Research Square

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Integrating Biology and Phytochemistry to Identify Antiparasitic Natural Products from *Gardenia imperialis* K. Schum (Rubiaceae)

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Abstract

Background

Gardenia imperialis is used in Cameroonian traditional medicine to treat malaria and related symptoms. This study aimed to conduct a phytochemical investigation of *G. imperialis* extracts through bioassay-guided fractionation to identify anti-parasitic compounds.

Methods

Crude methanolic extracts from the leaves and stem bark were subjected to bioassay-guided fractionation using liquid chromatography–mass spectrometry (LC–MS) and preparative high-performance liquid chromatography (HPLC). The structures of isolated natural products were elucidated by spectroscopic analysis, including NMR and MS. All extracts, fractions, subfractions, and pure compounds were evaluated for cytotoxicity against Vero and Raw264.7 cell lines and for anti-parasitic activity against *Plasmodium falciparum* (Dd2 and 3D7 strains), *Trypanosoma brucei brucei*, and *Leishmania donovani*.

Results

The leaf methanolic extract (GIIMeOH) exhibited broad-spectrum anti-parasitic activity (IC_{50} < 11 µg/mL) without cytotoxicity towards mammalian cell lines. Dereplication and isolation yielded fractions, subfractions, and compounds with improved activity (IC_{50} values ranging from 0.67 to 37.2 µg/mL). Among the isolated compounds, gradenin A, salvigenin, hispidulin, and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone showed potent antitrypanosomal activity with IC_{50} values of 2.8, 2.5, 16.6, and 9.7 µg/mL, respectively. The anti-parasitic activities of gradenin A, salvigenin, and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone are reported here for the first time.

Conclusion

The promising anti-parasitic profile of the *G. imperialis* leaf extract and its isolated constituents, particularly and 5,7,3′-trihydroxy-6,4′,5′-trimethoxyflavone, gradenin A, and salvigenin, supports further investigation into their potential as novel and selective agents against parasitic diseases.

1. Background

Malaria, Human African Trypanosomiasis (HAT), and leishmaniasis remain infectious diseases of significant public health concern, with a cumulative estimate of hundreds of millions of cases annually [1–3]. Malaria is a complex disease caused by protozoan parasites of the *Plasmodium* genus, which progress through multiple life stages, evading host immune responses and complicating treatment. Similarly, leishmaniasis (visceral and cutaneous) and trypanosomiasis are caused by various species of the *Leishmania* and *Trypanosoma* genera, respectively. Current therapies for these parasitic diseases are often inadequate, plagued by issues such as toxicity, complex administration regimens, and the increasing spread of drug resistance [4–6]. Consequently, there is an urgent need for new drug leads with novel mechanisms of action.

Natural products, informed by ethnopharmacological knowledge, represent a vital source for such new chemical entities [7]. The structural diversity of plant-derived metabolites can help renew the therapeutic arsenal in the continuous fight against resistance. The rich biodiversity of medicinal plants, combined with traditional use, continues to offer promising avenues for drug discovery, as exemplified by the historical success of quinine from *Cinchona* species and artemisinin from *Artemisia annua* [8].

Gardenia imperialis K. Schum (Rubiaceae) is a plant used in the traditional Cameroonian pharmacopoeia for the treatment of malaria and related symptoms. The genus *Gardenia* is known to produce a wide array of biologically active and structurally diverse secondary metabolites [9]. Therefore, this study was undertaken to perform a bioactivity-guided phytochemical investigation of *G. imperialis* using chromatographic and spectroscopic techniques, evaluating the extracts and isolated compounds against *Plasmodium falciparum*, *Leishmania donovani*, and *Trypanosoma brucei brucei*.

2. Methods

2.1 Plant Material

Leaves and stem bark of *Gardenia imperialis* K. Schum were collected in Sangmelima, Cameroon (2.9333° N, 11.9833° E), in August 2021. A voucher specimen (ID 58234/HNC) was deposited at the National Herbarium of Cameroon for identification.

