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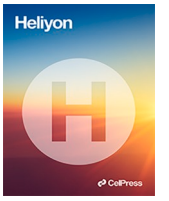

Evaluating phytochemical constituents and *in-vitro* antiplasmodial and antioxidant activities of *Fadogiella stigmatoloba*, *Hygrophylla auriculata*, *Hylodesmum repandum*, and *Porphyrostemma chevalieri* extracts

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ABSTRACT

Introduction: *Fadogiella stigmatoloba*, *Hygrophylla auriculata*, *Hylodesmum repandum* and *Porphyrostemma chevalieri* are used against malaria in traditional medicine in the Democratic Republic of the Congo (DRC). To evaluate their potential in the treatment of this disease, the *in vitro* antiplasmodial property of these four plants was evaluated. All experiments were conducted on methanolic extracts performed on selected organ parts of these plants.

Methods: The methanolic extracts, obtained by maceration, were firstly screened *in vitro* against the chloroquine sensitive (3D7) and resistant (W2) *Plasmodium falciparum* strains by the measurement of lactate dehydrogenase activity, and on human keratinocytes (HaCat) cells by the MTT assay to determine their selectivity indices (SI). Secondly, the antioxidant activity of the same extracts was evaluated using DPPH and FRAP assays. Finally, the presence of specific phytochemical constituents was evaluated using standard methods and tentatively identified by GC-MS.

Results: An optimum antiplasmodial activity ($IC_{50} = 3.4 \pm 0.7$ µg/mL, for 3D7, SI = 58.2; $IC_{50} = 7 \pm 1.0$ µg/mL, for W2, SI = 28.3) was obtained with the leave extract of *P. chevalieri*. The leaves (for *F. stigmatoloba* and *H. repandum*), and the aerial part (for *H. repandum*) extracts showed promising and moderate antiplasmodial activities against respectively the 3D7 strain (IC_{50}: <15 µg/mL), and W2 strain (IC_{50}:15–50 µg/mL). All extracts presented a weak cytotoxic effect (IC_{50}: >100 µg/mL) on HaCat cells. For the antioxidant test, the most interesting activity was obtained with the leaf extract of *P. chevalieri*. The GC–MS analysis of these four plants species extracts

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revealed the presence of various compounds, such as Ethyl 2-nonenoate, 2-(2-Hydroxy-2-phenylethyl)-3,5,6-trimethyl pyrazine, Palmitic Acid, Ethyl palmitate, Ethyl linolenate, and N-Acetyltyramine.
Conclusion: Based on the obtained results, *P. chevalieri* could be selected for further investigations or /and for the management of malaria after standardization.

1. Introduction

Malaria is a public health issue causing a high number of deaths in sub-Saharan Africa, particularly in the Democratic Republic of Congo (DR Congo) [1,2]. It is the second leading cause of death from infectious diseases in Africa, after HIV/AIDS [3]. According to a WHO estimation, 247 million cases of malaria were registered in the world in 2021 causing 619,000 deaths, with most of the victims being children under 5 years old from sub-Saharan Africa [3]. In developing countries, which account for more than 90% of malaria cases, 80% of the population does not have access to primary health care provided by modern medicine [4]. A similar condition is experienced by almost 78% of the population of Bukavu in the South-Kivu province of DR Congo, where the cost of acute malaria management is US $10.7 ± 5 for hospitalization and US$1.7 ± 1.3 for consultation while daily per capita income is less than US $ 1 [2]. In South-Kivu, this ineffective health system is increased by the existence of permanent conflicts within the region, with the consequence that access to health care is three times lower than in any other parts of the country [5].

In Africa, up to 90% of the population depend on traditional medicine to help meeting their health care needs [6] including the population of South-Kivu in DRC. However, the efficiency of the anti-malarial recipes used by this large proportion of population is often not scientifically established. The demand for medicinal plants is increasing in Africa as the population grows and pressure on medicinal plant resources becomes greater than ever. The efforts of finding out novel antimalarial drugs from plants has been intensively conducted during the last two decades by the world's searchers [7–10]. Moreover, drugs like quinine and artemisinin were isolated from plants. The resistance of the malaria parasite *P. falciparum* to existing antimalarial drugs is the most serious problem in modern malariology [11]. Because of this increased resistance to the established antimalarials, investigations on the potential presence of new chemical compounds with antimalarial properties within medicinal plants are necessary. Herbalists in Bukavu and Uvira have been commonly treating the recurrent fever typical of malaria with plant extracts. There is some literature dealing with the medical practices of South-Kivu population [2,12–14].

Due to the high diversity of botanicals, Congolese medicinal plants can be considered as very promising candidates for bioactive agents. Finding clinically useful antimalarial compounds in such plants could provide significant medical and economic benefits worldwide. The present study verified the *in vitro* effectiveness of the antiplasmodial activities of four Congolese medicinal plants: *Fadogiella stigmatoloba* (K.Schum.) Robyns (Rubiaceae), *Hygrophylla auriculata* (Schumach.) Heine (Acanthaceae), *Hylodesmum repandum* (Vahl) H. Ohashi & R.R.Mill (Fabaceae) and *Porphyrostemma chevalieri* (O.Hoffm.) Hutch. & Dalziel (Asteraceae) used in the management of malaria in traditional medicine in Bukavu and Uvira in DR Congo [2]. The study also evaluated the bioactive compounds and phytochemical groups of these plants and measured their cytotoxic and antioxidant effects. To the author's knowledge, no previous work appears to have investigated the potential antimalarial properties of these four plants.

2. Material and methods

2.1. Chemical and reagents

Albumax I, Dulbecco's Modified Eagle Medium (DMEM), Fetal bovine serum (FBS), Phosphate-buffered saline (PBS), Trypsin-EDTA solution, Saponin, Triton X-100 and Gentamicin were purchased from Gibco (Gibco, Waltham, MA, USA). Acetyl ester acetic 5-(chloromethyl)-2-(3,6-diacetoxy-2,7-dichloro-9H-xanthen-9-yl)benzoic anhydride (CMH2DCFDA), Aluminium tri-chloride ($AlCl_3$),

Table 1
List of selected plants, family, vernacular names, voucher number and specific parts used.

Plant species (Family)	Local name	Used part	Voucher specimens	Extraction yield (%)	Local of collection
Fadogiella stigmatoloba (K.Schum.) Robyns (Rubiaceae)	Mukubashenfero (Fuliru)	Leaves	[a]MANYA KIP 41 [b]BR0000020350017	21.7	Uvira
*Hygrophila auriculata (*Schumach.) Heine) (Acanthaceae)	Buganga bukali, Kanamafundwekazi (Mashi); Bunganga bukali (Rega)	Aerial part	[a]MANYA KIP 09 [b]BR0000020350000	4.4	Bukavu
Hylodesmum repandum (Vahl) H. Ohashi &R.R.Mill (Leguminosae)	Irhuza (Mashi); Kanyerekagoli (Fuliru)	Leaves Flowers	[a]MANYA KIP 30 [b]BR0000020350246	15.4 11.2	Uvira
Porphyrostemma chevalieri (O.Hoffm.) Hutch. & Dalziel (Asteraceae)	Nakwangi (Fuliru), Lunandu (Rega)	Leaves	[a]MANYA KIP 09 [b]BR0000020350116	7.4	Uvira

(a) Number of voucher specimens deposited in the Herbarium of INERA-Kipopo, DR Congo; (b) Number of voucher specimens deposited in the Herbarium of Meise Botanical Garden, Belgium

Anhydrous sodium carbonate (Na_2CO_3), Artemisinin, Chloromethyl-2′,7′-ichlorodihydrofluorescein diacetate, Chloroquine phosphate, Dimethyl sulfoxide (DMSO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), Ferric chloride ($FeCI_3$), Folin-Ciacalteu reagent, Gallic acid, Glacial acetic acid, Glucose, Giemsa, Hypoxanthine, Hydrochloric acid (HCl), Iodine, Methanol, Potassium iodide, Quercetin, Roswell Park Memorial Institute (RPMI)-1640, Rutin, Silica gel 60–120 mesh, Sodium bicarbonate, Sodium hydroxide (NaOH), Sorbitol and Sulfuric acid (H_2SO_4) were purchased from Sigma Chemica (Sigma-Aldrich, Netherlands).

