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Research article

Antiplasmodial activity and cytotoxicity of plant extracts from the Asteraceae and Rubiaceae families

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ABSTRACT

The increasing resistance of parasites to antimalarial drugs and the limited number of effective drugs are the greatest challenges in the treatment of malaria. It is necessary to search for an alternative medicine for use as a new, more effective antimalarial drug. Therefore, this study aimed to evaluate the *in vitro* antimalarial activity and cytotoxicity of extracts from plants belonging to the Asteraceae and Rubiaceae families. The phytoconstituents of one hundred ten ethanolic and aqueous extracts from different parts of twenty-three plant species were analyzed. Evaluation of their antimalarial activities against the chloroquine (CQ)-resistant *Plasmodium falciparum* (K1) strain was carried out using the lactate dehydrogenase (pLDH) assay, and their cytotoxicity in Vero cells was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric method. A total of 40.91% of the extracts were active antimalarial agents. Three extracts (2.73%) exhibited high antiplasmodial activity (IC_{50} < 10 μg/ml), twenty-four extracts (21.82%) were moderately active with IC_{50} values ranging from 10–50 μg/ml, and eighteen extracts (16.36%) were mildly active with IC_{50} values ranging from 50–100 μg/ml. The ethanolic leaf extract of *Mussaenda erythrophylla* (Dona Trining; Rubiaceae) exhibited the highest activity against *P. falciparum*, with an IC_{50} value of 3.73 μg/ml and a selectivity index (SI) of 30.74, followed by the ethanolic leaf extract of *Mussaenda philippica Dona Luz* x *M. flava* (Dona Marmalade; Rubiaceae) and the ethanolic leaf extract of *Blumea balsamifera* (Camphor Tree; Asteraceae), with IC_{50} values of 5.94 and 9.66 μg/ml and SI values of 25.36 and >20.70, respectively. GC–MS analysis of these three plant species revealed the presence of various compounds, such as squalene, oleic acid amide, β-sitosterol, quinic acid, phytol, oleamide, α-amyrin, sakuranin, quercetin and pillion. In conclusion, the ethanolic leaf extract of *M. erythrophylla,* the leaf extract of *M. philippica Dona Luz* x *M. flava* and the leaf extract of *B. balsamifera* had strong antimalarial properties with minimal toxicity, indicating that compounds from these plant species have the potential to be developed into new antiplasmodial agents.

1. Introduction

Malaria, a life-threatening infectious disease caused by *Plasmodium* parasites, may be caused by at least five different species [1, 2]. *Plasmodium falciparum* most often causes severe and life-threatening malaria, whereas *P. vivax* causes malaria relapse [2]. This disease is a major public health concern worldwide, especially in tropical and subtropical regions. Globally, there were an estimated 229 million cases and 409,000 deaths from malaria in 2019 [3]. The increase in the resistance of parasites to antiplasmodial drugs and the limited number of effective drugs are the greatest challenges in the treatment of malaria [4]. Therefore, it is necessary to search for a new alternative antimalarial drug that is more effective. Natural products, especially medicinal plant products, represent a potential source of pharmacologically active compounds since they contain a great variety of chemical structures [5]. There is increasing interest in the potential use of medicinal plants for pharmaceutical applications. These phytomedical compounds might be isolated and

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Table 1. Information about the plants utilized from the Asteraceae and Rubiaceae families.

No	Plant species	Family	Common name	Part used	Voucher number
1	*Acmella oleracea* (L.) R.K. Jansen.	Asteraceae	Para Cress	flowers/leaves/stems	SMD072001001
2	*Ageratum conyzoides* L.	Asteraceae	Goat Weed	leaves/stems	SMD072004001
3	*Blumea balsamifera* (L.) DC.	Asteraceae	Camphor Tree	leaves/stems	SMD072012003
4	*Chromolaena odorata* (L.) R.M. King & H. Rob.	Asteraceae	Christmas Bush	leaves/stems	SMD072020001
5	*Chrysanthemum morifolium* Ramat.	Asteraceae	Florist Chrysanthemum	flowers/leaves/stems	SMD072021003
6	*Cosmos sulphureus* Cav.	Asteraceae	Mexican Aster	flowers	SMD072010001
7	*Gerbera jamesonii* Bolus ex Hook.f.	Asteraceae	Gerbera	flowers/stems	SMD072038001
8	*Helianthus annuus* L.	Asteraceae	Sunflower	ray floret/leaves/stems/disc floret/sepals/seed shells	SMD072041002
9	*Praxelis clematidea* (Griseb.) R.M. King & H. Rob	Asteraceae	Praxelis	leaves/stems	SMD072071001
10	*Synedrella nodiflora* (L.) Gaertn.	Asteraceae	American Weed	leaves/stems	SMD072061001
11	*Tagetes erecta* L.	Asteraceae	Marigold	flowers/leaves/stems	SMD072063001
12	*Tridax procumbens* L.	Asteraceae	Coatbuttons	leaves/stems	SMD072066001
13	*Vernonia cinerea* (L.) Less.	Asteraceae	Little Ironweed	leaves/stems	SMD072067003
14	*Zinnia violacea* Cav.	Asteraceae	Zinnia	flowers/leaves/stem/pollen	SMD072070002
15	*Spermacoce laevis* Roxb.	Rubiaceae	Buttonweed	leaves/stems	SMD233074001
16	*Ixora lobbii* King & Gamble.	Rubiaceae	Glossy Ixora	flowers	SMD233033017
17	*Mussaenda erythrophylla* Schum. & Thonn.	Rubiaceae	Dona Trining	sepals/leaves/stems	SMD233048005
18	*Mussaenda philippica Dona Luz x M. flava*	Rubiaceae	Dona Marmalade	sepals/leaves	SMD233048016
19	*Morinda citrifolia* L.	Rubiaceae	Indian Mulberry	leaves/stems	SMD233046003
20	*Mussaenda philippica* Queen Sirikit	Rubiaceae	Dona Queen Sirikit	sepals/leaves	SMD233048015
21	*Mussaenda philippica* A. Rich.	Rubiaceae	Dona Aurora	sepals/leaves/stems	SMD233048010
22	*Paederia foetida* L.	Rubiaceae	Skunk-vine	leaves/stems	SMD233057003
23	*Paederia linearis* Hook.f.	Rubiaceae	Fever-vine	leaves/stems	SMD233057007

characterized from different plant parts, such as the roots, stems, bark, leaves, flowers, fruits and seeds [6].

Many studies have investigated the antimalarial properties of some plants as potential sources of new antimalarial agents. Accordingly, certain plant species in the Asteraceae and Rubiaceae families have been reported to be excellent sources of antimalarial agents [7]. Quinine was the first established antimalarial drug, and it has been used to treat malaria for centuries. This alkaloid was isolated from the bark of the *Cinchona* tree (Rubiaceae) [8, 9]. Artemisinin, an effective drug for the treatment of malaria, is derived from the leaves and floral buds of *Artemisia annua* (Asteraceae) [10]. Asteraceae (or Compositae) is the largest family of flowering plants, consisting of approximately 1,100 genera that comprise over 25,000 species. Many species of this family have been shown to have various pharmacological activities and are widely used for medicinal purposes. Several studies have demonstrated that Asteraceae species possess antibacterial, antifungal, anti-inflammatory, insecticide, antitumor and antimalarial activities [6, 11]. Rubiaceae is a flowering plant family containing 630 genera and more than 13,000 species, many of which are found in the tropics or subtropics [12]. Several plants of this family are used both ornamentally and in traditional medicine to treat several conditions, such as cough, constipation, abdominal irritation, anemia, arthritis, dermatitis, chicken pox and malaria [13].