2.2 Extraction

The plant materials were washed, air-dried in the shade, and pulverized. Powdered leaves and stem bark (500 g each) were macerated in 5 L of methanol for 72 h at room temperature. The extracts were filtered, and the filtrates were concentrated under reduced pressure at 50°C using a rotary evaporator. The resulting crude methanolic extracts from the leaves (GIIMeOH) and stem bark (GIsbMeOH) were stored at 4°C for subsequent bioactivity assays.

2.3 Bioassay-Guided Fractionation and Isolation

Preliminary antiparasitic screening identified the leaf extract (GIIMeOH) as the most active. Consequently, 78.6 g of GIIMeOH was subjected to flash chromatography [10] on silica gel (0.063–0.2 mm) eluted with a stepwise gradient of *n*-hexane-EtOAc (100:0 to 50:50, v/v), followed by methanol, to yield six

major fractions (GI1–GI6).

Fractions GI3, GI5, and GI6, which showed significant antiparasitic activity, were selected for further investigation. These were analyzed by LC–MS-Q-TOF, and the data were processed using the Global Natural Products Social (GNPS) molecular networking platform for dereplication. The fractions were subsequently purified by Medium/High Pressure Liquid Chromatography (M/HPLC). Fraction GI3 yielded four known flavonoids, while GI5 afforded one flavonoid. The structures of all isolated compounds were elucidated by extensive ^{1}H and ^{13}C NMR spectroscopy (Supplementary Information 1).

2.4 Antiparasitic Assays

2.4.1 Parasite Cultures

Plasmodium falciparum strains 3D7 (chloroquine-sensitive) and Dd2 (multidrug-resistant) were maintained in human O + erythrocytes at 4% hematocrit in complete RPMI 1640 medium (supplemented with 0.5% Albumax II, 25 mM HEPES, and 0.04% gentamicin) at 37°C under a 5% CO_2 atmosphere [5].

Leishmania donovani (strain 1S) promastigotes were cultured at 28°C in M199 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin.

Trypanosoma brucei brucei (bloodstream form, strain Lister 427) was cultivated at 37°C under 5% CO_2 in HMI-9 medium supplemented with 10% FBS.

2.4.2 Sample Preparation

Stock solutions of extracts, fractions, and isolated compounds (100 mg/mL) and reference drugs were prepared in DMSO. Working concentrations were prepared by serial dilution in the respective culture media, ensuring the final DMSO concentration did not exceed 0.1%.

2.4.3 Antiplasmodial Assay

Anti-*Plasmodium* activity was evaluated against synchronized ring-stage cultures [11] of *P. falciparum* (3D7 and Dd2) using the SYBR Green I fluorescence method [12]. Parasites (1% parasitemia, 2% hematocrit) were incubated with test samples for 72 h. Artemisinin and chloroquine (10 µM) served as positive controls, while 0.1% DMSO was the negative control. After lysis with SYBR Green I buffer, fluorescence was measured (excitation/emission: 485/530 nm). The half-maximal inhibitory concentration (IC_{50}) was determined from dose-response curves.

2.4.4 Antileishmanial and Antitrypanosomal Assays

Activity against *L. donovani* promastigotes and *T. b. brucei* trypomastigotes was assessed using a resazurin-based assay [13, 14]. Parasites were incubated with test samples for 24 h (*L. donovani*) or 68 h (*T. b. brucei*), after which resazurin solution was added. Following further incubation, fluorescence was measured (excitation/emission: 530/590 nm). Amphotericin B and pentamidine were used as positive controls for *Leishmania* and *Trypanosoma*, respectively.

2.5 Cytotoxicity Assay

Cytotoxicity against mammalian Vero (ATCC® CRL-1586) and Raw264.7 (ATCC® TIB-71) cell lines was determined using a resazurin assay [14]. Cells were seeded at 1 × 10^4 cells/well, allowed to adhere, and then treated with samples for 48 h. Resazurin was added, and after 4 h, fluorescence was measured. The half-maximal cytotoxic concentration (CC_{50}) was calculated. The selectivity index (SI) was defined as SI = CC_{50} (mammalian cells) / IC_{50} (parasites).

