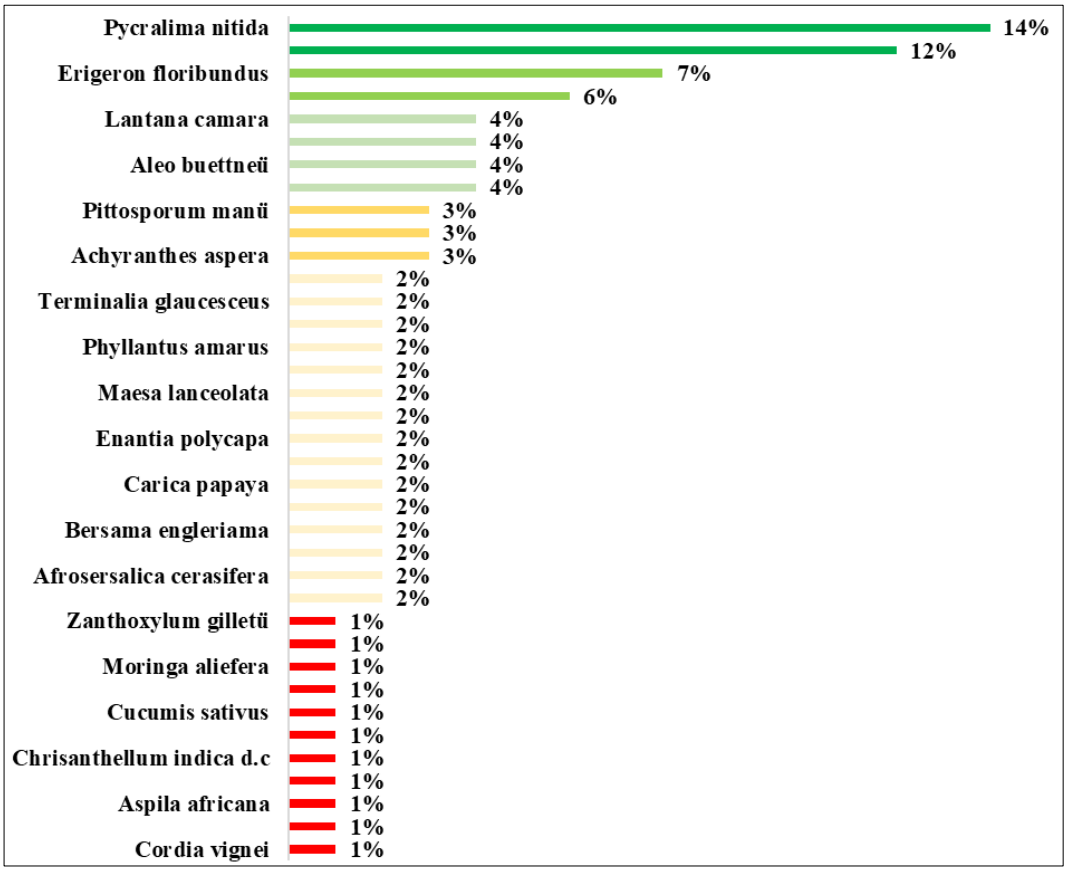


Fig 3: Frequency of plant species used for antimalarial treatment

The most commonly used plant parts (Figure 4) were bark (32%), leaves (29%), whole plant (19%) and fruit (14%). The other parts that are poorly represented were stems, flowers and seeds (2%). They were mostly used in fresh form.