2.2. Plant collection

Fadogiella stigmatoloba (K.Schum.) Robyn (Leaves, Rubiaceae), *Hygrophylla auriculata* Schumach.) Heine (Aerial part, Acanthaceae), *Hylodesmum repandum* (Vahl) H. Ohashi & R.R.Mill (Leaves and flowers, Fabaceae) and *Porphyrostemma chevalieri* (O.Hoffm.) Hutch. & Dalziel (Leaves, Asteraceae) were collected (Oct 2014) in Bukavu and Uvira, two towns of the South-Kivu province of DR Congo (Table 1). The plant materials were identified by comparison with samples preserved in the herbarium of the Institut National d'Etudes et de Recherches en Agronomie (INERA-Kipopo) in Lubumbashi by Mr. D. Mbangu. Voucher specimens were kept at INERA, and at the National Botanical Garden of Belgium (Meise, Belgium). Samples were cleaned and air dried at room temperature (25 °C) for one week and separately ground into fine powder using an electric stainless-steel grinder.

2.3. Plant extraction

For each plant sample, 10 g of powdered plant material were submerged in 100 mL of methanol under constant shaking at room temperature for 24 h (days?). All plant extracts were filtered through Grade 1 filter paper and dried at 40 °C under vacuum (Rotavapor®, Buchi, Switzerland). Finally, the dry extracts were weighed (Table 1), transferred into airtight containers and stored at −20 °C until further analysis.

2.4. Phytochemical studies

2.4.1. Preliminary phytochemical screening

The major secondary metabolite classes such as alkaloids, anthocyanins, anthraquinones, coumarins, flavonoids, saponins, steroids, tannins, and terpenes contained in the four plants were identified using previously described methods [15–17].

2.4.2. Determination of total flavonoid content

Total flavonoid content was determined according to the methods described by previous studies [18,19]. One hundred microliters of sample and 100 μl of $AlCl_3$ (2%) were mixed in 96 micro-well plate and incubated for 10 min. The absorbance was measured at 415 nm with a microplate reader. The standard curve was generated using variable concentrations of quercetin ($y = 0.0162x + 0.0704$, $r^2 = 0.99$) and the results were expressed as mg QE/g of sample.

2.4.3. Determination of total phenolic content

Total phenolic content was evaluated by using Folin-Ciocalteu colorimetric assay described by Compaoré et *al.* [18]. Twenty-five microliters of the sample were added to 125 mL of Folin–Ciocalteu reagent (0.2 mol/L) and incubated for 5 min, followed by the addition of 100 mL of sodium carbonate (75 g/L). After 1 h of dark incubation, the absorbance was recorded at 760 nm with a microplate reader. Gallic acid was used to produce the standard curve ($y = 0.0149\ x + 0.14442$; $r^2 = 0.99$) and the results were expressed as mg GAE/g of extract.

2.4.4. GC-MS investigation

The GC-MS analyses were performed only in the methanolic extracts of the plants with an interesting antiplasmodial activity on the 3D7 *P. falciparum* strain. Potential interesting compounds present in these samples were analyzed using a Thermo Scientific Trace GC Ultra with DSQ II GC/MS system equipped with a 20 m long Zebron ZB-5ms column with an internal diameter of 0.18 mm and a stationary phase film thickness of 0.18 μm. One microliter of the sample in methanol was injected in split mode with a split ratio of 20:1. Helium gas (99.99% of purity) was used as the carrier gas at a constant flow rate of 1 ml/min, and the temperatures of the injector and transfer line were kept constant at 220 and 240 °C, respectively. The initial column temperature was set at 60 °C for 2 min and increased to 150 °C with an increasing rate of 10 °C/min. Spectroscopic detection by GC-MS involved an electro-ionization system with a high ionization energy of 70 eV, an ion source temperature of 250 °C, and a mass scan range of 50–650 a.m.u in full scan. Finally, the temperature was brought to 300 °C at 5 °C/min. Identification of the chemical compounds of the tested samples was done by comparing the mass spectra of manually integrated chromatogram peaks with the known compound spectral database in the National Institute of Standards and Technology (NIST, version 2.2) structural library. Each analysis was performed in duplicate. Only compounds from the peaks with at least 55% similarity with NIST library compounds were considered.

2.5. Antioxidant activities

2.5.1. Inhibition of radical DPPH assay

The assay was performed as described by Refs. [18,19]. Briefly, from 100 μg/mL extracts diluted in methanol, a series of eight successive dilutions was made. For each concentration, three tests were carried out by mixing 100 μL of extract and 200 μL of DPPH

(20 mg/L in methanol). After 15 min of incubation in the dark, the absorbance was measured at 517 nm using a UV–Vis 96-microplate spectrophotometer (BioTek Instruments, USA). Methanol (blank) and standards (Gallic acid and quercetin solutions at different concentrations: 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78) were run simultaneously with the plant methanolic extract solutions in triplicates. The inhibition percentage was determined according to the following formula:

$$\text{DPPH scavenging effect } (\%) = \text{Abs Control} - \text{Abs Sample}/\text{Abs Control x } 100$$

The data expressed in IC_{50}, which represents the concentration that inhibits 50% of the DPPH activity, were the averages of 3 independent triplet tests.

2.5.2. Reduction of iron III assay

The reducing power of the methanolic extracts was determined according to the FRAP method previously described [18,19]. In a test tube, 0.5 mL of extract was mixed with 1.25 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 1.25 mL of 1% aqueous potassium hexacyanoferrate. This mixture was incubated for 30 min, followed by an addition of TCA and centrifugated at 3000 r/min for 10 min. In 96 micro-wells, the upper layer solution (125 µl) was mixed with 125 µl of H_2O and 25 mL of $FeCl_3$ fresh solution. Ascorbic acid was used to produce the calibration curve by reading the absorbance at 700 nm ($y = 0.014x + 0.1442$, $r^2 = 0.99$). The iron (III) reducing activity of each sample was obtained from 3 independent triplet determinations and expressed in mg AAE/g of extract. Quercetin and gallic acid were used as positive controls (0.01 mg/mL).

2.6. In vitro antiplasmodial activity

2.6.1. Parasite strains

Chloroquine sensitive (3D7 clone) and resistant (W2 clone) *P. falciparum strains* (MRA-156, MR4, ATCC® Manassas Virginia) were kindly provided by the MR4/American Type Culture Collection.