2.6 Data Analysis

All experiments were performed in duplicate and independently repeated twice. Dose-response curves were plotted using GraphPad Prism 8.0, and IC_{50}/CC_{50} values were derived by nonlinear regression. Data processing was performed using Microsoft Excel.

3. Results

3.1 Bioassay-Guided Fractionation and Identification of Compounds

Initial screening of the methanolic extracts from *Gardenia imperialis* revealed that the leaf extract (GIIMeOH) exhibited potent and broad-spectrum antiparasitic activity with IC_{50} values ranging from 5.5 to 10.7 µg/mL and good selectivity (SI > 9) (Table 3). It was consequently selected for bioassay-guided fractionation.

Fractionation of GIIMeOH by flash chromatography yielded several fractions, of which GI3, GI5, and GI6 showed significant activity. The GI3 fraction (eluted with *n*-hexane-EtOAc, 80:20) was particularly active against *P. falciparum* Dd2 (IC_{50} = 6.9 µg/mL) and *T. b. brucei* (IC_{50} = 4.8 µg/mL) and was therefore subjected to further purification.

Subsequent HPLC separation and spectroscopic analysis of fraction GI3 (Fig. 1) led to the isolation and identification of four flavonoids (Fig. 2, Tables 1&2). The structures of these compounds were established as hispidulin (**1**), 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone (**2**), gradenin A (**3**), and salvigenin (**4**) based on ^{1}H and ^{13}C NMR and MS data (Supplementary Information). The ^{1}H and ^{13}C NMR data for these compounds are summarized in Tables 1&2. Compound 5,7,3',trihydroxy-6-4'5'-trimethoxyflavone along with 6 subfractions (GI3.2f, GI3.2 g, GI3.2 h, GI3.2i, GI3.3a, and GI3.3g) exhibited a broad-spectrum antiparasitic activity with IC_{50} values ranging from 0.6 µg/mL to 23.9 µg/mL and good selectivity. Salvogenin, gradenin A, and hispidulin potently inhibited *T. b. brucei* with IC_{50} values of 2.5 µg/mL, 2.8 µg/mL, and 15.6 µg/mL, respectively. Fractionation of fraction GI5 led to one inactive flavonoid, cirsimaritin (**5**) (Fig. 2).

Table 1

^{1}H NMR data for compounds (1–5) compared with literature data.

Hispidulin	1	Flavone	2	Gradenin A	3	Savigenin	4	Cirsimaritin	5
6.61	6.59	12.99	12.98	7.09	7.09	12.76	12.77	12.76	12.76
6.79	6.77	10.70	10.76	6.61	6.63	7.84	7.85	7.86	7.86
7.93	7.93	9.60	9.63	6.56	6.57	7.02	7.02	6.92	6.92
6.93	6.93	7.17	7.16	3.94	3.93	6.54	6.56	6.60	6.61
3.76	3.75	6.92	6.91	3.97	3.97	6.58	6.60	6.52	6.52
13.09	13.07	6.60	6.59	3.99	3.99	3.97	3.98	3.92	3.91
10.5	10.5	3.80	3.88			3.92	3.93	3.87	3.87
		3.70	3.75			3.89	3.90		

Table 2

^{13}C NMR data for compounds (1–5) compared with literature data.

Hispidulin	1	Flavone	2	Gradenin A	3	Salvigenin	4	Cirsimaritin	5
182.1	182.1	182.2	182.3	182.6	182.6	182.5	182.7	182.1	182.2
163.7	163.8	165.3	163.3	163.8	163.9	163.8	164.1	163.8	163.7
161.2	161.2	153.6	153.6	158.9	158.9	162.4	162.6	161.2	161.1
157.7	157.3	152.8	152.5	153.7	153.6	158.6	158.7	158.9	158.9
152.7	152.8	150.9	150.9	153.2	153.2	152.9	153.7	152.7	152.7
152.4	152.4	139.6	139.7	153.1	153.0	127.9	128.0	152.4	152.5
131.3	131.4	125.9	125.9	141.6	141.4	123.5	123.5	131.3	131.1
121.1	121.2	107.7	107.7	132.9	132.7	114.4	114.5	128.4	128.4
103.9	104.0	104.2	104.2	126.5	126.5	104.1	104.1	121.2	121.2
128.4	128.5	102.1	102.2	106.3	106.2	90.5	90.5	115.9	115.8
115.9	115.9	60.1	60.1	105.4	105.4	60.5	60.9	104.1	104.0
94.2	94.2	59.9	59.9	103.9	103.7	56.3	56.3	102.4	102.4
102.3	102.3	56.2	56.2	90.7	90.7	55.6	55.5	94.3	94.2
59.7	59.9	157.5	157.5	61.0	61.1			59.9	59.9
		152.5	152.5	60.8	60.9			56.4	56.4
				56.5	56.4				
				56.4	56.4				