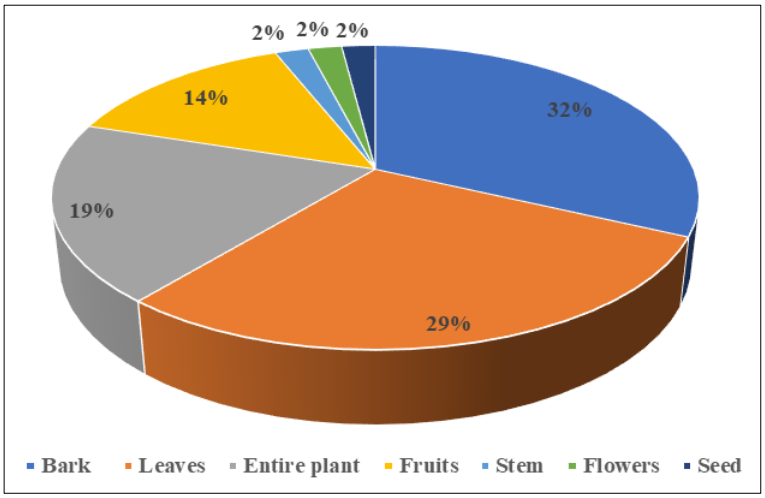


Fig 4: Parts of plants used

Botanical characteristics and diversity of plants with anti-malarial properties in the Haut-Plateaux Division

In Figure 5, 37 plant species with anti-malarial properties were displayed. They belong to 25 families. The most represented families were the Asteraceae with nine species: *Bidens pilosa*, *Chrysanthellum indica*, *Mikirria cordata*, *Conyza aegyptiaca*, *Agerantum conyzoides*, *Aspila africana*, *Atemisia annua*, *Erigeron florubundus* and *Laggera alata*. The Apocynaceae were represented with six species each, followed by the *Euphorbiaceae* with four species. The *Liliaceae*, the *Hypericaceae* with three species each. Seven families were represented by two species each and six represented by one species each (Figure 5).

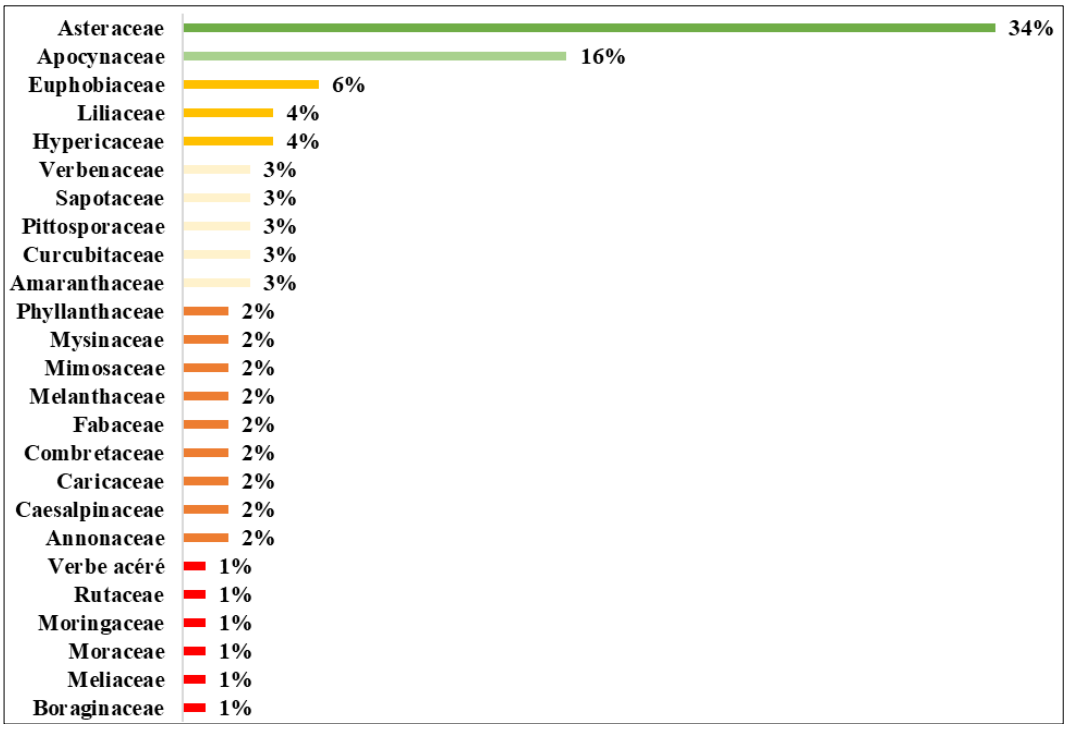


Fig 5: Distribution of the plant families listed and the number of plant species cited

The plants listed below (Table II) were grouped by family. Each plant is followed by its vernacular name, the name of the collector and its harvest number, the reference to the National Herbarium of Cameroon and its therapeutic properties as cited in the literature.