2.6.2. Culture of Plasmodium falciparum and antiplasmodial test

Both 3D7 and W2 clones were maintained in continuous culture in human erythrocytes suspended in RPMI 1640 (Gibco, USA) culture medium supplemented with 10% human B serum and 25 mM HEPES (at 37 °C under a gas mixture of 5% CO_2, 5% O_2 and 90% N_2) according to the standard method as described by Ref. [20].

All assays were performed using 2% parasitemia and 1% hematocrit. For each crude extract, a serie of 8 threefold dilutions (from 200 to 0.09 µg/mL) was prepared, placed in two rows of a 96-well microplate, and tested in triplicate. Artemisinin (98%, Sigma-Aldrich) and chloroquine diphosphate salt (Sigma-Aldrich) were used as standards. Infected and uninfected erythrocytes were added as positive and negative controls respectively. After 48 h of incubation at 37 °C, the level of parasitemia was estimated by measuring lactate dehydrogenase activity, as previously described [21]. The results were expressed as the mean IC_{50} (the concentration of a drug that reduces the level of parasitemia to 50%) using the software GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA).

2.7. In vitro cytotoxic assay

Cytotoxicity of each plant extract was assessed on immortal human keratinocyte (HaCat) cells. The cells were cultured in Dulbecco's modified Eagle's medium which was supplemented with 10% foetal bovine serum (Roth, Germany), 5% of L-glutamate and 5% (10 mg/mL) of penicillin-streptomycin (Sigma-Aldrich). The cells were then seeded in 96-well microtiter plates at 10,000 cells per well in 100 µl culture medium. The cells were incubated for 48 h at 37 °C in a CO_2 incubator until they reached a level of confluence. After 48 h, the medium was completely removed from the wells by inverting the microtiter plate and by tapping it on a sterile filter paper. The medium was then replaced with 100 µl of fresh culture medium, followed by 100 µl of crude plant extract (200 µg/mL) in row H of the 96 well plate and serially diluted twofold to give concentrations ranging from 200 to 0.09 µg/ml. Row A of the 96 well plate served as control wells, whereby the negative control contained culture medium and HaCat cells without plant extract (100% growth). The cells were maintained at 37 °C in a CO_2 incubator before determining their viability by using the MTT assay as previously described [22]. The absorbance was measured at 570 nm using the microplate reader (Labsystems iEMS reader/dispenser MF, Finland). The mean of IC_{50} values were calculated using the software GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA).

2.8. Selectivity index (SI)

In this study, the selectivity index (SI) was calculated to determine the selectivity of samples to *P. falciparum*. This value corresponds to the ratio between the cytotoxic and antiparasitic activities of each tested plant extract.

2.9. Statistical analysis

Data were presented as mean of three determinations ± SEM. Statistical analysis was carried out using one-way analysis of variance (ANOVA).

3. Results

3.1. Antiplasmodial activity and cytotoxicity effect

According to the *in vitro* antiplasmidal test results (Table 2), the leaves extract of *P. chevalieri* was the most active on 3D7 strain with IC_{50} value 3.4 ± 0.7 μg/mL and showed a promising activity on W2 with IC_{50} value 7 ± 1 μg/mL. The three other extracts (leaves of *F. stigmatoloba*, aerial part of *H. repandum* and leaves of *H. repandum*) showed a promising antiplasmodial activity (IC_{50} < 15 μg/mL) against the 3D7 strain of *P. falciparum*. A moderate activity on the 3D7 strain was observed with the extract from the flowers of *H. repandum* (IC_{50} between 15 and 50 μg/mL). However, extracts from aerial part of *H. auriculata* (IC_{50} = 16.7 ± 1.3 μg/mL) and leaves of *H. repandum* (IC_{50} = 17.4 ± 2.1 μg/mL), and *F. stigmatoloba* (IC_{50} = 39.2 ± 4.8 μg/mL) were moderately active on W2 *P. falciparum* strain.

All analyzed extracts presented a weak cytotoxic effect on HaCat cells with IC_{50} value > 100 μg/mL (Table 2). With the two plasmodial strains (3D7 and W2) used in this study, the highest selective antiplasmodial activity was found with extracts from the leaves of *P. chevalieri* (SI > 25), followed by leaves of *H. repandum* (SI > 9.5).

3.2. Antioxidant activities

3.2.1. Inhibition of radical DPPH

The DPPH radical scavenging activities of extracts are shown in Table 3. According to a previous study [23], the antioxidant activities of plant extracts are significant when IC_{50} < 20 μg/mL, moderate when 20 μg/mL ≤ IC_{50} ≤ 75 μg/mL and weak when IC_{50} > 75 μg/mL. In this investigation, gallic acid and quercetin gave antioxidant activity with respective IC_{50} values of 2.5 ± 0.2 and 1.9 ± 0.1 μg/mL. The extracts of leaves of *P. chevalieri* and *H. repandum* exhibited noteworthy antioxidant activities with an IC_{50} value of 3.2 ± 0.1 and 4.8 ± 0.1 μg/mL, respectively. The extracts of other plants showed a moderate activity (>20 μg/mL) against the radical DPPH.

3.2.2. Ferric reducing power

The leaves extract of *P. chevalieri* showed FRAP value of 13.0 ± 2.8 AAE/g of dried extracts, which was higher than the other extracts (Table 3). The leaves and flowers extracts of *H. repandum* were found to have FRAP values of 9.7 ± 0.9 AAE/g and 7.8 ± 0.5 AAE/g of dried extracts, respectively. The positive control gallic acid was found to have FRAP value of 15.0 ± 0.2 AAE/g of dried extracts, which was not significantly different from leaves extract of *P. chevalieri* ($p < 0.01$).

3.3. Preliminary phytochemical screening

The preliminary phytochemical screening of the crude extracts revealed the presence of some secondary metabolites such as alkaloids, anthocyanins, anthraquinones, flavonoids, coumarins, saponins, steroids, tannins, and terpenes (Table 4). Methanolic extracts of the four plants showed the presence of flavonoids and terpenes. Anthocyanins were present in *H. auriculata*, *H. repandum* and *P. chevalieri,* while anthraquinones were observed in three plants except in *H. auriculata*. The presence of steroids was revealed in *F. stigmatoloba*, *H. auriculata* and *H. repandum*. The results showed that saponins and tannins were present in two plants, *F. stigmatoloba* and *H. repandum*. However, the presence of alkaloids was only observed in *F. stigmatoloba* and coumarins only in *P. chevalieri*. The leaves of *F. stigmatoloba* and *H. repandum* contained more metabolites (with the presence of seven observed metabolites) compared to the tested organs of *H. auriculata* (aerial part) and *P. chevalieri* (leaves).

3.4. Phenolic and flavonoid contents

Polyphenols and flavonoids present in plants are responsible for many biological activities [15]. In this study, we have determined the total phenolic and flavonoid contents of the four studied plants. The total concentrations of phenolic and flavonoid contents were

Table 2
In vitro antiplasmodial and cytotoxic activities of methanolic extracts from four tested plants.