Plants contain different types of phytochemical compounds, also known as secondary metabolites. These compounds are useful in the treatment of certain disorders due to their individual, additive, or synergic effects that improve health [14]. Phytochemical investigation of the extracts and the identification of compounds are important for screening new lead compounds for the development of new drugs [15]. Gas chromatography–mass spectrometry (GC–MS) is an analytical technique used to identify compounds present in plant samples. GC–MS plays an important role in the phytochemical analysis of medicinal plants containing biologically active components [16].

The Asteraceae and Rubiaceae families have been reported to be excellent sources of antimalarial agents, and *in vitro* screening is an essential part of antimalarial drug development. Therefore, the present study aimed to evaluate the antiplasmodial activity and cytotoxic effects of crude extracts of plants belonging to the Asteraceae and Rubiaceae families.

2. Materials and methods

2.1. Plant material collection

Fourteen plant species belonging to the Asteraceae family and nine plant species belonging to the Rubiaceae family (Table 1) were collected from Thasala District (8°40′0″N, 99°55′54″E), Nakhon Si Thammarat Province and Khuan Khanun District (7°44′6″N, 100°0′36″E), Phatthalung Province, Thailand, between March and April 2020. The plants were botanically identified by Assoc. Prof. Tanomjit Supavita, the School of Pharmacy, Walailak University. Voucher specimens of the plants were deposited at the School of Medicine, Walailak University. Prior to extraction, the freshly harvested plant parts of twenty-three species were cut into small pieces, which were dried separately in the shade at room temperature for 48 h and further dried in a circulating air oven at 50 °C for 5 days. The dry plant materials were pulverized to a fine powder using a grinder (Taizhou Jincheng Pharmaceutical Machinery Co., Ltd, Model; SF, Jiangsu, China).

2.2. Plant extraction

Plant extraction was carried out by maceration and reflux techniques. Sixty grams of each powdered plant part was macerated in 600 ml of ethanol for 72 h at room temperature. Another sixty grams was extracted with 600 ml of distilled water using the reflux method for 2 h. Each extract was filtered through Whatman filter paper No. 1, and the residue was extracted an additional two times. Then, all the filtrates were combined and concentrated to dryness under reduced pressure at 50 °C using a rotary evaporator (Rotavapor, Buchi, China). The extracts were further concentrated to dryness with a freeze-dryer (Christ Gamma 2-16 LSCplus, Germany) to obtain the ethanolic and aqueous extracts. The dried extracts were weighed to calculate the percent yield and stored in screw cap containers at 4 °C until use to prevent contamination.

2.3. Phytochemical screening

The extracts were subjected to qualitative phytochemical screening for the identification of plant secondary metabolites, including flavonoids, terpenoids, alkaloids, tannins, anthraquinones, cardiac glycosides, saponins and coumarins, according to standard methods with some modifications [17, 18, 19]: Shinoda's test for the detections of flavonoids, Salkowski's test for terpenoids, Dragendorff's test for alkaloids, the ferric chloride test for tannins, the froth test for saponins, Keller Killiani's test for cardiac glycosides, modified Borntrager's test for anthraquinones and the NaOH paper test for coumarins.

2.3.1. Test for flavonoids

A 0.5 g aliquot of each dry extract was added to 5 ml of ethanol, and the mixture was slightly heated and then filtered. The filtrate was added to some fragments of magnesium ribbon, and a few drops of concentrated HCl was then added to the mixture. The appearance of pink, orange, or red to purple coloration indicated the presence of flavonoids.

2.3.2. Test for terpenoids

Five milliliters of each extract solution were mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. The appearance of a reddish-brown color at the interface indicated the presence of terpenoids.

2.3.3. Test for alkaloids

A 0.5 g aliquot of each dry extract was dissolved in 5 ml of ethanol and then filtered. The filtrate was mixed with 5 ml of 1% HCl. A few drops of Dragendorff's reagent were added to the tube. The appearance of orange or orange red precipitates indicated the presence of alkaloids.

2.3.4. Test for tannins

A 0.5 g aliquot of each dry extract was boiled in 5 ml of water in a test tube and then filtered. A few drops of 1% ferric chloride solution were added to the filtrate. The appearance of a brownish green color indicated the presence of tannins.

2.3.5. Test for anthraquinones

A 5 ml aliquot of each extract solution was dried and shaken with 3 ml petroleum ether. The filtrate was added to 2 ml of a 10% ammonia solution, and the mixture was shaken. The appearance of a red color indicated the presence of anthraquinones.

2.3.6. Test for cardiac glycosides

A 5 ml aliquot of each extract solution was mixed with 2 ml of glacial acetic acid, and a few drops of 5% ferric chloride were added. This was under layered with 1 ml of concentrated H_2SO_4. The formation of a brown ring at the interface indicated the presence of cardiac glycosides.

2.3.7. Test for saponins

A 0.5 g aliquot of each extract was boiled in 5 ml of distilled water in a water bath and filtered. The filtrate was mixed again with 3 ml of distilled water and shaken to mix vigorously to obtain a stable persistent froth. The appearance of foam indicated the presence of saponins.

2.3.8. Test for coumarins

Five milliliters of extract solution were placed in a small test tube and covered with filter paper moistened with 10% NaOH solution. The test tube was placed for a few minutes in boiling water, and then, the filtered paper was observed under long wavelength UV at 365 nm. A greenish blue color indicated the presence of coumarins.

2.4. In vitro cultivation and maintenance of P. falciparum

The *P. falciparum* culture was maintained following the method described by Trager and Jensen with some modifications [20]. The CQ-resistant *P. falciparum* (K1) strain obtained from Dr. Rapatbhorn Patrapuvich, Department of Drug Research Unit for Malaria, Faculty of Tropical Medicine, Mahidol University, Thailand, was cultivated in 2% hematocrit (noninfected human type O-positive red cells) and maintained in RPMI-1640 supplemented with 2 mg/ml sodium bicarbonate, 10 μg/ml hypoxanthine (Sigma–Aldrich, New Delhi, India), 4.8 mg/ml HEPES (HiMedia, Mumbai, India), 0.5% Albumax II (Gibco, Waltham, MA USA) and 2.5 μg/ml gentamicin (Sigma–Aldrich, New Delhi, India). The culture was maintained at 37 °C in a CO_2 incubator. The culture medium was changed, and Giemsa-stained slides were prepared daily to monitor parasitemia.

2.5. In vitro antiplasmodial activity

The antiplasmodial activity of the crude extracts was evaluated according to an *in vitro Plasmodium* lactate dehydrogenase (pLDH) assay as described by Makler with some modifications [21]. Briefly, parasitized red blood cells (2% hematocrit, 2% parasitemia) were added to a 96-well cell culture plate, and then, the infected red cells were exposed. To evaluate the antiplasmodial activity, twofold serial dilutions of the extracts (dissolved in dimethyl sulfoxide (DMSO) at final concentrations ranging from 0.8 to 2000 μg/ml and a final DMSO concentration of 0.5%) were added to 96-well plates and incubated for 72 h at 37 °C in a CO_2 incubator. Artesunate (Sigma–Aldrich, New Delhi, India) was used as a positive control. At the end of incubation, the plates were subjected to three freeze/thaw cycles (frozen at -20 °C and thawed at 37 °C) for complete hemolysis. The lysed cells were transferred to a new 96-well plate containing a mixture of 100 μl of Malstat reagent and 20 μl of nitroblue tetrazolium/phenazine ethosulfate solution (Calbiochem®, Sigma–Aldrich, New Delhi, India) and incubated for 1 h in the dark. Each extract was tested in triplicate. These solutions were used to determine the lactate dehydrogenase (LDH) enzyme activity in the cultures. When LDH was present, a purple product was formed, and the optical density was measured using a microplate reader at a wavelength of 650 nm. The percent inhibition and half maximal inhibitory concentration (IC_{50}) were calculated by using a nonlinear dose–response curve. The protocol for this study was approved by the Human Research Ethics Committee of Walailak University (Approval number: WUEC-20-147-01). Informed consent was obtained before participant recruitment and blood collection for the maintenance of *P. falciparum* strains in human red blood cells.