Table 2: Directories of anti-malarial plant species listed

Family	Scientific name	Vernacular name	Coll & Num	Ref HNC	Some known therapeutic properties
Asteraceae	*Agertum conyzoides*	Chekgneu	Berger No 467	16345 / HNC	Antimalarial
	Aspilia africana		J.Monama No 7 F5	51881 / HNC	Anti-ulcer, Anti-inflammatory, Antimalarial
	Bridens pilosal	Leulianoc	AJM leeven berger No 2883	9509 / SRF	Antimalarial
	Chrisanthellum Indica, D,C	Tchiopres	Geerling No 4732	488371 / HNC	Antimalarial
	Conyza aegyptiaca (1)	Meveuvetcheu	bretcler No 2502	36311 / HNC	Antifungal, Antimicrobial
	Erigeron florubundus		SCA No 3184	36014 / HNC	Antimicrobial, Antidiabetic
	Artemisia annua			3589/HNC	Antimalarial
	Mikiria cordata	Ngouguonck	Boss No 5291	29456/ HNC	Anti-ulcer
Amaranthaceae	*Achyranthes Aspera*	Nkepyok	Collecteur No 2949	2919/ SRFK	Antimalarial
Annonaceae	*Enantia polycarpa*		westphall 10090	43143 /HNC	Antimalarial
Apocynaceae	*Catharanthus roseus*	Houn-kong	Dang D No 80	12567 / HNC	Antimalarial
	Funtumia elastica	Tutchem	D.W thomas N° 5920	55353 / HNC	Antimalarial
	Pycralima nitida	ntom	letousey 11804	28741 /HNC	Antimalarial
Boraginaceae	*Cordia vignei*		check 9021	61597 /HNC	Anti-inflammatory
Caesalpinaciae	*Senna alata*	Datrier	SB Manning No 321	57704 / HNC	Antimalarial
Combretaceae	*Terminalia mantaly*		Tabras Mbenkun No 246	24672 /HNC	Antimicrobial
	Terminalia glaucesceus	Vuvup	Tabras Mbenkun No 383	32194 / HNC	*Antimalarial*
Curcubitaceae	*Cucumis savitus*		Thomas D,W 3286	50812 /HNC	
	zehneria scabra		AJM leevenberg No 6040	19668 / HNC	Antimicrobial, Anti-inflammatory
Euphobiaceae	*Bridelia micrantha*	Chonguouang	SB Making No 631	57602 / HNC	Antidiabetic
	Jatropha curcas		sonke 189	6575 / HNC	Antimalarial
Fabaceae	*Cassia ocudentalis*		Letouzey11804	19797/SRF/CAM	Antimalarial
Hypericaceae	*Harangana madagascariensis*	Nkom	JN. Assonganyi No 539	48488 / HNC	Antimalarial