3.2. Experimental Section

Hispidulin (**1**): Yellow compound. ^{1}H and ^{13}C NMR spectra (DMSO-d_6), see Tables 2 and 3; (+)-HRESIMS: *m/z* [M + H] $^{+}$ calc. for $C_{16}H_{12}O_6$ 301.0700 (calcd for $C_{16}H_{12}O_6$, 301.0634; Δ3.47ppm) calc. [M + Na] 323.0516,

5,7,3′-Trihydroxy-6,4′,5′-trimethoxyflavone (**2**): Yellow powder, ^{1}H and ^{13}C NMR spectra (DMSO-d_6), see Tables 2 and 3; (+)-HRESIMS: *m/z* [M + H] $^{+}$ calc. for $C_{18}H_{16}O_8$ 361.0895 (calcd for $C_{18}H_{16}O_8$, 361.0923; Δ1.36ppm), calc. [M + Na] 383.0703.

Gradenin A (**3**): White powder, ^{1}H and ^{13}C NMR spectra (DMSO-d_6), see Tables 2 and 3; (+)-HRESIMS: *m/z* [M + H]$^{+}$ calc. for $C_{20}H_{20}O_8$ 389.1215 (calcd for $C_{20}H_{20}O_8$, 389.1236; Δ2.40ppm), calc. [M + Na] 411.1034.

Salvigenin (**4**): White powder, ^{1}H and ^{13}C NMR spectra ($CDCl_3$), see Tables 2 and 3; (+)-HRESIMS: *m/z* [M + H] $^{+}$ calc. for $C_{18}H_{16}O_6$ 329.1028 (calcd for $C_{18}H_{16}O_6$, 329.1025; Δ0.91ppm), calc. [M + Na] 351.0849.

Cirsimaritin (**5**): White powder, ^{1}H and ^{13}C NMR spectra ($CDCl_3$), see Tables 2 and 3; (+)-HRESIMS: *m/z* [M + H] $^{+}$ calc. for $C_{17}H_{14}O_6$ 315.0872 (calcd for $C_{17}H_{14}O_6$, 315.0868; Δ1.27ppm), calc. [M + Na] 337.0689.

Table 3
Antiparasitic activity and selectivity of *Gardenia imperialis* extracts, fractions, and compounds