Samples	Used part	IC_{50} ± SD (μg/mL)				
		HaCat	3D7	SI	W2	SI
F. stigmatoloba	Leaves	118.5 ± 6.2***	11.4 ± 3.3*	10.4	39.2 ± 4.8***	3.02
H. auriculata	Aerial part	132.7 ± 3.3***	9.8 ± 0.7 NS	13.5	16.7 ± 1.3***	7.9
H. repandum	Leaves	168.3 ± 6.8***	5.4 ± 0.8 NS	31.2	17.4 ± 2.1***	9.7
	Flowers	nd	41.5 ± 7.9***	nd	nd	nd
P. chevalieri	Leaves	197.9 ± 1.8***	3.4 ± 0.7 NS	58.2	7 ± 1.0***	28.3
Artemisinin		nd	0.002 ± 0.00013	nd	0.03 ± 0.008	nd
Chloroquine		nd	0.02 ± 0.1	nd	nd	nd
Doxorubicin		0.42 ± 0.01	nd	nd	nd	nd

Comparison with positive controls (Artemisinin chloroquine and doxorubicin): *** = ($p < 0.0001$); ** = ($p < 0.01$); * = ($p < 0.05$). nd: not determined; HaCat: human keratinocyte cells; 3D7: chloroquine-sensitive strain of *P. falciparum*; W2: chloroquine-resistant strain of *P. falciparum*; SI: selectivity index.

Table 3
Antioxidant activity of methanolic extracts of four selected plants.

Samples	Used part	DPPH radical scavenging capacity (IC_{50} en µg/mL) (n = 3)	FRAP Reducing power (mg AAE/g) (n = 3)
Fadogiella stigmatoloba	Leaves	70.9 ± 15.6***	3.4 ± 0.5***
Hylodesmum repandum	Leaves	4.8 ± 0.1 ^{NS}	9.7 ± 0.9***
	Flowers	22.4 ± 1.7*	7.8 ± 0.5***
Hygrophila auriculata	Aerial part	51.1 ± 4.9***	3.2 ± 0.4***
Porphyrostemma chevalieri	Leaves	3.2 ± 0.1 ^{NS}	13.0 ± 2.8**
Quercetin		1.9 ± 0.1	19.5 ± 1.0
Gallic acid		2.5 ± 0.2 ^{NS}	15.0 ± 0.2**

Comparison with quercetin: *** = ($p < 0.0001$); ** = ($p < 0.01$); * = ($p < 0.05$); NS (not significant) = ($p < 0.001$).

Table 4
Results of preliminary phytochemical screening.

Plant name (Used part)	Phytochemical compound groups								
	alkaloids	anthocyanins	anthraquinones	flavonoids	coumarins	saponins	steroids	tannins	terpenes
F. stigmatoloba (leaves)	+	–	+	+	–	+	+	+	+
H. auriculata (aerial part)	–	+	–	+	–	–	+	–	+
H. repandum (leaves)	–	+	+	+	–	+	+	+	+
H. repandum (flowers)	–	+	+	+	–	–	–	–	+
P. chevalieri (leaves)	–	+	+	+	+	–	–	–	+

estimated from calibration curves performed respectively using gallic acid and quercetin. The results from these investigations (Table 5) revealed a great variability in the levels of total polyphenols and flavonoids contents, with very significant differences in the evaluated extracts. The most relevant results of total phenolic content were obtained with the leaves of *P. chevalieri* and *H. repandum*: the content of phenolic compounds was respectively 849.4 ± 1.2 and 588.0 ± 8.9 g gallic acid/100 g of dried extracts. The leaves of *P. chevalieri* contained also the highest flavonoid content (29.9 ± 1.2 mg QE/100 g of dried extracts).

3.5. GC-MS analyses

Based on the antiplasmodial activity, four plant species with IC_{50} values < 15 µg/ml on the 3D7 plasmodial strain, were selected for a tentative identification of their phytochemical's contents by GC-MS.

3.5.1. Methanolic leaf extract of F. stigmatoloba

According to the NIST library spectral database, 9 compounds were identified and characterized in the methanolic leaf extract of *F. stigmatoloba* (Table 6). The most abundant compound was Ethyl palmitate, a fatty acid ester with a retention time of 38.75 min (14.51%), followed by Ethyl linolenate (8.48%), Palmitic Acid (7.14%) and 2-(2-hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine (6.41%). The peak areas of other identified compounds were less than 5%. Fig. 1 illustrates the GC–MS chromatogram of this extract.

3.5.2. Methanolic aerial part extract of H. auriculata

GC-MS analysis of the methanolic extract from the aerial part of *H. auriculata* allowed the identification and characterization of 10 compounds (Table 7). The most abundant compound was Ethyl 2-nonenoate, with a retention time of 34.58 min (21.3%). This compound is followed by 2-(2-Hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine (19.73%), Palmitic Acid (10.06%) and Ethyl palmitate (9.38%) such as shows the chromatogram in Fig. 2.

3.5.3. Methanolic leaf extract of H. repandum

Fourteen compounds were identified and characterized from the leaf extract of *H. repandum* (Table 8). This extract contains in majority Palmitic Acid (12.95%), whith a retention time of 37.98 min, N-Acetyltyramine (8.28%), Propan one, 1-hydroxy-3(4-hydroxy-3-methoxyphenyl)- (4.99%), and Tyrosol (3.41%). Other compounds were found in low amounts below 3%. The GC–MS

Table 5
Total content of flavonoids and polyphenols in the four plants studied.

Samples	Used part	Total flavonoids (mg QE/g) (n = 3)	Total polyphenols (mg GAE/g) (n = 3)
Fadogiella stigmatoloba	Leaves	3.1 ± 0.8^{a}	131.5 ± 4.2^{b}
Hylodesmum repandum	Leaves	11.5 ± 0.01^{c}	588.0 ± 8.9^{d}
	Flowers	14.7 ± 0.9^{d}	29.3 ± 5.5^{a}
Hygrophila auriculata	Aerial part	8.6 ± 0.8^{b}	294.7 ± 18.1^{c}
Porphyrostemma chevalieri	Leaves	29.9 ± 1.2^{e}	849.4 ± 1.2^{f}

Values with the same letters do not have a statistically significant difference ($p \leq 0.05$).

Table 6
Results of GC-MS analysis of the methanolic extract from leaf of *Fadogiella stigmatoloba*.

N°	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	22.00	Malic acid	$C_4H_6O_5$	134	2.63
2	33.70	2-(2-hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine	$C_{15}H_{18}N_2O$	242	6.41
3	37.94	Palmitic Acid	$C_{16}H_{32}O_2$	256	7.14
4	38.75	Ethyl palmitate	$C_{18}H_{36}O_2$	284	14.51
5	42.86	Ethyl linolenate	$C_{20}H_{34}O_2$	306	8.48
6	43.95	Ethyl stearate	$C_{20}H_{40}O_2$	312	1.73
7	46.97	Stearic acid	$C_{18}H_{36}O_2$	284	1.28
8	53.80	Stigmasterol	$C_{29}H_{48}O$	412	2.38
9	58.59	β-Sitosterol	$C_{29}H_{50}O$	414	2.88

RT: Retention time; MW: Molecular weight.