Liliaceae	*Aleo buettneii*	Aleoverra	C.B No 6392	59062 / HNC	Antifungal, Analgesics
Melanthaceae	*Bersama engleriana*	Ngack	CDAD No 1802	23829 / HNC	Antifungal, Antimicrobial
Meliaceae	*Trichilia dregeana*		De wilde WJJO 2361	26936/HNC	Antimicrobial
Minosaceae	*Samanea Saman*		Dang D No 80	18681/HNC	Antifungal
Moraceae	*Ficus exasperata*	Lemsum	GA Zenker	446 / HNC	Antimalarial
Moringaceae	*Moringa aliefera*		Etuge 3459		Antimalarial, Anti-inflammatory
Mysinaceae	*maesa lanceolata*		J2A Raynal No 13054	13140 / SRFCam	Antimalarial
Phyllantaceae	*Phyllantus amarus*		B.A Nkongmeneck	24876 / HNC	Antimalarial
Pittosporaceae	*Pittosporum manii*	Metong		50179 / HNC	Antimicrobial
Rutaceae	*Zanthroxylum gillitii*		D.W Thomas No 6790	56599 / HNC	Antimalarial, Antifungal
Sapotaceae	*Afrosersalica cerasifera*	Mbeubok coup	Rletorizey No 241	3831 / HNC	Antimicrobial, Antifungal
	Chrysophyllum africanisme		B.Pollard	60481 / HNC	Anti-inflammatory
Verbinaceae	*Lantana camara*	Nkiemeusang	Boss J.J No 2955	30440 / HNC	Antimicrobial, Anti-inflammatory
Caricaceae	*Carica papaya*		Mbom.B No 312	No 2884 SRFK	Antimalarial, Hepatoprotector

Extraction rate

The rates of aqueous and hydro-ethanolic extracts of *C. aegyptiaca* are described in Table III. The aqueous leaf extract (black glossy paste) obtained by decoction of *C. aegyptiaca* recorded the highest extraction yield (9.69%) while the hydroethanolic extract (green glossy paste) had the least (7.79%).

Table 3: Extraction yields of aqueous and hydro-ethanolic leaf extracts of *C. aegyptica*

	Maceration-based aqueous Extract (MAE)	Decoction-based aqueous extract (DAE)	Hydroethanolic extract (HE)
Weight of raw material (g)	500	100	100
Weight of crude extract (g)	29.53	9.69	7.79
Extraction rate (%)	5.9	9.69	7.79

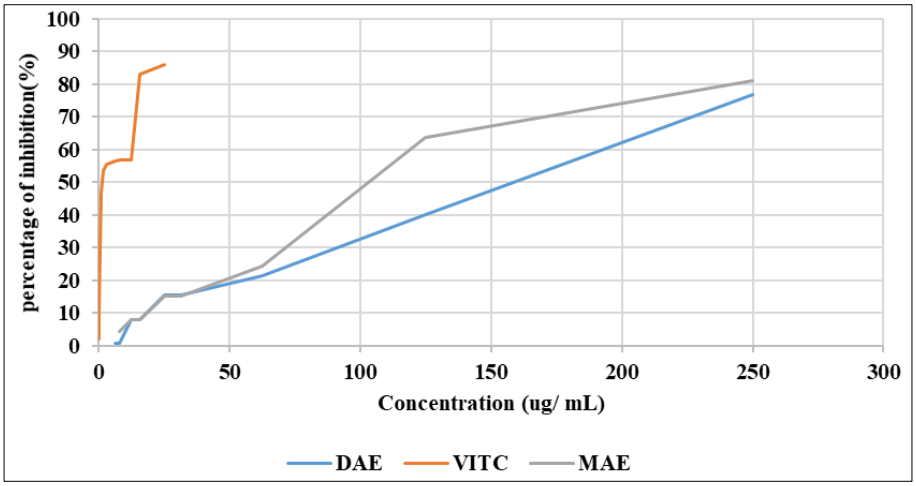
MAE: Maceration-based aqueous Extract; DAE: Decoction-based aqueous extract; HE: Hydroethanolic extract

Qualitative phytochemical screening

The phytochemical screening of the leaf extracts of *C. aegyptiaca* in Table 4 reveals the presence of flavonoids, saponosides, phenols, steroids, tannins, triterpenes and coumarins and the absence of anthocyanins and Anthaquinone irrespective of the extraction methods. While the alkaloids were only absent in the hydro-ethanolic extract the glycosides were only present in the maceration-based aqueous leaf extract of *C. aegyptiaca*.

Antioxidant activity of *C. aegyptiaca* extracts

Figure 6 below describes the anti-radical activity of the leaf extracts of *C. aegyptiaca*. Results show that at a concentration equal to 250 µg/mL, MAE registered the highest anti-radical activity (81.21%) followed by the aqueous extract (76.93%). However, both extracts had lower anti-radical activity than the control (86.09%), i.e. the ascorbic acid.