2.6. In vitro assessment of cytotoxicity

The toxicity of each extract was assessed in Vero cells (Elabscience, Wuhan, Hubei, China) using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The cells were cultured in Dulbecco's modified Eagle's medium (CaissonLab, Smithfield, UT) supplemented with 10% fetal bovine serum (CaissonLab, Smithfield, UT). The Vero cell line was seeded into a 96-well flat-bottom plate at a density of 10^4 cells/ml and incubated for 24 h at 37 °C with 5% CO_2. The ethanolic and aqueous extracts were added to plates after twofold serial dilutions with final concentrations ranging from 5–200 μg/ml. Wells containing doxorubicin (Sigma–Aldrich, New Delhi, India) were used as a toxic control. The plates were incubated at 37 °C with 5% CO_2 in a CO_2 incubator, and cell viability was determined by the MTT assay as previously described [22]. The 50% cytotoxic concentration (CC_{50}) was determined by using a dose–response curve.

2.7. Selectivity index

To estimate the potential of the extracts to inhibit the growth of parasites without toxicity, the selectivity index (SI) was calculated as Eq. (1):

$$SI = CC_{50} \text{ Vero cells}/IC_{50} \textit{ P. falciparum} \quad (1)$$

Table 2. Extraction yields and phytochemical screening of the ethanolic and aqueous extracts of plants from the Asteraceae and Rubiaceae families.

No	Plant species	Part used	Extract	Yield (% w/w)	Phytochemical constituents							
					FL	TN	AL	TA	AN	CG	SA	CM
1	*Acmella oleracea*	flowers	ethanolic	20.6	+	++	-	-	-	-	++	-
			aqueous	38.6	+	+	-	-	-	-	++	-
		leaves	ethanolic	26.7	-	+	+++	-	-	-	-	+
			aqueous	38.8	-	-	++	-	-	-	+	-
		stems	ethanolic	6.1	+	+	+++	-	-	-	-	+
			aqueous	37.5	+	-	+	-	-		+	-
2	*Ageratum conyzoides*	leaves	ethanolic	17.1	-	+++	-	-	-	-	++	+
			aqueous	30.1	+	+++	-	+	-	-	++	-
		stems	ethanolic	8.2	+	+	+	-	-	-	-	-
			aqueous	30.9	+	+	+	-	-	-	-	+
3	*Blumea balsamifera*	leaves	ethanolic	10.0	+++	+++	-	-	-	-	++	-
			aqueous	19.9	+	+++	-	++	-	-	++	-
		stems	ethanolic	3.8	+	+	+	-	-	-	-	-
			aqueous	14.0	+	-	++	+++	-	-	+	-
4	*Chromolaena odoratum*	leaves	ethanolic	18.3	-	-	-	+	-	-	+	-
			aqueous	37.6	+	+++	-	+	-	-	+++	-
		stems	ethanolic	4.8	+	+	++	-	-	-	-	-
			aqueous	12.6	+	-	+	+	-	-	+	+
5	*Chrysanthemum morifolium*	flowers	ethanolic	29.2	++	+	-	-	-	-	-	-
			aqueous	49.2	+	+		++	-	-	+	+
		leaves	ethanolic	7.7	+	++	-	-	-	-	+	-
			aqueous	29.7	+	-	+	+	-	-	++	-
		stems	ethanolic	4.1	+	+	++	-	-	-	+	-
			aqueous	16.8	+	-	++	-	-	-	+	-
6	*Cosmos sulphureus*	flowers	ethanolic	23.3	++	+	++	+	-		-	+
			aqueous	39.9	+	-	+	++	-	-	-	-
7	*Gerbera jamesonii*	flowers	ethanolic	36.0	++	-	++	-	-	-	-	-
			aqueous	51.8	++	-	+	+	-	-	-	-
		stems	ethanolic	9.7	+	+	++	-	-	-	++	-
			aqueous	29.8	+	-	+	-	-	-	-	-
8	*Helianthus annuus*	ray floret	ethanolic	27.7	+++	++	-	-	-	-	-	-
			aqueous	91.2	++	-	-	++	-	-	++	-
		disc floret	ethanolic	8.1	+	+	+	-	-	-	-	+
			aqueous	25.7	+	+	++	+	-	-	++	-
		leaves	ethanolic	8.1	+	-	-	-	-	-	-	-
			aqueous	31.6	+	++	-	-	-	-	-	
		stems	ethanolic	4.9	++	+	-	-	-	-	-	+
			aqueous	21.3	++	+	-	-	-	-	-	-
		sepals	ethanolic	7.0		-	-	-	-	-	-	-
			aqueous	9.0		-	++	-	-	-	++	+
		seed shells	ethanolic	3.6	+	+	+	-	-	-	-	-
			aqueous	14.1	+	+	+	-	-	-	+	-
9	*Praxelis clematidea*	leaves	ethanolic	19.4	+	+	-	++	-	-	++	-
			aqueous	40.2	+	++	-	++	-	-	++	-
		stems	ethanolic	5.6	+	+	+++	+	-	-	-	-
			aqueous	18.3	+	+	+	++	-	-	-	+
10	*Synedrella nodiflora*	leaves	ethanolic	12.4	-	-	++	-	-	-	-	-
			aqueous	3.6	+	++	-	-	-	-	++	-
		stems	ethanolic	3.2	+	+	-	-	-	-	-	-
			aqueous	28.4	-	-	+	-	-	-	-	-
11	*Tagetes erecta*	flowers	ethanolic	24.6	+++	+++	-	++	-	-	-	-
			aqueous	22.8	++	-	-	++	-	-	++	-
		leaves	ethanolic	8.2	+	-	+++	-	-	-	-	+
			aqueous	34.3	+	-	+	+	-	-	-	-
		stems	ethanolic	2.9	+	++	++	+	-	-	-	-
			aqueous	11.5	+	-	+	+	-	-	-	+
12	*Tridax procumbens*	leaves	ethanolic	13.3	+	+	+	-	-	-	-	-

(*continued on next page*)

Table 2 (*continued*)