Codes		Vero/Raw264.7 Cells	*P. falciparum IC50 (µg/mL) ± SD*					*Kinetoplastids IC50 (µg/mL) ± S*		
Crude extracts		**CC50 (µg/mL) ± SD**	*Pf*Dd2	*SIVero/Dd2*	*Pf*3D7	*SIVero/3D7*	**RI**	*L. donovani*	*SIRaw264.7*	*T. b bru*
GIsbMeOH		> 100	18.9 ± 4.4	> 5.3	32.1 ± 4.5	> 3.1	0.6	> 50		> 5
GIlMeOH		> 100	5.5 ± 0.0	> 18.2	8.1 ± 1.2	> 12.3	0.7	10.7 ± 0.2	> 9.3	9.4 0.4
Fractions										
GI3:Hex- EtOAc (80:20)		> 100	6.9 ± 0.4	> 14.5	> 50		ND	> 50		4.8 0.3
	Subfractions/compounds									
	GI3.2a (Gradenin A)	> 100	> 50		> 50		ND	> 50		2.8 0.3
	GI3.2b (Salvigenin)	> 100	> 50		> 50		ND	> 50		2.5 0.2
	GI3.2c (Hispidulin)	> 100	23.8 ± 2.7	> 4.2	> 50		< 1.0	4.3 ± 0.10	> 23.2	15. ± 1
	GI3.2d (5,7,3'-Trihydroxy-6-4',5'-trimethoxyflavone)	> 100	20.2 ± 3.4	> 4.9	20.4 ± 3.2	> 4.9	1	9.7 ± 0.05	> 10.3	9.7 0.4
	G31.2e	> 100	22.9 ± 0.6	> 4.4	> 50		< 0.9	6.2 ± 0.09	> 16.1	8.2 0.3
	GI3.2f	> 100	9.2 ± 0.6	> 10.9	18.4 ± 2.2	> 5.4	0.5	2.8 ± 0.10	> 35.7	9.9 0.0
	GI3.2 g	> 100	4.9 ± 0.4	> 20.4	6.8 ± 0.1		0.7	8.0 ± 0.12	> 12.5	1.5 0.5
	GI3.2 h	> 100	4.7 ± 0.1	> 21.3	9.0 ± 0.6	> 11.1	0.5	11.5 ± 0.23	> 8.7	1.3 0.2
	GI3.2i	> 100	10.7 ± 0.9	> 9.3	23.9 ± 0.0	> 4.2	0.4	3.0 ± 0.10	> 33.3	12. ± 2
	GI3.2j	> 100	14.1 ± 0.8	> 7.1	> 50		< 0.6	> 50		21. ± 0
	GI3.2k	> 100	7.0 ± 0.1	> 14.3	24.9 ± 0.0	> 4	0.3	> 50		13. ± 2
	GI3.2 l	> 100	16.4 ± 0.3	> 6.1	> 50		< 0.7	> 50		23. ± 1
	GI3.3a	> 100	11.4 ± 0.4	> 8.8	14.5 ± 0.3	> 6.9	0.8	6.7	> 14.9	8.4 0.1
	GI3.3b	> 100	24.0 ± 0.0	> 4.2	> 50		< 1.0	> 50		5.3 07
	GI3.3c	> 100	23.4 ± 0.3	> 4.3	> 50		< 0.9	> 50		5.5 7
	GI3.3d	> 100	14.6 ± 0.9	> 6.8	12.0 ± 0.6	> 8.3	1.2	> 50		4.8 0.6
	GI3.3e	> 100	11.9 ± 0.1	> 8.4	17.0 ± 1.8	> 5.9	0.7	> 50		2.2 0.4
	GI3.3 f	> 100	17.8 ± 0.7	> 5.6	> 50		< 0.7	> 50		1.1 0.1
	GI3.3 g	> 100	13.0 ± 0.1	> 7.7	21.4 ± 0.3	> 4.7	0.6	9.1 ± 0.03	> 11	0.6 0.0
	GI3.3h	> 100	14.7 ± 0.0	> 6.8	13.7 ± 0.8	> 7.3	1.1	> 50		3.0 0.9
GI5: Hex- EtOAc (1:1)		> 100	18.1 ± 0.1	> 5.5	> 50		ND	> 50		> 5
	GI5.1	> 100	> 50		> 50		ND	> 50		> 5
	GI5.2	> 100	11.4	> 8.8	> 50		ND	> 50		5.8