MAE: Maceration-based aqueous Extract; DAE: Decoction-based aqueous extract; HE: Hydroethanolic extrac

Fig 6: Percentage entrapment of DPPH- at different concentrations of the extract

The IC_{50} values (168.42) of the decoction-based aqueous extract shown in the Table 5 were greater that the IC_{50} values (104.13) of MAE. Equally, both leaf extracts had lower antioxidant activity than the control (Vitamin C) that registered an IC_{50} value of 9.8.

Results also show that the maceration-based aqueous extract had the highest anti-radical power (14.516×10^{-5}) followed by the ethanol extract (9.092×10^{-5}). The aqueous leaf extracts of *C. aegyptiaca* showed a low anti-radical activity compared to the standard Vitamin C ($156{,}961 \times 10^{-5}$) irrespective of the extraction techniques. The anti-radical power values of extracts were inversely proportion to the their CE_{50} values.

Table 4: IC_{50}, EC_{50} and PA values of *C. aegyptiaca* leaf extracts

Samples	IC_{50}	EC_{50} ($x10^3$)	PA ($x10^{-5}$)
DAE	168.42 ± 0.007^c	11.238 ± 0.045^c	9.092 ± 1.608^a
MAE	104.13 ± 0.011^b	6.941 ± 0.0397^b	14.516 ± 1.556^b
HE	/	–	–
VitC	9.7767 ± 0.490^a	0.652 ± 0.029^a	156.961 ± 29.959^c

MAE: Maceration-based aqueous Extract; DAE: Decoction-based aqueous extract; HE: Hydroethanolic extract

Iron Reduction Method (FRAP: Ferric Reducing Antioxidant Power)

The Fe^{3+} Reduction activity of leaf extracts of of *C. aegyptiaca* was described in Figure 7 below. Generally, the absorbance of the Fe^{2+}-orthophenanthroline complex increases with the concentration of extract and this absorbance is proportional to the amount of reduced Fe^{3+} ions. At concentration equal to 250 µg/ mL, the maceration-based aqueous extract exhibited the highest Fe^{3+} Reduction activity (OD = 3.4367) followed by the decoction-based aqueous extract (OD = 2.62375). The least Fe^{3+} Reduction activity was exhibited by the hydro-ethanolic extract (OD= 2.0905)

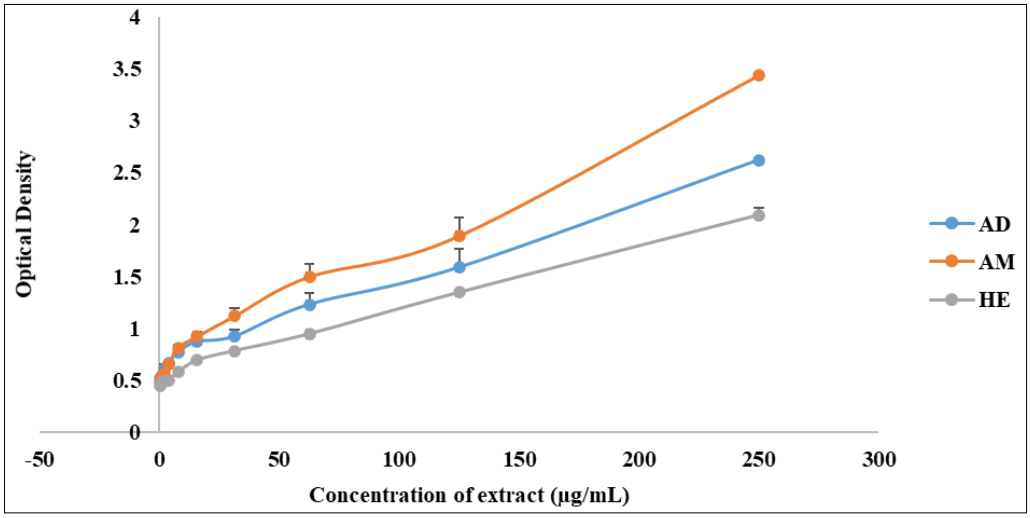


Fig 7: Absorbance of the Fe^{2+}-orthophenanthroline complex as a function of extract concentration

Antihemolytic Activity

Figure 8 displays the antihemolytic activity of leaf extracts of *C aegyptiaca* through the evaluation of haemolysis inhibition rate (%) at different concentrations. Results indicate that the inhibition of haemolysis is concentration-dependent, i.e. as the concentration of the extract increased with the of haemoysis inhibition.