No	Plant species	Part used	Extract	Yield (% w/w)	Phytochemical constituents							
					FL	TN	AL	TA	AN	CG	SA	CM
			aqueous	42.5	++	-	++	+++	-	-	-	-
		stems	ethanolic	3.9	+	+	+	-	-	-	-	-
			aqueous	23.9	+	-	++	++	-	-	+	-
13	*Vernonia cinerea*	leaves	ethanolic	18.8	-	-	++	-	-		-	-
			aqueous	32.2	+	-	+	++	-	-	+	-
		stems	ethanolic	5.3	-	+	-	-	-	-	-	-
			aqueous	20.2	+	-	-	++	-	-	++	-
14	*Zinnia violacea*	flowers	ethanolic	12.4	+	+++	-	-	-	-	++	-
			aqueous	26.4	++	-	+	++	-	-	++	-
		leaves	ethanolic	9.7	-	-	+	-	-	-	+	-
			aqueous	33.3	++	-	+	++	-	-	++	+
		stems	ethanolic	5.8	+	+	+++	-	-	-	-	+
			aqueous	17.5	+	-	++	-	-	-	-	-
		pollen	ethanolic	6.4	+	+	-	-	-	-	-	+
			aqueous	26.5	+	-	+++	++	-	-	++	+
15	*Spermacoce laevis*	leaves	ethanolic	16.5	++	++	++	+	-	-	-	-
			aqueous	78.0	++	+	+	+	-	-	-	-
		stems	ethanolic	4.1	-	+++	-	-	-	-	-	-
			aqueous	55.2	+	-	-	-	-	-	+	-
16	*Ixora lobbii*	flowers	ethanolic	28.1	-	++	-	+	-	-	-	-
			aqueous	41.8	++	+++	-	++	-	-	+	-
17	*Mussaenda erythrophylla*	sepals	ethanolic	22.0	+++	++	-	++	-	-	-	-
			aqueous	47.5	+++	++	-	++	-	-	+	-
		leaves	ethanolic	8.0	++	++	-	+	-	-	-	-
			aqueous	26.4	++	++	-	+	-	-	-	-
		stems	ethanolic	2.5	++	++	-	+	-	-	-	-
			aqueous	5.7	++	+	-	+	-	-	-	-
18	*Mussaenda philippica*	sepals	ethanolic	29.8	+++	+	-	++	-	-	-	-
	Dona Luz x M. flava		aqueous	58.0	++	++	-	++	-	-	+	-
		leaves	ethanolic	8.1	++	++	-	++	-	-	-	-
			aqueous	32.7	+	++	-	+	-	-	+	-
19	*Morinda citrifolia*	leaves	ethanolic	11.3	++	++	+	-	-	-	-	-
			aqueous	31.0	++	++	+	-	-	-	+	-
		stems	ethanolic	4.4	++	+	+	-	-	-	-	-
			aqueous	9.5	++	+	+	-	-	-	-	-
20	*Mussaenda philippica*	sepals	ethanolic	16.2	+++	++	-	++	-	-	-	-
	Queen Sirikit		aqueous	47.5	++	+	-	++	-	-	+	-
		leaves	ethanolic	10.3	++	+	-	++		-		-
			aqueous	6.9	++	+	-	++		-		-
21	*Mussaenda philippica*	sepals	ethanolic	23.2	+++	+	-	++	-	-	-	-
			aqueous	39.9	+	+	-	++	-	-	+	-
		leaves	ethanolic	13.7	++	++	-	++	-	-	-	-
			aqueous	33.5	++	++	-	++	-	-	-	-
		stems	ethanolic	5.6	++	++	-	-	-	-	-	-
			aqueous	8.7	++	+	-	+	-	-	-	-
22	*Paederia foetida*	leaves	ethanolic	15.9	-	+++	-	++	-	-	-	-
			aqueous	61.8	+	-	-	++	-	-	-	-
		stems	ethanolic	6.4	-	++	-	-	-	-	-	-
			aqueous	18.6	+	+	-	-	-	-	-	-
23	*Paederia linearis*	leaves	ethanolic	10.4	-	+++	-	-	-	-	+	-
			aqueous	36.2	+	-	-	++	-	-	+	-
		stems	ethanolic	4.8	+	+++	-	-	-	-	-	-
			aqueous	16.2	+	-	-	+	-	-	-	-

%w/w: percentage weight/weight.
FL: flavonoids; TN: terpenoids; AL: alkaloids; TA: tannins; AN: anthraquinones; CG: cardiac glycosides; SA: saponins; CM: coumarins.
+++: highly abundant; ++: moderately abundant; +: present in a low amount, -: absent.

Table 3. *In vitro* antiplasmodial activity and cytotoxicity of the ethanolic and aqueous extracts of plants from the Asteraceae and Rubiaceae families.

No	Plant species	Part used	Ethanolic extract			Aqueous extract		
			IC_{50}(μg/ml)	CC_{50}(μg/ml)	SI	IC_{50}(μg/ml)	CC_{50}(μg/ml)	SI
1	*Acmella oleracea*	flowers	21.5 ± 1.9	187.8 ± 1.3	9.3	47.0 ± 0.5	49.8 ± 0.8	1.1
		leaves	28.9 ± 0.8	66.2 ± 4.2	2.3	110.7 ± 3.5	>200	>1.8
		stems	28.2 ± 1.2	83.0 ± 6.1	3.0	536.7 ± 0.6	>200	>0.4
2	*Ageratum conyzoides*	leaves	31.4 ± 0.1	129.9 ± 18.7	4.1	78.4 ± 11.5	91.3 ± 18.0	1.16
		stems	99.7 ± 0.6	42.2 ± 1.4	0.4	196.7 ± 3.3	>200	>1.0
3	*Blumea balsamifera*	leaves	9.7 ± 0.7	>200	>20.7	30.0 ± 3.1	134.8 ± 5.9	4.5
		stems	35.5 ± 0.4	158.1 ± 12.7	4.5	206.0 ± 3.0	>200	>1.0
4	*Chromolaena odoratum*	leaves	42.8 ± 4.7	>200	>4.7	137.30 ± 8.2	1.2 ± 0.2	0
		stems	112.3 ± 1.0	48.8 ± 0.8	0.4	488.9 ± 3.3	>200	>0.1
5	*Chrysanthemum morifolium*	flowers	54.4 ± 1.8	>200	4.5	110.2 ± 2.8	44.1 ± 2.1	0.4
		leaves	27.6 ± 0.1	>200	>7.2	97.7 ± 1.7	118.2 ± 17.8	1.2
		stems	107.5 ± 7.8	>200	>1.7	503.2 ± 2.9	>200	>0.4
6	*Cosmos sulphureus*	flowers	41.2 ± 1.5	>200	>4.9	515.3 ± 5.3	>200	>0.4
7	*Gerbera jamesonii*	flowers	122.4 ± 1.3	>200	>1.6	270.8 ± 1.3	>200	>0.7
		stems	123.3 ± 1.6	87.9 ± 2.4	0.7	479.4 ± 1.7	150.0 ± 18.2	0.3
8	*Helianthus annuus*	ray floret	126.1 ± 7.8	50.5 ± 4.1	0.40	132.0 ± 12.0	128.4 ± 5.0	1.0
		disc floret	37.3 ± 0.8	34.6 ± 1.9	0.9	719.5 ± 1.1	141.1 ± 3.1	0.2
		leaves	42.6 ± 1.1	88.3 ± 2.4	2.1	60.4 ± 2.5	>200	>3.3
		stems	105.7 ± 6.7	>200	>1.9	140.2 ± 6.8	>200	>1.4
		sepals	40.8 ± 1.0	49.2 ± 6.9	1.2	606.2 ± 2.1	>200	>0.3
		seed shell	118.9 ± 2.3	>200	>1.7	225.9 ± 6.0	146.4 ± 33.4	0.6
9	*Praxelis clematidea*	leaves	173.8 ± 29.8	38.7 ± 3.9	0.2	417.3 ± 1.9	129.7 ± 0.9	0.3
		stems	12.8 ± 2.3	>200	>15.7	308.3 ± 0.7	>200	>0.7
10	*Synedrella nodiflora*	leaves	37.8 ± 3.0	117.0 ± 18.2	3.1	153.9 ± 8.7	>200	0.3
		stems	142.2 ± 1.8	137.4 ± 20.7	1.0	539.9 ± 1.5	>200	>0.5
11	*Tagetes erecta*	flowers	32.8 ± 1.7	101.1 ± 6.6	2.6	35.6 ± 5.1	>200	>5.6
		leaves	70.6 ± 0.9	66.3 ± 2.4	0.9	229.5 ± 5.3	83.5 ± 0.3	0.7
		stems	86.6 ± 0.5	73.5 ± 1.3	0.9	450.9 ± 3.0	103.1 ± 8.3	0.2
12	*Tridax procumbens*	leaves	57.9 ± 1.9	>200	>3.5	461.6 ± 11.5	>200	0.4
		stems	52.6 ± 0.9	79.1 ± 2.0	1.5	775.4 ± 5.9	>200	0.3
13	*Vernonia cinerea*	leaves	30.4 ± 1.0	154.9 ± 7.0	5.1	63.0 ± 3.5	111.0 ± 11.6	1.8
		stems	143.4 ± 18.2	168.3 ± 19.0	1.2	917.1 ± 7.4	>200	0.2
14	*Zinnia violacea*	flowers	112.5 ± 3.5	23.7 ± 8.5	1.1	428.7 ± 24.2	45.3 ± 1.7	0.1
		leaves	22.4 ± 0.2	31.0 ± 1.3	1.4	197.0 ± 2.8	107.0 ± 0.9	0.5
		stems	111.3 ± 0.5	120.0 ± 14.0	1.1	823.7 ± 2.8	116.2 ± 14.7	0.1
		pollen	87.8 ± 1.5	>200	>2.3	202.80 ± 3.2	59.4 ± 6.6	0.3
15	*Spermacoce laevis*	leaves	13.7 ± 1.9	>200	>14.7	159.2 ± 2.0	>200	>13
		stems	79.8 ± 6.7	>200	2.5	176.0 ± 1.2	>200	>0.1
16	*Ixora lobbii*	flowers	343.3 ± 5.9	>200	>0.6	813.3 ± 8.4	>200	>0.3
17	*Mussaenda erythrophylla*	sepals	258.3 ± 17.8	59.6 ± 3.1	0.2	202.3 ± 1.0	>200	>1.0
		leaves	3.7 ± 2.6	114.7 ± 4.5	30.7	222.1 ± 3.0	>200	>0.9
		stems	29.6 ± 0.7	198.1 ± 6.0	7.4	209.9 ± 3.6	>200	>1.0
18	*Mussaenda philippica*	sepals	338.9 ± 20.8	43.9 ± 0.1	0.1	146.5 ± 43.7	>200	>1.4
	Dona Luz x M. flava	leaves	5.9 ± 0.4	150.7 ± 2.1	25.7	52.2 ± 1.9	>200	>3.8
19	*Morinda citrifolia*	leaves	54.7 ± 2.1	>200	>3.7	83.4 ± 3.2	170.4 ± 4.2	2.0
		stems	147. ± 7.7	>200	>1.4	63.3 ± 1.5	>200	>3.2
20	*Mussaenda philippica*	sepals	343.7 ± 38.5	101.7 ± 7.7	0.3	54.1 ± 6.7	>200	>3.7
	Queen Sirikit	leaves	11.3 ± 0.7	184.7 ± 0.5	16.3	46.0 ± 1.8	169.5 ± 0.1	3.7
21	*Mussaenda philippica*	sepal	287.3 ± 35.6	119.2 ± 7.1	0.4	260.3 ± 8.8	>200	>0.8
		leaves	47.0 ± 3.7	39.7 ± 0.4	0.8	348.3 ± 2.5	106.7 ± 7.1	0.3
		stems	90.6 ± 2.6	111.4 ± 1.2	1.2	384.8 ± 1.2	175.2 ± 6.0	0.5
22	*Paederia foetida*	leaves	80.1 ± 4.4	54.4 ± 4.3	0.7	137.3 ± 8.2	>200	>1.5
		stems	216.5 ± 11.2	180.7 ± 10.4	0.8	309.4 ± 2.8	>200	>0.7
23	*Paederia linearis*	leaves	212.5 ± 8.1	132.8 ± 2.2	0.6	238.3 ± 3.4	168.3 ± 4.8	0.7
		stems	233.5 ± 18.5	113.9 ± 12.1	0.5	421.1 ± 2.3	>200	>0.5
	Artesunate	IC_{50} = 1.3 ± 0.5 ng/ml						
	Doxorubicin	CC_{50} = 1.6 ± 0.2 μg/ml						