Codes		Vero/Raw264.7 Cells	*P. falciparum IC50 (µg/mL) ± SD*					*Kinetoplastids IC50 (µg/mL) ± S*		
Crude extracts		**CC50 (µg/mL) ± SD**	*Pf*Dd2	*SIVero/Dd2*	*Pf*3D7	*SIVero/3D7*	**RI**	*L. donovani*	*SIRaw264.7*	*T. b.*
			± 2.2							0.1
	GI5.3	> 100	> 50		> 50		ND	> 50		4.8 0.6
	GI5.4 (Cirsimaritin)	> 100	> 50		> 50		ND	> 50		> 5
	GI5.5	> 100	9.1 ± 0.8	> 11	> 50		> 15.9	> 50		> 5
GI6: Residue (MeOH)		> 100	37.2 ± 1.5	> 2.7	> 50		ND	> 50		> 5
Reference drugs										
Artemisinin (µM)		ND	0.03 ± 0.0		0.04 ± 0.0		0.7	ND		ND
Chloroquine (µM)		ND	0.70 ± 0.2		0.03 ± 0.0		22.6	ND		ND
Amphotericin B (µM)		ND	ND		ND		ND	0.02 ± 0.0		ND
Pentamidine (µM)		ND	ND		ND		ND	ND		0.0 ± 0
Podophyllotoxin (µM)		0.19 ± 0.01	ND		ND		ND	ND		ND

IC_{50}: 50% inhibitory concentration; CC_{50}: 50% cytotoxic concentration; Activity values were derived for sigmoidal dose-response curves fitted by non-linear regression using GraphPad Prism 8.0 software; SD: standard deviation; Plant samples were tested in triplicate experiments against parasites in normal culture conditions; GIsbMeOH: Methanolic extract from stem bark of *G. imperialis*; GIIMeOH: Methanolic extract from leaves of *G. imperialis*; RI: Resistance index = IC_{50}*Pf*Dd2/IC_{50}*Pf*3D7; SIVero: Selectivity index when tested on Vero cells; SIRaw264.7: Selectivity index when tested on Raw264.7 cells.

4. Discussion

This study provides the first comprehensive report on the antiparasitic properties of *Gardenia imperialis*. While the fractionation process successfully concentrated the antitrypanosomal activity, it did not significantly enhance the potency against *Plasmodium* or *Leishmania* compared to the crude leaf extract (GIIMeOH). A key finding was that all active samples, including the crude extract, fractions, and isolated compounds, demonstrated an antiplasmodial resistance index (RI) of less than 1. This indicates a consistent and promising preferential inhibition of the multidrug-resistant *P. falciparum* Dd2 strain over the sensitive 3D7 strain.

The observed antiplasmodial activity aligns with previous studies on other *Gardenia* species. Extracts from *G. sokotensis*, *G. lutea*, *G. ternifolia*, and *G. saxatilis* have all shown activity against various *Plasmodium* strains [15–17], supporting the genus as a source of anti-malarial compounds. Previous phytochemical investigations of these species have identified flavonoids and triterpenoids [17], compound classes consistent with our findings.

Our bioassay-guided approach led to the isolation of several flavonoids. Among them, hispidulin confirmed its known broad-spectrum antiprotozoal potential, showing activity against all three parasites tested [18, 19]. The most significant contributions of this work, however, are the first reports of antiparasitic activity for three compounds: gradenin A, salvigenin, and 5,7,3’-trihydroxy-6,4’,5’-trimethoxyflavone.

Notably, gradenin A and salvigenin exhibited potent antitrypanosomal activity (IC_{50} = 2.8 and 2.5 µg/mL, respectively). While salvigenin is known for its anti-inflammatory, analgesic, and anticancer properties [20, 21], its activity against *T. b. brucei* is described here for the first time. In contrast, gradenin A appears to be a largely unexplored natural product. It is listed in the ChEMBL database (CHEMBL485823) but, to the best of our knowledge, no biological activity has been previously reported. Its potent antitrypanosomal activity identified in this study positions it as a promising novel scaffold for future investigation.

Similarly, the broad-spectrum antiparasitic activity of 5,7,3’-trihydroxy-6,4’,5’-trimethoxyflavone against *P. falciparum*, *L. donovani*, and *T. b. brucei* is reported here for the first time. Its structural similarity to other bioactive trimethoxyflavones [22] suggests a potential mechanism of action worthy of further exploration.