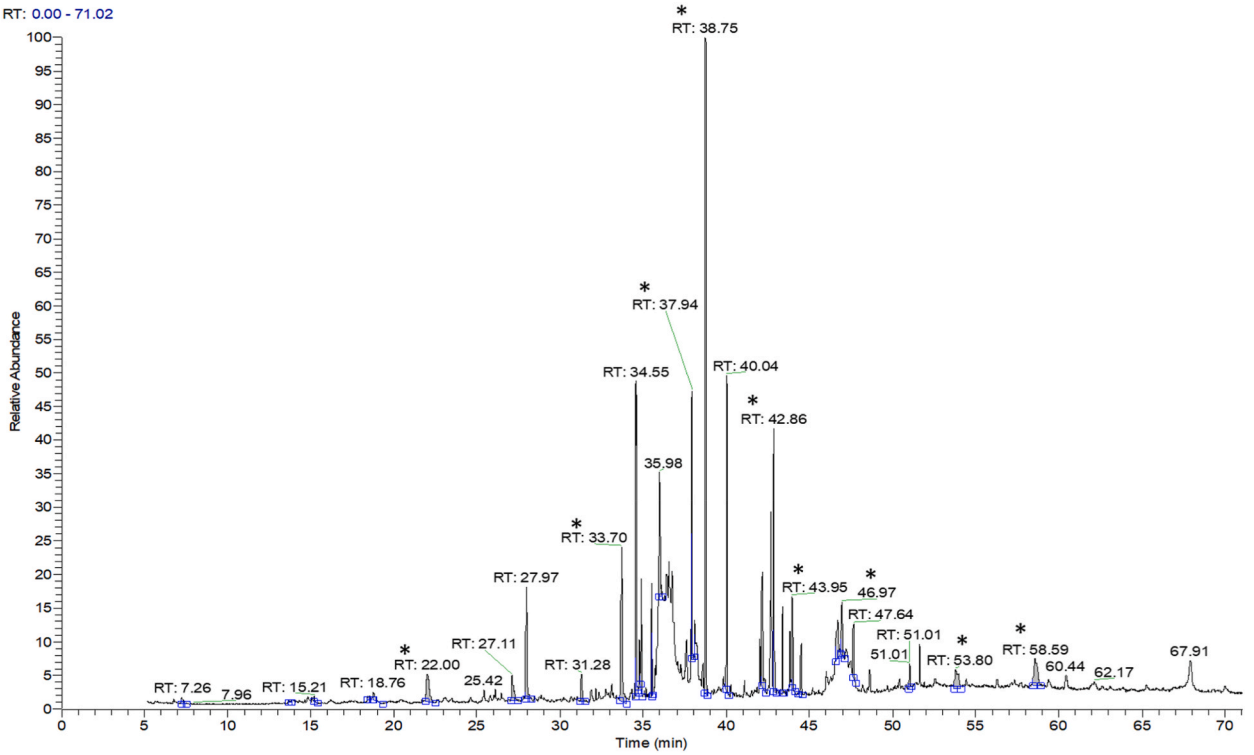

Fig. 1. GC–MS chromatogram of the methanolic leaf extract of *Fadogiella stigmatoloba*; * Peaks of selected compounds.

Table 7
Results of GC-MS analysis of the methanolic extract aerial part leaf of *Hygrophila auriculata*.

No.	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	9.21	Propylene glycol 1-acetate	$C_5H_{10}O_3$	118	0.57
2	11.02	Propylene glycol diacetate	$C_7H_{12}O_4$	160	0.31
3	24.97	5-Hydroxy-2,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	$C_{14}H_{22}O_2$	222	0.74
4	26.74	4-Aminobutyramide, N-methyl-N-(4-(1-pyrrolidinyl)-2-butyryl)-N′-aminoacetyl	$C_{15}H_{26}N_4O_2$	294	0.81
5	27.27	N-acetyltyramine	$C_{10}H_{13}NO_2$	179	3.97
6	33.67	2-(2-Hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine	$C_{15}H_{18}N_2O$	242	19.73
7	34.58	Ethyl 2-nonenoate	$C_{11}H_{20}O_2$	184	21.3
8	36.70	2-ethoxycarbonyl-succinic acid diethyl ester	$C_{11}H_{18}O_6$	246	2.97
9	37.88	Palmitic Acid	$C_{16}H_{32}O_2$	256	10.06
10	38.73	Ethyl palmitate	$C_{18}H_{36}O_2$	284	9.38

RT: Retention time; MW: Molecular weight.

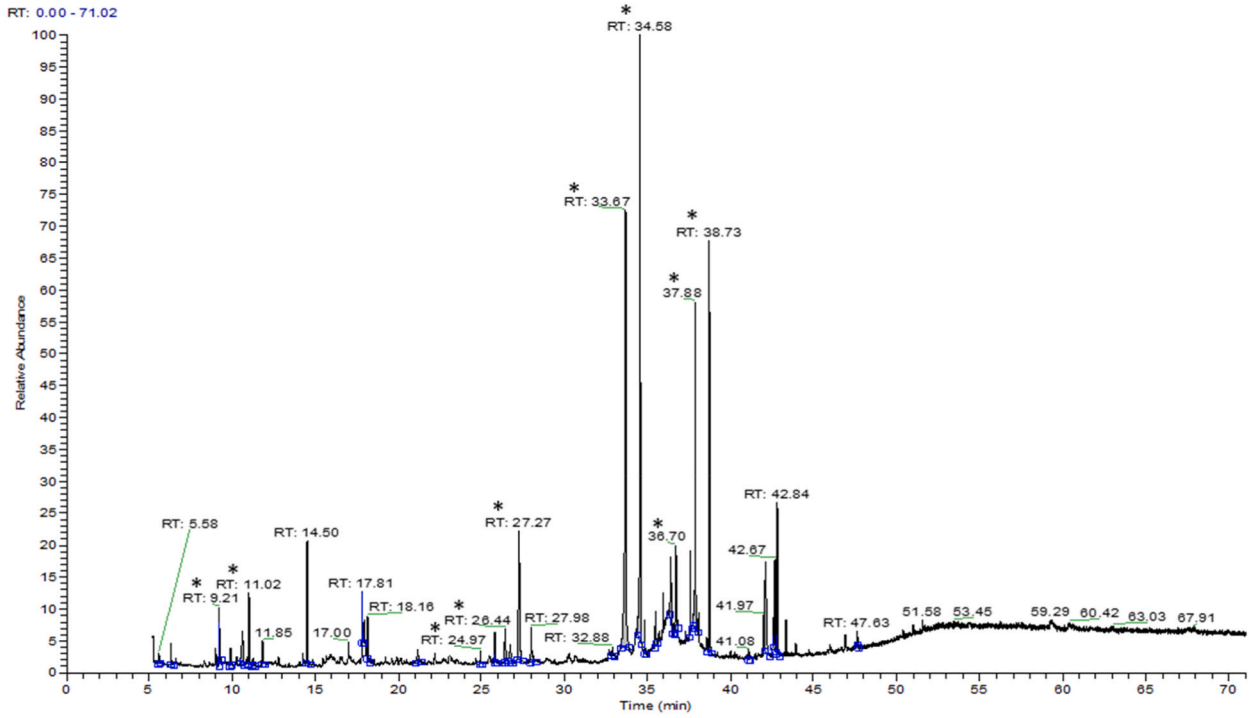

Fig. 2. GC–MS chromatogram of the methanolic aerial part extract of *Hygrophila auriculata*; * Peaks of selected compounds.

chromatograms of the methanolic leaf extract of *H. repandum* are shown in Fig. 3.