IC_{50}: 50% inhibitory concentration, CC_{50}: 50% cytotoxic concentration.

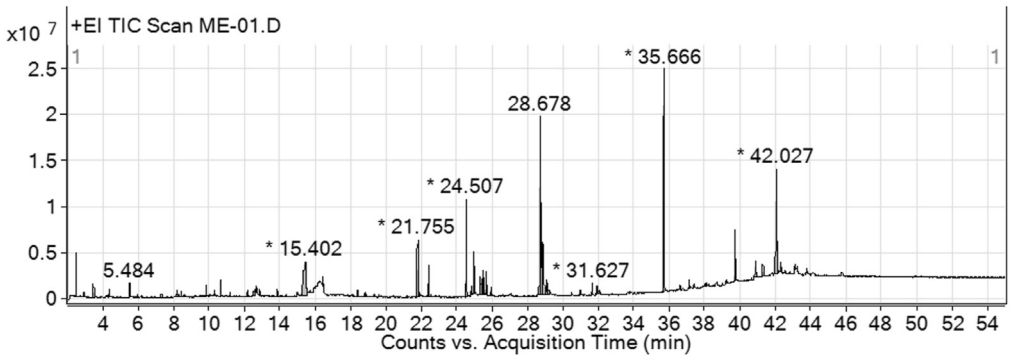

Figure 1. GC–MS chromatogram of the ethanolic leaf extract of *Mussaenda erythrophylla*.

2.8. The GC-analysis

GC–MS analysis of compounds in the extracts that possessed high antiplasmodial activity was carried out using Agilent Technologies GC systems with a 7000C GC/MS Triple Quad model (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS column (30 m × 0.25 mm; 0.25 μm). Spectroscopic detection by GC–MS involved an electron ionization system with a high ionization energy of 70 eV, ion source temperature of 250 °C and mass scanning range of 33–600 amu in full scan. Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 ml/min, and the injector temperature was maintained at a constant of 250 °C. The initial column temperature was set to 60 °C for 2 min and increased to 150 °C with an increasing rate of 10 °C/min. Finally, the temperature was increased to 300 °C at 5 °C/min. One microliter of the sample in ethanol was injected in split mode with a split ratio of 20:1. The identification of the phytochemical compounds in the test samples was performed by comparing their mass spectra with the spectral database of known compounds in the National Institute of Standards and Technology (NIST2011) structural library. Only selected peaks with 80% similarity and above with NIST libraries were chosen and identified.

3. Results

3.1. Phytochemical screening

Phytochemical screening of the extracts of plants from the Asteraceae and Rubiaceae families revealed the presence of various constituents, including flavonoids, terpenoids, alkaloids, tannins, anthraquinones, saponins and coumarins (Table 2). Among them, flavonoids were present in all the plants and present at high levels in six plants, including the flowers of *H. annuus*, flowers of *T. erecta*, sepals of *M. erythrophylla*, sepals of *M. philippica Dona Luz* x *M. flava*, and sepals of *M. philippica* Queen Sirikit and *M. philippica* (Dona Aurora). Flavonoids are the most common and widely distributed group of plant phenolic compounds. Flavonoids are present in all plant parts, particularly photosynthesising plant cells, and are major coloring components of flowering plants [23]. Flavonoids also occur abundantly in plants as glycosides in which one or more phenolic hydroxyl groups are combined with sugar residues. They are water soluble and thermostable compounds possessing acidic properties due to the aromatic phenol groups [24]. Phytochemical analysis of a methanolic extract of *T. erecta* showed that it contained several polyphenolic compounds, including flavonoids, especially the glycosides quercetagetin, quercetin, kaempferol, and patuletin [25].

Terpenoids were also detected in all the plants, and abundant contents were detected in nine plants, including the flowers of *T. erecta*, the flowers of *Z. violacea*, the flowers of *I. lobbii*, the stems of *S. laevis,* the leaves of *A. conyzoides*, the leaves of *B. balsamifera*, the leaves of *C. odoratum*, the leaves of *P. foetida* and the leaves and stems of *P. linearis.* Alkaloids were present at high levels in only three plants, including the leaves and stems of *A. oleracea,* the leaves of *T. erecta* and the stems and pollen of *Z. violacea*.