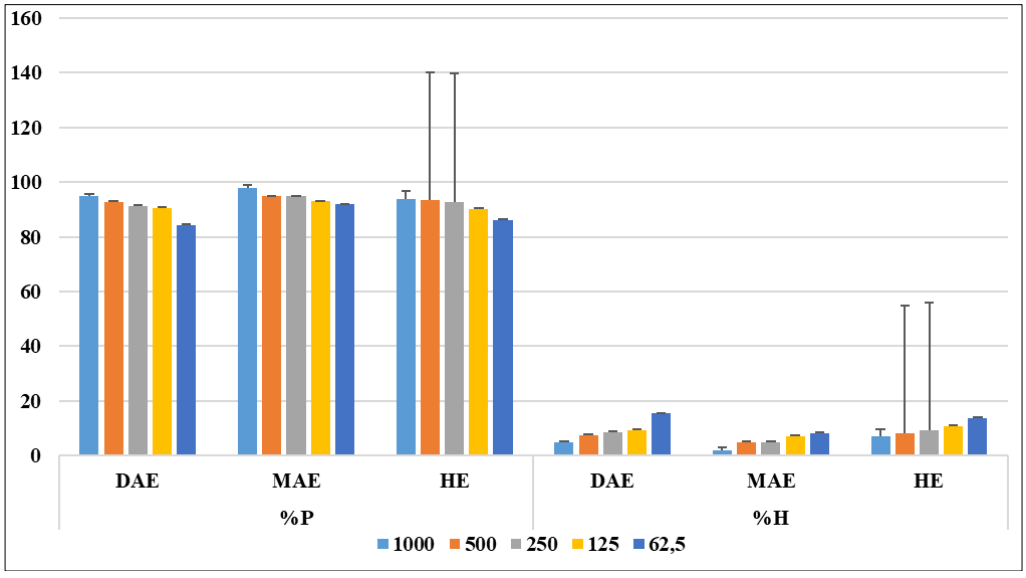


Fig 8: Antihemolytic activity of *C. aegyptiaca* extracts

Cytotoxicity Test

The cytotoxic profile of plant hydro-ethanolic extract on the Vero cell line was presented in Figure 9 below. Results show that the CC_{50} values of the hydro ethanolic leaves of *C. aegyptiaca* were 853.95 µg/mL

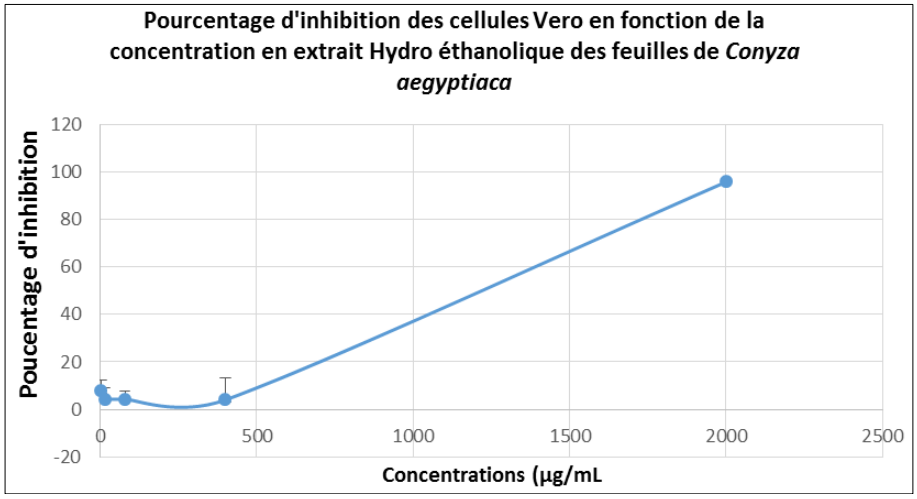


Fig 9: Percentage of inhibition of Vero cells according to the concentration of hydroethanolic extract of *C. aegyptiaca* leaves.