3.5.4. Methanolic leaf extract of P. chevalieri

According to Table 9, 22 compounds were identified and characterized in the methanolic leaf extract of *P. chevalieri*. The most abundant compound was 2-(3,4-Dimethoxyphenyl) ethanol, with a retention time of 33.68 min (8.27%), followed by Palmitic Acid (4.43%), and Methylamine (4.28%). Additionally, phenolic compounds such as Caffeic acid, Protocatechoic acid, Syringic acid and Tyrosol have also been identified in this extract. GC–MS chromatograms of the methanolic extract of *P. chevalieri* leaves are shown in Fig. 4.

4. Discussion

This study reported for the first time the antiplasmodial properties and the cytotoxic effect of *F. stigmatoloba*, *H. auriculata*, *H. repandum* and *P. chevalieri*, four plants that were selected following an ethnobotanical survey focusing on plants traditionally used to treat malaria in Bukavu and Uvira in D.R. Congo [2]. The remarkable activity of quinine and related drugs and the success of

Table 8
Results of GC-MS analysis of the methanolic extract from leaf of *Hylodesmum repandum*.

No.	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	5.57	Furfural	$C_5H_4O_2$	96	2.17
2	10.41	DL-Pantolactone	$C_6H_{10}O_3$	130	1.7
3	11.35	Levulinic acid	$C_5H_8O_3$	116	1.68
4	11.47	2-Furoic acid	$C_5H_4O_3$	112	1.26
5	18.99	Salicylic Acid	$C_7H_6O_3$	138	1.31
6	23.45	Tyrosol	$C_8H_{10}O_2$	138	3.41
7	26.48	1-hydroxy-3-(4-hydroxy-3-methoxyphenyl) propan-2-one	$C_{10}H_{12}O_3$	180	2.81
8	27.39	N-Acetyltyramine	$C_{10}H_{13}NO_2$	179	8.28
9	32.23	Propanone, 1-hydroxy-3(4-hydroxy-3-methoxyphenyl)-	$C_{10}H_{12}O_4$	196	4.99
10	34.76	Gallic acid trimethyl ether	$C_{10}H_{12}O_5$	212	0.19
11	37.98	Palmitic Acid	$C_{16}H_{32}O_2$	256	12.95
12	39.09	2-Myristynoyl pantetheine	$C_{25}H_{44}N_2O_5S$	484	0.53
13	46.96	Stearic acid	$C_{18}H_{36}O_2$	284	1.69
14	61.65	9-Desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol	$C_{28}H_{40}O_{10}$	536	0.16

RT: Retention time; MW: Molecular weight.

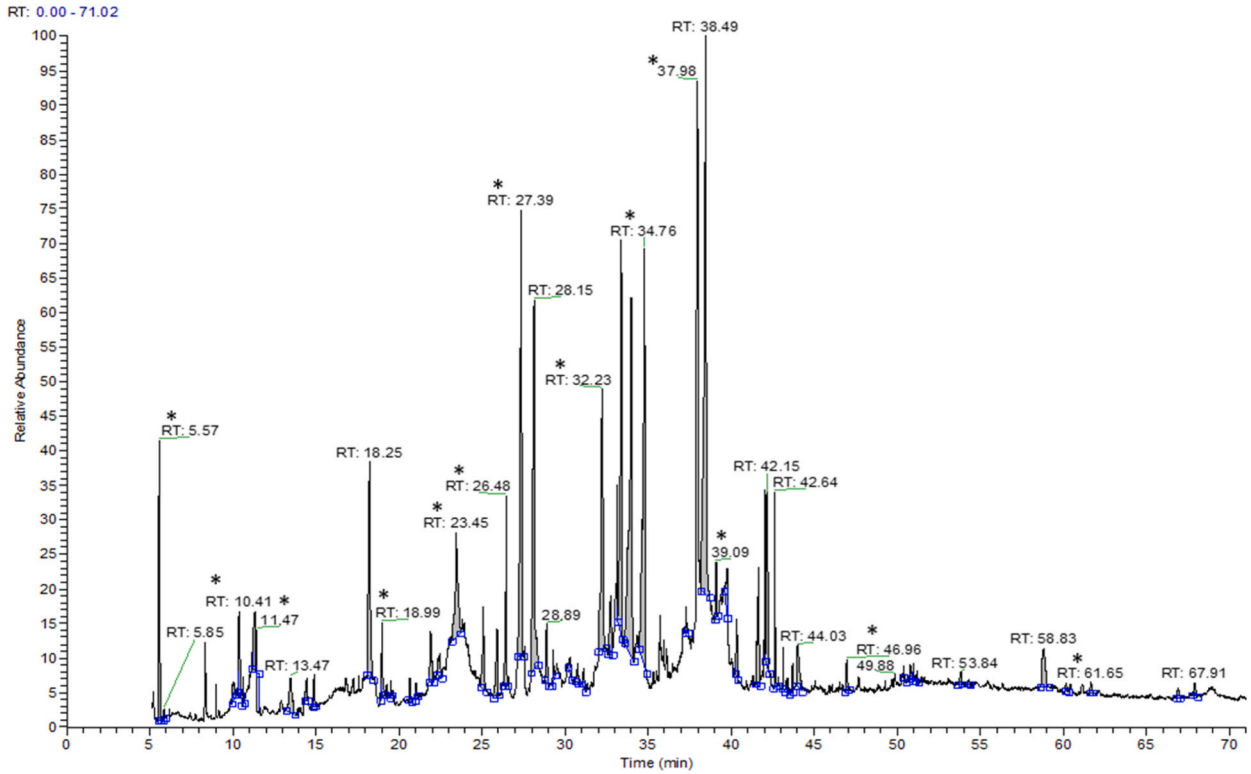

Fig. 3. GC–MS chromatogram of the methanolic leaf extract of *Hylodesmum repandum*; * Peaks of selected compounds.

Table 9
Results of GC-MS analysis of the methanolic extract from leaf of *Porphyrostemma chevalieri*.

N°	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	6.44	Methylamine	CH_3NH_2	31	4.28
2	6.61	Ethylene glycol	$C_2H_6O_2$	62	1.24
3	11.28	2-Ethoxyethanol	$C_4H_{10}O_2$	90	0.77
4	13.47	Oxalic acid	HO_2CCO_2H	90	2.74
5	15.34	Pentolactone, 3R	$C_6H_{10}O_3$	130	0.33
6	15.92	Propanedioic acid	$C_3H_4O_4$	104	1.46
7	19.54	Butanedioic acid	$C_4H_6O_4$	118	0.9
8	20.33	Glyceric acid	$C_3H_6O_4$	106	1.31
9	20.60	2-Butenedioic acid	$C_4H_4O_4$	116	0.66
10	21.17	Tartronic acid	$C_3H_4O_5$	120	0.18
11	22.30	Malic acid	$C_4H_6O_5$	134	2.29
12	23.36	Tyrosol	$C_8H_{10}O_2$	138	2.6
13	32.81	Azelaic acid	$C_9H_{16}O_4$	188	0.44
14	33.68	2-(3,4-Dimethoxyphenyl) ethanol	$C_{10}H_{14}O_3$	182	8.27
15	34.73	Protocatechoic acid	$C_7H_6O_4$	154	2.37
16	36.71	Syringic acid	$C_9H_{10}O_5$	198	0.34
17	37.92	Palmitic Acid	$C_{16}H32O_2$	256	4.43
18	39.19	2-Hydroxysebacic acid	$C_{10}H_{18}O_5$	218	0.55
19	42.41	Caffeic acid	$C_9H_8O_4$	180	1.92
20	43.99	α-Linolenic acid	$C_{18}H_{30}O_2$	278	1.24
21	46.24	4-Nitrophenyl-beta-D-galacturonide	$C_{24}H_{45}NO_9Si_4$	315	0.45
22	51.78	Rhodoxanthin	$C_{40}H_{50}O_2$	562	1.75