In particular, different phytochemical constituents were found in samples of different plant parts and in different solvent extracts. These results clearly show the flavonoid levels in *T. erecta*: The ethanolic flower extract had high levels of flavonoids, and the aqueous flower extract showed moderate levels, whereas the ethanolic and aqueous leaf and stem extracts had low flavonoid levels. In addition, the flowers of *T. erecta* were also rich in terpenoids, whereas its leaves did not contain this substance. Alkaloids were found in high amounts in the leaves and in moderate amounts in the stems of *T. erecta* but were not present in the flowers. Saponins were found only in the flowers. Moreover, differences in phytochemical constituent contents were also found among the different parts of *Z. violacea*. Flavonoids and terpenoids were discovered in all the plant parts but at different levels, as flavonoids were present at high levels in the flowers, alkaloids were present at high levels in the stems, and terpenoids were present at high levels in the flowers but not present in the leaves.

3.2. In vitro antiplasmodial activity

A total of one hundred ten ethanolic and aqueous extracts from different parts of twenty-three plant species of the Asteraceae and Rubiaceae families were tested against the CQ-resistant *P. falciparum* (K1) strain *in vitro*. The IC_{50} values, CC_{50} values and SI of each extract are shown in Table 3. These plant extracts exhibited varying degrees of antiplasmodial activity, and 40.91% were active (IC_{50} < 100 μg/ml). Three extracts (2.73%) exhibited high antiplasmodial activity (IC_{50} < 10 μg/ml), twenty-four extracts (21.82%) were moderately active with IC_{50} values ranging from 10–50 μg/ml, and eighteen extracts (16.36%) were mildly active with IC_{50} values ranging between 50–100 μg/ml. Artesunate, the reference antiplasmodial drug, was used as a positive control (IC_{50} = 1.25 ng/ml). Of the tested total plant extracts, the ethanolic leaf extract of *M. erythrophylla*, which belongs to the Rubiaceae family, was found to be the most active against *P. falciparum*, with the lowest IC_{50} value of 3.73 μg/ml and minimal toxicity with a CC_{50} value of 114.65 μg/ml (SI of 30.74). The next most active extracts were the ethanolic leaf extract of *M. philippica Dona Luz x M. flava* (Dona Marmalade) and the ethanolic leaf extract of *B. balsamifera* (Camphor Tree), with IC_{50} values of 5.94 and 9.66 μg/ml and SI values of 25.36 and >20.70, respectively. Among the twenty-four extracts with moderate activity, the ethanolic leaf extract of *M. philippica* Queen Sirikit, the ethanolic stem extract of *P. clematidea* and the ethanolic leaf extract of *S. laevis* showed the highest activity, as they possessed promising antiplasmodial activity with IC_{50} values of 11.31, 12.78 and 13.65 μg/ml, respectively. It should be noted that the ethanolic extracts of most of the plants were more active than the corresponding aqueous extracts, except for those of the sepals of *M. erythrophylla*, the sepals of *M. philippica Dona Luz* x *M. flava*, the stems of *M. citrifolia*, the sepals of *M. philippica* Queen Sirikit and the sepals of *M. philippica* (Dona Aurora).

Table 4. Compounds identified in the ethanolic leaf extract of *Mussaenda erythrophylla* by GC–MS.

No.	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	2.472	Acetal	$C_6H_{14}O_2$	118	1.92
2	5.484	Glycerin	$C_3H_8O_3$	92	0.90
3	10.605	Salicylic acid	$C_7H_6O_3$	138	0.69
4	12.818	Coumarin	$C_9H_6O_2$	146	0.31
5	15.402	Quinic acid	C_7H12O_6	192	7.98
6	18.059	Myristic acid	$C_{14}H_{28}O_2$	228	0.09
7	19.322	Isopropyl myristate	$C_{17}H_{34}O_2$	270	0.20
8	19.579	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.24
9	21.755	Palmitic acid	$C_{16}H_{32}O_2$	256	3.95
10	22.389	Ethyl palmitate	$C_{18}H_{36}O_2$	284	1.75
11	22.941	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.11
12	24.507	Phytol	$C_{20}H_{40}O$	296	5.51
13	24.810	Linoleic acid	$C_{18}H_{32}O_2$	280	0.50
14	24.930	Linolenic acid	$C_{18}H_{30}O_2$	278	3.23
15	25.304	Stearic acid	$C_{18}H_{36}O_2$	284	1.15
16	25.362	Ethyl linoleate	$C_{20}H_{36}O_2$	308	1.06
17	25.482	Ethyl linolenate	$C_{20}H_{34}O_2$	306	1.62
18	25.626	Palmitamide	$C_{16}H_{33}N_O$	255	1.41
19	25.908	Ethyl stearate	$C_{20}H_{40}O_2$	312	0.54
20	28.580	12-Methyl-E,E-2, 13-octadecadien-1-ol	$C_{19}H_{36}O$	280	0.52
21	28.678	Oleic acid amide	$C_{18}H_{35}NO$	281	12.62
22	29.074	Stearic amide	$C_{18}H_{37}NO$	283	1.37
23	31.906	Cis-11-Eicosenamide	$C_{20}H_{39}NO$	309	0.64
24	35.666	Squalene	$C_{30}H_{50}$	410	13.74
25	38.392	Quercetin	$C_{15}H_{10}O_7$	302	0.98
26	40.856	Campesterol	$C_{28}H_{48}O$	400	1.31
27	41.270	Stigmasterol	$C_{29}H_{48}O$	412	1.00
28	42.027	γ-Sitosterol	$C_{29}H_{50}O$	414	8.62

RT = Retention time, MW = Molecular weight.

Among fifty-five aqueous extracts, none exhibited high antiplasmodial activity, four exhibited moderate activity, seven exhibited mild activity, and the others were inactive, with IC_{50} values >100 μg/ml and low SI values. Among those with moderate activity, the leaf extract of *B. balsamifera* showed the highest activity (IC_{50} = 29.98 μg/ml), followed by the leaves of *T. erecta* and the flowers of *A. oleracea,* with IC_{50} values of 35.61 and 47.00 μg/ml, respectively. In particular, the extracts of different plant parts exhibited different antiplasmodial effects; for example, the ethanolic extracts of different parts of *H. annuus* showed antiplasmodial activity ranging from 37.29 to 126.10 μg/ml. Its ethanolic disc floret extract exhibited the highest activity, followed by extracts of the sepals, leaves, stems, seed shells and ray floret, with IC_{50} values of 37.29, 40.79, 42.60, 105.70, 118.93 and 126.10 μg/ml, respectively.

Table 5. Compounds identified in the ethanolic leaf extract of *Mussaenda philippica Dona Luz* x *M. flava* by GC–MS.