Results indicate that DAE and MAE had similar CC50 values (>2000 µg/mL) indicating their non-cytoxicity.

Table 5: Cytotoxic concentrations 50 of the leaf extracts of *C. aegyptiaca*

Codes	CC50 (µg/mL)
HE	853.95±116.74
DAE	>2000
MAE	>2000

MAE: Maceration-based aqueous Extract; DAE: Decoction-based aqueous extract; HE: Hydroethanolic extract

Discussion

The present study focused on the ethnopharmacological investigation related to the management of malaria by traditional healers using plants species with potential antimalarial properties in the Western Highlands of Cameroon, with special focus on the biological (antioxidant, antihemolytic and cytotoxic) activities of the leaves of *C. aegyptiaca* (Asteraceae).

Most traditional healers (68%) in the Hauts-Plateaux Division were males. Similarly, in a Recent publication underlined the predominance of men in the practice of traditional medicine in the other areas of the west region of Cameroon [28, 20]. In contrast, the current study contradicts the findings of previous studies that reported more involvement of women in the activities of traditional medicine in Kenya, Nigeria and Togo [30, 31, 32]. Additionally, it seems that the knowledge of traditional medicine necessitates a certain degree of maturity and trustworthiness. This may explain why this profession is practiced mostly by older people who were initiated into it within the family circle in this study. Equaly, Simbo previously indicated that knowledge and practice of plants as medicines in Babungo, West region of Cameroon lie mostly in the hands of the older generation [33]. Therefore the knowledge of a recipe in traditional medicine is a family secret that is passed on from generation to generation through customs and oral tradition. According to Bagwana, parents and grand-parents are the key guardians of traditional knowledge who are assigned to transmit it to their sons and daughters [34]. In the present study, it was observed that the diagnosis of malaria by traditional practitioners in the Western Highlands of Cameroon is based primarily on clairvoyance, symptoms of the disease, but also on the medical doctors 'diagnosis. The present study showed a good diversity of plants used in the treatment of malaria in the Haut-Plateaux Division. A total of 37 medicinal plant species from 25 families used for treating malaria were identified in the survey with Asteraceae (34%) being the most represented family. The predominance of Asteraceae has also been reported by other studies carried in other areas of the West and North-West regions of Cameroon [35, 36, 33, 28, 29]. The wide spread of Asteraceae is owing to its large spectrum of biologically active compounds comprising a large number of plants [35, 36]. On the other hand, the Apocynaceae was the second most common family in this study has also been reported among the most abundant plant families in terms of medicinal plant diversity in the South and North-West regions [37, 37].

Other ethnobotanical studies have revealed similar diversity of antimalarial plants in different settings in Cameroon [14, 36, 37, 34, 39].

This work indicates that the majority of antimalarial plants were trees. This result is similar to the conclusion of a number of previous ethnopharmacological studies [40, 41, 42] that reported a predominance of trees among plants with antimalarial properties. For the traditional healers in the western highlands of Cameroon, malaria management consisted of the prescription of some hygienic and dietary measures associated with treatment with medicinal plants. The use of plant as single recipe or a combination of many plants is claimed to cure malaria with the preponderance of monospecific recipes. This result is similar to past studies carried out in different parts of Cameroon [43, 44]. The preponderance of monospecific recipes described in the current study is to the advantage of patients because poorly matched combinations of plants are sometimes dangerous. In contrast, the World Health Organization (WHO) now recommends combinations of drugs in the treatment of malaria because of the resistance of the parasite [45, 46]. This can be explained by the synergy of action that may exist between the different plants associated with them, thus helping to maximise the patient's chances of recovery.