RT: Retention time; MW: Molecular weight.

artemisinin continue to stimulate the search for new plant-derived antimalarials. To achieve this, many plants continue to be screened for their antiplasmodial activity. In this type of investigation, the selection of plant species to be studied is a crucial step for the ultimate success of the research. Three strategies are currently pursued: random collection of plant material, targeted collection based on consideration of chemotaxonomic relationships and the exploitation of ethnomedical information [24]. Identification of new plant-derived antimalarial using an ethnopharmacological approach appears to be more predictive compared with random screening

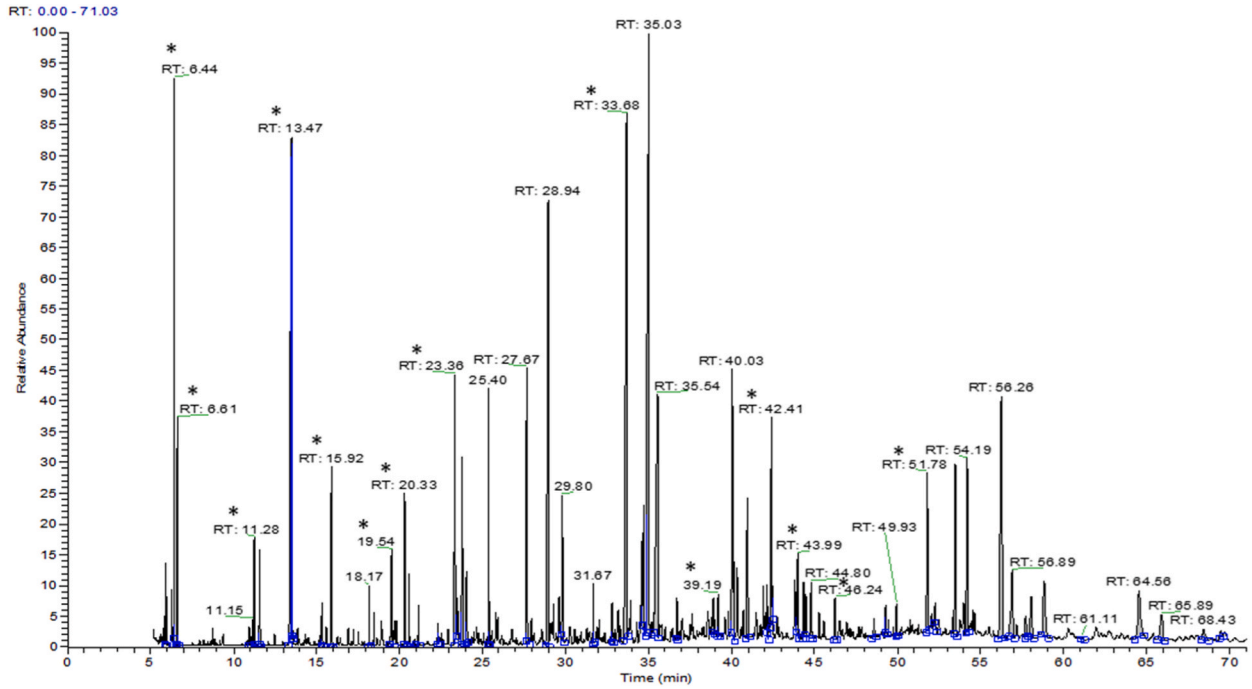

Fig. 4. GC–MS chromatogram of the methanolic leaf extract of *Porphyrostemma chevalieri*; * Peaks of selected compounds.

[25].

According to previous studies [9,26], the *in vitro* antiplasmodial activity was categorized as high when IC_{50} was <5 μg/ml, promising when IC_{50} was 5–15 μg/ml, moderate when IC_{50} was 15–50 μg/ml and inactive when IC_{50} was >50 μg/ml. Usually, a selectivity index higher than 10 is considered as interesting *in vitro* [27]. Among the five evaluated methanolic extracts, four showed a significant antiplasmodial activity with an IC_{50} < 15 μg/ml against the 3D7 strain of *Plasmodium falciparum* (Leaves of *P. chevalieri*, leaves of *H. repandum*, aerial part of *H. auriculata* and leaves of *F. stigmatoloba*). Only one extract (Leaves of *P. chevalieri*) was highly active against this 3D7 *Plasmodium falciparum* strain with IC_{50} < 5 μg/ml and promising active against W2 *P. falciparum* strain with IC_{50} between 5 and 15 μg/ml. The moderate activity on the 3D7 strain was observed with the extract from the flowers of *H. repandum*. The results showed also that the extracts from aerial part of *H. auriculata* and leaves of *H. repandum* and *F. stigmatoloba* were moderately active on W2 *P. falciparum* strain. With the two plasmodial strains (3D7 and W2) used in this study, the highest selective antiplasmodial activity was found in extracts from the leaves of *P. chevalieri*. The *in vitro* activity of this plant seems to be interesting by considering his selectivity index. To the best of our knowledge, no previous work appears to have investigated the potential antimalarial activity and the cytotoxic effect of these four plants.

In this investigation, the extract of *P. chevalieri* also exhibits the highest antioxidant activity compared to the other tested extracts. It is reported that a malaria infection generates a great deal of oxidative stress, even in the occurrence of its complications [28]. During a *Plasmodium* infection, oxidative stress comes either from digestion by phagocytic cells which contain iron-rich compounds causing an overproduction of reactive oxygen species [29,30] or from a decrease in antioxidant enzymes and compounds such as catalase, peroxidase, SOD (superoxide dismutase), GSH and many others [31]. This overproduction of EROs is responsible for neuro-malaria and anaemia [32,33]. Besides these two harms, several other damages due to oxidative stress are known leading to pathologies such as atherosclerosis, type 2 diabetes, genetic mutations, obesity, and aging [28,34]. In this context, antioxidant substances, such as those contained in *P. chevalieri*, could bring benefits by protecting people from most oxidative stress damages.

The preliminary phytochemical screening of plants under investigation revealed the presence and absence of different secondary metabolic compounds. Beside *H. auriculata*, being the only species previously reported that contains alkaloids, saponins, flavonoids, steroids, tannins, and triterpenoids [35], the three other plants (*F. stigmatoloba*, *H. repandum* and *P. chevalieri*) have been evaluated and reported for the first time in this study. These four plants are used to treat a lot of afflictions, including malaria, by traditional healers in DRC [2]. The phytochemical compound groups identified in these plants may be responsible for their biological activities, as shown in this study, and probably for other medicinal properties. Indeed, the different groups of phytochemical compounds found in our extracts are known for their numerous pharmacological properties, including the antimalarial effect [36,37]. Several investigations have been published in the field of antiplasmodial plants related to different bioactive phytochemical groups classified as alkaloids [38–40], terpenoids [41,42], and phenolic compounds including flavonoids [36] and quinones [43,44]. In this phytochemical screening, only flavonoids and terpenoids were present in all extracts. Previous studies have reported that flavonoids are antiallergic, anti-inflammatory, antioxidant, cardiovascular, anti-cancer, anti-diabetic, anti-fungal, hepatoprotective, diuretic, vasodilator, chemoprotective, anti-tumor, anti-thyroid, anti-thrombotic, antiparasitic and antiprotozoal [37,45–50], while terpenoids such as

triterpenes, sesquiterpenes and diterpenes are known to treat several pathologies including congestive heart failure, cardiac arrhythmia, and malaria [36,51]. However, it was noticed that in all plant samples more than one group of constituents were found in each morphological tested part. Biological activity may be due to one or more than one group of constituents [40].