No.	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	2.469	Acetal	$C_6H_{14}O_2$	118	2.39
2	10.599	Salicylic acid	$C_7H_6O_3$	138	0.50
3	12.395	Cinnamic acid	$C_9H_8O_2$	148	0.15
4	13.839	Phenol, 2,4-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.36
5	15.436	Quinic acid	$C_7H_{12}O_6$	192	10.15
6	19.322	Isopropyl myristate	$C_{17}H_{34}O_2$	270	0.13
7	19.579	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.46
8	19.686	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	0.24
9	21.093	7-Methyl-Z-tetradecen -1-ol acetate	$C_{17}H_{32}O_2$	268	0.14
10	21.749	Palmitic acid	$C_{16}H_{32}O_2$	256	4.38
11	22.389	Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.60
12	22.938	Isopropyl palmitate	$C_{19}H_{38}O_2$	298	0.13
13	24.510	Phytol	$C_{20}H_{40}O$	296	9.25
14	24.814	Linoleic acid	$C_{18}H_{32}O_2$	280	0.79
15	24.939	Linolenic acid	$C_{18}H_{30}O_2$	278	6.34
16	25.212	Oleic acid	$C_{18}H_{34}O_2$	282	0.19
17	25.301	Stearic acid	$C_{18}H_{36}O_2$	284	1.77
18	25.359	Ethyl linoleate	$C_{20}H_{36}O_2$	308	0.52
19	25.482	Ethyl linolenate	$C_{20}H_{34}O_2$	306	0.61
20	25.629	Palmitamide	$C_{16}H_{33}NO$	255	2.06
21	25.908	Ethyl stearate	$C_{20}H_{40}O_2$	312	0.25
22	26.987	Oleic acid	$C_{18}H_{34}O_2$	282	0.46
23	28.580	E,E,Z-1,3,12 -Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	0.77
24	28.679	Oleic acid amide	$C_{18}H_{35}NO$	281	16.47
25	29.074	Stearic amide	$C_{18}H_{37}NO$	283	1.87
26	30.968	Glyceryl palmitate	$C_{19}H_{38}O_4$	330	1.05
27	31.624	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	1.88
28	31.906	cis-11-Eicosenamide	$C_{20}H_{39}NO$	309	1.32
29	35.667	Squalene	$C_{30}H_{50}$	410	1.91
30	38.392	Quercetin	$C_{15}H_{10}O_7$	302	1.01
31	40.856	Campesterol	$C_{28}H_{48}O$	400	1.08
32	41.270	Stigmasterol	$C_{29}H_{48}O$	412	0.93
33	42.027	β-Sitosterol	$C_{29}H_{50}O$	414	7.63

RT = Retention time, MW = Molecular weight.

3.3. GC–MS analysis of plant extracts

Based on antiplasmodial activity, three plant species that possessed high effects with IC_{50} values < 10 μg/ml were selected to identify the phytochemical compounds by GC–MS.

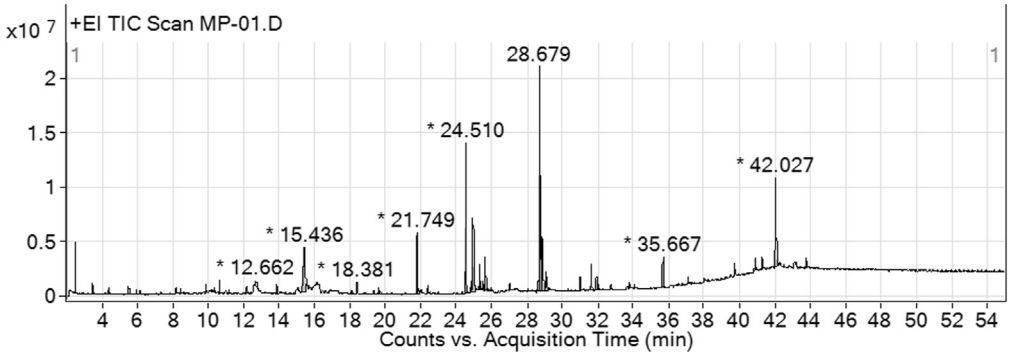

Figure 2. GC–MS chromatogram of the ethanolic leaf extract of *Mussaenda philippica Dona Luz* x *M. flava*.

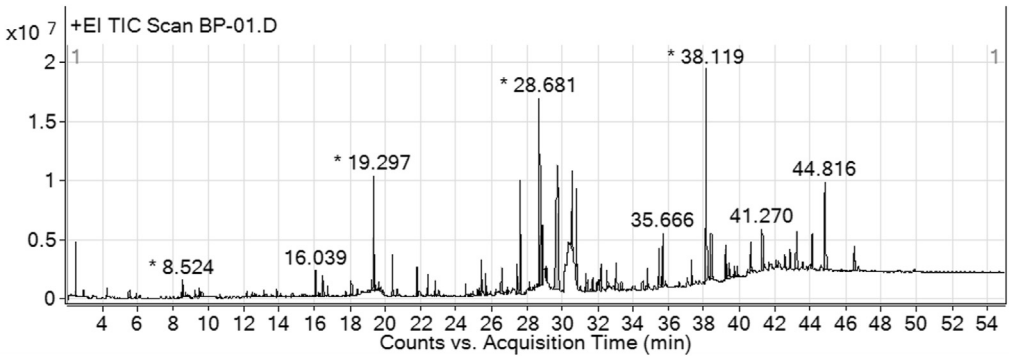

Figure 3. GC–MS chromatogram of the ethanolic leaf extract of *Blumea balsamifera*.

3.3.1. GC–MS analysis of ethanolic leaf extract of M. erythrophylla

The GC–MS chromatograms of the ethanolic leaf extract of *M. erythrophylla* are shown in Figure 1. The mass spectra of phytochemical compounds were compared with the spectral database of known compounds of the NIST library. Twenty-eight compounds were identified and characterized, as listed in Table 4. The most abundant compound was squalene, an unsaturated terpenoid with a retention time of 35.666 min (13.74%), followed by oleic acid amide (12.62%), β-sitosterol (8.62%), quinic acid (7.98%), and phytol (5.51%). Other compounds were present at less than 5%.

3.3.2. GC–MS analysis of the ethanolic leaf extract of M. philippica Dona Luz x M. Flava

The GC–MS chromatograms of the ethanolic leaf extract of *M. philippica Dona Luz x M. flava* are shown in Figure 2. Thirty-three compounds were identified and characterized, as listed in Table 5. The *M. philippica Dona Luz x M. flava* extract was mainly composed of an oleic acid amide (16.47%), which was found at a retention time of 28.679 min, followed by quinic acid (10.15%), phytol (9.25%), β-sitosterol (7.63%), and linolenic acid (6.34%). Other compounds were found in low amounts of less than 5%.

3.3.3. GC–MS analysis of the ethanolic leaf extract of B. balsamifera

The GC–MS chromatograms of the ethanolic leaf extract of *B. balsamifera* are shown in Figure 3. Thirty-nine compounds were identified and characterized, as listed in Table 6.

The most abundant compound was an unknown compound with a retention time of 38.119 min, followed by oleamide (7.04%) with a retention time of 28.681 min, α-amyrin (4.95%), β-eudesmol (3.40%), 3,3a epoxydicyclopenta [a,d]cyclo octan-4.beta.-ol, and 9,10a-dimethyl-6-methylene-3.beta.-isopropyl- (3.32%). In addition, many flavonoid compounds were also identified, such as sakuranin, quercetin, pilloin, 5,7-dihydroxy, 3′,4′,5′-trimethoxyflavone, retusin and 7,3′-dimethylquercetin, which were in accordance with results of phytochemical screening that found a high abundance of terpenoids and flavonoids.

4. Discussion

One hundred ten crude extracts from twenty-three different plant species from the Asteraceae and Rubiaceae families were screened *in vitro* to measure their antiplasmodial activity and cytotoxicity. All the plant parts were used to calculate the extraction yield, which is a measure of solvent efficiency, in order to extract specific components from the original material [26]. The results from the extraction of all of the different plant parts revealed that almost all of the aqueous extracts provided a higher yield than the corresponding ethanolic extracts, except for the leaves of *S. nodiflora,* the flowers of *T. erecta* and the leaves of *M. philippica* Queen Sirikit, where the yield of the aqueous extract was lower than that of the ethanolic extract. This difference may be due to the parameters of the extraction process and the chemical constituents; sugars and oligosaccharides are more soluble in water than ethanol, so the yield of the aqueous extract would be higher than that of the ethanolic extract [27, 28]. Therefore, an effective method for the extraction process is one of the important parameters to obtain a high total yield of an extract.