Additionally, other plant species with antimalarial properties identified during our investigation, were namely *Picralima nitida*, *conyza aegyptiaca* and *bidens pilosa*. Several studies carried out in other countries including Indonesia, Nigeria, Uganda have demonstrated the anti-malarial activity of *Ageratum conyzoides; Aspilia africana*; *Senna alata*; *Arungana madagascariensis*; *Ficus exasperata*; *Artemisia annua* cited in our study [47, 48, 49]. Though 98% of the plants described in this study have already been cited at least in one scientific publication, the mode of action of most of these

phytomedicines against plasmodium parasites remains poorly understood.

The preference of barks in the herbal preparations of traditional healers in this study could be related to the abundance of chemical groups they contain but also due to their easy post-harvest conservation. Plant barks are also known to be the site of accumulation of secondary metabolites in the plant [50]. These contradict the findings of several authors in Cameroon and other African countries who reported that the leaves were the most popular plant parts used in the various herbal preparations in traditional medicine [39, 45, 34, 51]. Traditional medicines were mainly prepared as decoction or maceration but administered preferably by oral route in the western highlands of Cameroon. While decoction eases, the extraction of a large number of active ingredients under the effect of boiling temperature, maceration on the other hand, preserves the integrity of active compounds. Similarly, several previous ethnobotanical studies reported the preparation and administration of medicinal plants for the management of malaria by decoction and oral route, respectively [39, 34, 45, 52].

Extracts of *C. aegyptiaca* irrespective of the extraction methods are poor antioxidants compared to the standard (vitamin C). These results are in line with a previous work carried out in Benin [53] which, indicated that the antiradical activity of the leaf extract of *C. aegyptiaca* was 10 times lower than that of vitamin C [53]. The poor DPPH of leaf extracts does correspond to the phytochemical analysis that demonstrates their rich content of flavonoids, terpenoids, phenolics, and alkaloids. Terpenoids. Previously, other studies attributed antioxidant activity to *Conyza* because its high content of phenolic and flavonoid compounds [54, 55].

On other hand, the Fe^{3+} reduction activity of leaf extracts of *C. aegyptiaca* could be attributed to its phenolic content would result in the reducing of iron (III) to iron (II) by donating an electron. In fact, the presence of hydroxyl groups in the phenolic compounds could serve as electron donors as documented [56, 57] shown that iron reduction leads to a rapid decrease in DNA synthesis, an increase in oxidative stress via the loss of activity of Superoxide Dismutase in parasites thereby causing parasite death [58, 59]. Results demonstrate that the leaf extracts of *C aegyptiaca* showed non-toxic and antihemolytic activity. The antihaemolytic effect of *C. aegyptiaca* could be explain though the addition of hydrogen peroxide that increases the permeability of the erythrocyte membrane and the thermal decomposition of the latter producing free radicals that attacks the lipids of the membrane. As reported by previous studies, the protection of red blood cells could be attributed to the presence of polyphenols (flavonoids) in the plant [60, 61].

Conclusion

The survey shows that a large number of medicinal plants are used in the Western highlands (Haut-Plateaux division) of Cameroon to manage malaria traditionally. The knowledge of traditional medicine remains a family secret and lies mostly in the hands of the older generation. The youth should also be encouraged to learn the traditional medicinal knowledge to preserve it from being lost with the older generation. The present study showed a good diversity of plants used in the treatment of malaria in the Haut-Plateaux Division with Asteraceae being the most represented plant family. The management of malaria in this region of the country is characterised by the preponderance of monospecific recipes obtained via decoction or maceration and taken mainly orally. However, this practice should be standardized and their side effects deeply studied. Though the phytochemical profile and antioxidant and antihemolytic activities of *Conyza aegyptiaca* (one of the plant species cited during the survey), further research is warranted to identify and isolates active compounds of all the antimalarial plants with higher performance indices in the Haut-plateaux division of the West region of Cameroon.

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