Different levels of phenols and flavonoids from the different tested extracts were observed in this study. Extensive data show that the flavonoids and phenols synthesis in plants is influenced by different abiotic and biotic factors including UV light radiation, drought, ozone, phytopathogens, insect-deterrent [52], and environmental factors [53]. Globally the results show that the leaves of *P. chevalieri* had the highest flavonoids and phenol contents compared to the other plant extracts. Previous studies have reported that phenolic and flavonoids compounds are well-known antioxidant and antiplasmodial compounds, and that they contain many other important bioactive agents known for their benefits in human health, by curing and preventing many diseases [54]. It can be hypothesized that the high concentration of phenols and flavonoids in *P. chevalieri* is responsible for its antiplasmodial and antioxidant properties. To our knowledge, this study is the first report of the total phenol and flavonoid contents results for *F. stigmatoloba, H. repandum* and *P. chevalieri*. The total phenolic contents of *H. auriculata* was previously reported [35].

The GC-MS analysis was done to explore the likely bioactive principles in the four plants investigated. Among the main compounds potentially contained in these extracts, Palmitic Acid was identified in all the tested plants. This fatty acid could play a role in the antimalarial and antioxidant activities observed in our plant extracts. According to the literature, Palmitic Acid is known for its antioxidant, antipsychotic, hypocholesterolemic, hemolytic, inhibition prostate cancer cell growth, and 5-α-reductase inhibitor properties [55–57]. Several other major compounds with various biological properties have been identified in our plants. Ethyl palmitate, for example, which was present in both *F. stigmatoloba* and *H. auriculata*, was reported as antiandrogenic, antioxidant, hemolytic, hypocholesterolemic, and nematicide [57]. On the other hand, Ethyl linolenate from *F. stigmatoloba*, was reported to possess antiarthritic, anticancer, hepatoprotective, antimicrobial, and antiasthma biological activities [58]. Propanone, 1-hydroxy-3 (4-hydroxy-3-methoxyphenyl)-, identified in *H. repandum*, has shown antiplasmodial effect on D6 *Plasmodium falciparum* strain with an IC_{50} value of 23.5 mM [59]. In the same plant species Tyrosol, a phenolic compound, was found. This compound is a strong antioxidant, anti-arrhythmia, and cardiovascular drug with a protective effect [60]. Based on the consulted literature, the pharmacological properties of some other identified major compounds, such as Ethyl 2-nonenoate, 2-(2-Hydroxy-2-phenylethyl)-3,5,6-trimethylpyrazine, 2-(3,4-Dimethoxyphenyl) ethanol and N-acetyltyramine have not been established [61]. However, in the list of identified minor compounds (Peak area <3.41%), some are known to have antiplasmodial properties, such as β-Sitosterol identified in *F. stigmatoloba*, which was reported to show a high activity against a chloroquine-sensitive (3D7) strain with an IC_{50} value of 5.51 μM [62]. In this category of compounds, phenolic acids such as Caffeic acid, Protocatechoic acid, Syringic acid and Tyrosol were identified in *P. chevalieri*. Indeed, Caffeic acid and Protocatechoic acid have been confirmed to have antimalarial and antioxidant activities [63–65]. Protocatechoic acid is also well known to exhibit anti-inflammatory, antihyperglycemic, antibacterial, anticancer, and antispasmodic properties [65]. Syringic acid is used in a wide range of therapeutic applications to prevent diabetes, CVDs, cancer, cerebral ischemia; in addition to its antioxidant, antimicrobial, anti-inflammatory, antiendotoxic, neuro and hepatoprotective activities [66]. Briefly, the antimalarial and antioxidant activities of the four methanolic plant extracts may be produced by either a single phytoconstituent or by the synergistic effect of the cited compounds.

According to the consulted literature, there is little or no scientific work on the phytochemistry and biological aspects of the four plants evaluated in this study. *F. stigmatoloba* is an herb harvested in Uvira, province of South-Kivu. The phytochemical study and biological activities of this plant being completely unexplored, this work is the first report which goes into this direction. The *in vitro* pharmacological properties of this plant exhibited a promising activity on 3D7 *Plasmodium falciparum* strain and a moderate activity on W2 *Plasmodium falciparum strain*. The methanolic extract of this plant showed a low selectivity index and a weakness antioxidant activity. *H. auriculata*, a wild shrub, was collected in Bukavu where an aqueous decoction of aerial part is used to treat dermatoses and malaria [2]. The plant is also known as diuretic and a remedy against asthma, bronchitis, coughing, fever, gonorrhoea, fracture, headache, lumbago, and skin infection [67–70]. This work is the first report for the promising and moderate antiplasmodial activities respectively on 3D7 and W2 *P. falciparum* strains. A previously study [71] reports the phytochemical screening and the evaluation of other biological activities conducted on this plant. *H. repandum*, a shrub, is a wild plant in D.R. Congo. An aqueous decoction or infusion of leaves or flowers of the plant is used to cure dysmenorrhea, female sterility, malaria, and new-born colic in Uvira region [2]. It is also used to treat anorexia, asthenia, diarrhoea, dehydration, gastric ulcers, and liver inflammation [69]. The leaves extract of *H. repandum* showed a promising activity against the 3D7 strain of *Plasmodium falciparum* with a good selectivity index. No pharmacology and phytochemical studies of this plant were previously reported. *P. chevalieri* is a wild shrub collected in Uvira. The plant is used both by the people of Bukavu and Uvira to treat amoeba and malaria [2]. Only one study [72] reports the use of this plant in the pasture of livestock.

Based on the results presented and discussed above, this work has certain strong points. This is the first scientific report on the *in vitro* antimalarial activity and cytotoxic effect of the four evaluated medicinal plants, in particular the *P. chevalieri* species. This plant showed high and promising activity against *Plasmodium falciparum* strains 3D7 and W2 respectively, with a high selectivity index. The study also reports for the first time the preliminary phytochemical composition and interesting antioxidant activity of this plant. However, the results obtained in this work should be supplemented by *in vivo* studies and further phytochemical analysis.

5. Conclusion

The result of this study supports the usual claim that some plants are useful malaria treatment. Promising antiplasmodial and antioxidant activities with an interesting selectivity index have been demonstrated for the first time in *P. chevalieri*. Further studies are in progress in our laboratory to isolate and characterize the relevant bioactive components in the leaves of *P. chevalieri*.

Data availability statement

Data will be made available on request.

Author contribution statement

Henry Manya Mboni: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marie Faes: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Stéphanie Fraselle: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Salvius Bakari Amuri: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Moussa Compaoré: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Joh Kahumba Byanga: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Pierre Duez: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lumbu Simbi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Caroline Stévigny: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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