5. Conclusion

This bioactivity-guided investigation of *Gardenia imperialis* validates its traditional use and identifies its leaves as a source of potent antiparasitic compounds. The study successfully isolated several flavonoids, with the antitrypanosomal activities of gradenin A and salvigenin being particularly notable. Furthermore, the broad-spectrum activity of 5,7,3’-trihydroxy-6,4’,5’-trimethoxyflavone against *Plasmodium falciparum*, *Leishmania donovani*, and *Trypanosoma brucei brucei* is reported here for the first time. The consistent preference for inhibiting the multidrug-resistant *P. falciparum* Dd2 strain is

another promising finding. These results warrant further investigation into these natural products as novel and selective lead compounds for the development of new treatments for malaria and neglected tropical diseases.

Abbreviations

ACT: Artemisinin-based Combination Therapy; **ATCC**: American Type Culture Collection; **BEI Resources**: Biodefense and Emerging Infections Research Resources Repository; **CC_{50}** : 50% Cytotoxic Concentration; **DMEM**: Dulbecco's Modified Eagle's Medium; **DMSO**: DiMethylSulfOxyde; **EDTA**: Tetracyclic Ethylene Diamine; **FBS**: Fœtal Bovin Serum; **HEPES**: 4-(2-hydroxyethyl) -1-piperazineethanesulfonic acid; **Hex**: hexane; **IC_{50}** : 50% Inhibitory Concentration; **MeOH**: Methanol; **NMR**: Nuclear Magnetic Resonance Spectrometer; ***P. falciparum***: *Plasmodium falciparum* ; ***Pf*3D7**: *Plasmodium falciparum* 3D7 ***Pf*Dd2** : *Plasmodium falciparum* Dd2; **RPMI 1640**: Roswell Park Memorial Institute 1640; **SD**: Standard Deviation; **SI**: Selectivity index (CC_{50}/IC_{50}); ***SIRaw264.7***: Selective Index for Raw264.7 cells; ***SIVero***: Selective Index for Vero cells; GIsbMeOH: Methanolic extract from stem bark of *G. imperialis*; GIlMeOH: Methanolic extract from leaves of *G. imperialis*; RI: Resistance index = IC_{50}*Pf*Dd2/IC_{50}*Pf*3D7.

Declarations

Data Availability

All data generated or analysed during this study are included in this published article and its supplementary information file.

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The authors have no competing interests to declare that are relevant to the content of this article.

Authors' contributions

Christelle Wayoue Kom: Investigation, Methodology, Software, Visualization, Validation, Writing – original draft, Writing – review & editing;

Sylvester Osei Bobbie: Investigation, Methodology, Writing – review & editing;

Darline Dize: Investigation, Methodology, Writing – review & editing;

Mariscal Brice Tchatat Tali: Investigation, Methodology, Validation, review & editing;

Cyrille A.N. Ngansop: Investigation, Methodology, Software;

Lauve R.T. Yamthe: Methodology, Writing – review & editing;

Bill Baker: Validation, Supervision, Writing – review & editing;

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All the authors approved the final version of the manuscript

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Figures

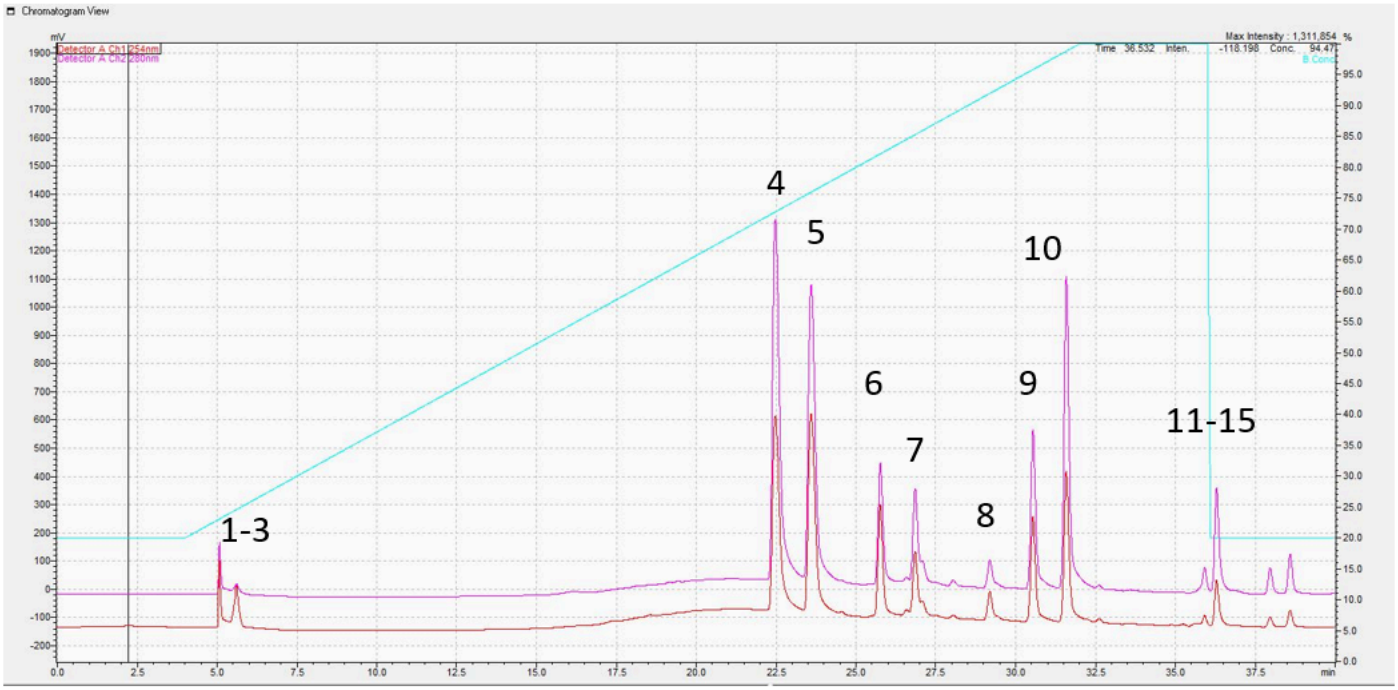
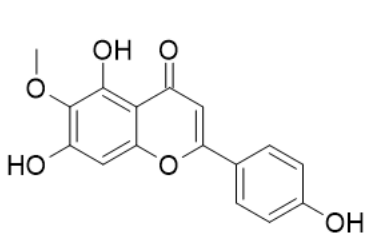


Figure 1

High Pressure Liquid Chromatography (HPLC) of GI3. HPLC conditions: Solvent water (A) Acetonitrile (B). Gradient: 0 to 4 mins 10%B; 32mins 100%B; 36mins 100%B; 40mins 10%B; Wavelength: 254 and 280 nm; Flow rate: 4ml/min, Column: Luna 5u C18(2) 100A 250 x 10.00mm, 5 micron. Numbers shown indicate the pooling of fractions into 15 sub fractions.



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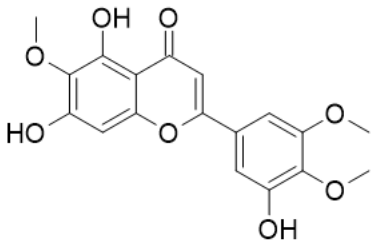
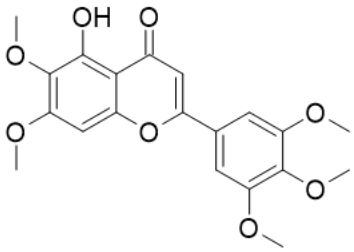
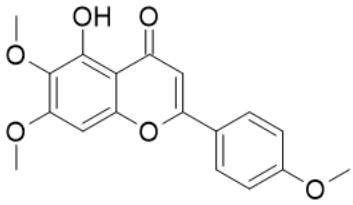


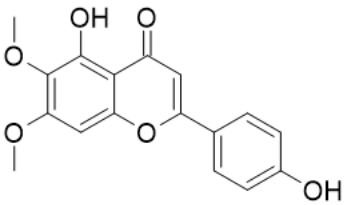
5,7,3'-Trihydroxy-6,4',5'-trimethoxyflavone (2)



Gradenin A (3)



Salvigenin (4)



Cirsimaritin (5)

Figure 2

Flavonoids found in *Gardenia imperialis* from Cameroon, Africa.

Supplementary Files

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