Qualitative screening of the phytochemicals in the plants was performed using chemical reactions and color tests to determine the classes of secondary metabolites. The screening results of the twenty-three plants indicated that the Asteraceae and Rubiaceae families contained flavonoids, terpenoids, alkaloids, tannins, anthraquinones, saponins and coumarins. In particular, most plants contained flavonoids, as shown by the appearance of a yellow color in Shinoda's test, and they also contained terpenoids, as shown by the appearance of a reddish-brown color in Salkowski's test. In the present study, the plants with promising antiplasmodial activity contained high levels of flavonoids, terpenoids, and alkaloids. These findings are consistent with previous studies reporting that these phytoconstituents are responsible for antiplasmodial activity [29]. However, dissimilarity of the phytochemical constituents was found in the different solvent extracts and in the extracts from different plant parts. This dissimilarity of constituents is based on a number of intrinsic and extrinsic factors, specific metabolic activities and endogenous physiological changes in the plants [30].

The antiplasmodial activity of the extracts was tested *in vitro* against CQ-resistant *P. falciparum* using the pLDH assay. LDH is an important enzyme in the glycolytic pathway in malaria parasites that is produced during the sexual and asexual stages of the parasite [31]. The production and accumulation of LDH are used as reliable markers to determine parasite viability [32]. Therefore, the detection of LDH is specific for the parasitic enzyme and has been used for antimalarial drug screening during the asexual stages in high-throughput screening platforms [33, 34]. Here, the antiplasmodial activity of the extracts was considered high if the IC_{50} was <10 μg/ml, moderately active if the IC_{50} ranged from 11–50 μg/ml, mildly active if the IC_{50} ranged from 51–100 μg/ml and inactive if the IC_{50} was >100 μg/ml [35, 36]. In the present study, three plants exhibited high antiplasmodial activity. The ethanolic leaf extract of *M. erythrophylla* (Rubiaceae) possessed the most potent antiplasmodial activity (IC_{50} = 3.73 μg/ml, SI = 30.74), followed by the ethanolic leaf extract of *M. philippica Dona Luz* x *M. flava* (Rubiaceae) (IC_{50} = 5.94 μg/ml, SI = 25.36) and the ethanolic leaf extract of *B. balsamifera* (Asteraceae) (IC_{50} = 9.66 μg/ml, SI > 20.70). The SI value was used to estimate the potential of each extract to inhibit the growth of parasites without toxicity. A high SI value offers potential antiplasmodial activity and safer therapy. In contrast, a low SI indicates that the extract probably presents cytotoxicity rather than possesses antiplasmodial activity against the parasite [37]. Usually, an SI higher than 10 is considered interesting with respect to *in vitro* antimalarial activity [38]. Therefore, this study suggested that the three plant extracts that showed an IC_{50} < 10 μg/ml and an SI value >10 had potential antiplasmodial activity against CQ-resistant *P. falciparum*, that is, the extracts of *M. erythrophylla*, *M. philippica Dona Luz* x *M. flava* and *B. balsamifera.* Regarding

Table 6. Compounds identified in the ethanolic leaf extract of *Blumea balsamifera* by GC–MS.

No.	RT (min)	Name of the compounds	Molecular formula	MW	Peak area (%)
1	2.472	Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$	118	1.26
2	4.295	α-Methylchrotonic acid	$C_5H_8O_2$	100	0.31
3	8.411	Pyranone	$C_6H_8O_4$	144	0.13
4	8.524	(+)-Camphor	$C_{10}H_{16}O$	152	0.35
5	12.588	Caryophyllene	$C_{15}H_{24}$	204	0.08
6	13.838	Phenol, 2,4-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O$	206	0.20
7	14.068	(-)-Spathulenol	$C_{15}H_{24}O$	220	0.10
8	14.718	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.12
9	15.907	Tricyclo [6.3.0.0 (1,5)]undec-2-en-4-one, 2,3,5,9-tetramethyl-	$C_{15}H_{22}O$	218	0.07
10	16.039	γ- Eudesmol	$C_{15}H_{26}O$	222	0.78
11	16.281	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	$C_{14}H_{22}O_3$	238	0.13
12	16.398	β- Eudesmol	$C_{15}H_{26}O$	222	0.56
13	16.440	α-Eudesmol	$C_{15}H_{26}O$	222	0.39
14	16.474	Juniper camphor	$C_{15}H_{26}O$	222	0.48
15	16.713	Longipinocarveol, trans-	$C_{15}H_{24}O$	220	0.29
16	19.297	β-Eudesmol	$C_{15}H_{26}O$	222	3.40
17	20.648	Acetic acid, 7-(1-hydroxymethyl-vinyl)-1,4a-dimethyl-3-oxo-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl ester	$C_{17}H_{24}O_4$	292	0.43
18	21.742	Palmitic acid	$C_{16}H_{32}O_2$	256	1.09
19	22.392	Ethyl palmitate	$C_{18}H_{36}O_2$	284	0.62
20	24.507	Phytol	$C_{20}H_{40}O$	296	0.43
21	24.798	Linoleic acid	$C_{18}H_{32}O_2$	280	0.11
22	24.905	6,9,12,15-Docosatetraenoic acid, methyl ester	$C_{23}H_{38}O_2$	346	0.29
23	25.628	Palmitamide	$C_{16}H_{33}NO$	255	0.80
24	26.542	Anticopalic acid	$C_{20}H_{32}O_2$	304	1.29
25	28.681	Oleamide	$C_{18}H_{35}NO$	281	7.04
26	30.526	3,3a-Epoxydicyclopenta [a,d] cyclooctan-4.beta.-ol, 9,10a-dimethyl-6-methylene-3.beta.-isopropyl-	$C_{20}H_{32}O_2$	304	3.32
27	31.311	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	324	0.61
28	31.912	cis-11-Eicosenamide	$C_{20}H_{39}NO$	309	0.37
29	32.151	Dihydroxanthin	$C_{17}H_{24}O_5$	308	1.32
30	34.759	Sakuranin	$C_{22}H_{24}O_{10}$	448	0.85
31	35.666	Squalene	$C_{30}H_{50}$	410	1.9
33	38.391	Quercetin	$C_{15}H_{10}O_7$	302	1.64
34	39.191	Pilloin	$C_{17}H_{14}O_6$	314	1.46
35	39.384	5,7-Dihydroxy, 3′,4′,5′-trimethoxyflavone	$C_{18}H_{16}O_7$	344	1.00
36	39.835	Retusin	$C_{19}H_{18}O_7$	358	0.86
37	40.601	7,3′-Dimethylquercetin	$C_{17}H_{14}O_7$	330	1.11
38	41.270	Stigmasterol	$C_{29}H_{48}O$	412	1.68
39	44.816	α-Amyrin	$C_{30}H_{50}O$	426	4.95

RT = Retention time, MW = Molecular weight.

M. erythrophylla and *M. philippica Dona Luz* x *M. flava,* phytochemical analysis of their ethanolic leaf extracts revealed the presence of flavonoids, terpenoids and tannins, which may be responsible for their antiplasmodial activity. These findings correspond with a previous study that reported that flavonoids, triterpenoids and iridoids are the common chemical constituents distributed in *Mussaenda* species [39].

The genus *Mussaenda* contains flowering plants and is one of the largest genera in the Rubiaceae family. *M. erythrophylla,* which has the most potent antiplasmodial effects, is a widely known plant due to its ornamental properties [40]. The leaves and stems of this plant have hepatoprotective activity, while the roots have been found to have anthelmintic and diuretic properties, are used for the treatment of cough and jaundice, and possess antitumor and antimicrobial activities [41, 42].

GC–MS analysis of ethanolic leaf extracts of *M. erythrophylla* and *M. philippica Dona Luz x M. flava* identified similar compounds. The major compounds of *M. erythrophylla* were squalene, oleic acid amide, β-sitosterol, quinic acid and phytol. The major compounds of *M. philippica Dona Luz x M. flava* were oleic acid amide, quinic acid, phytol, β-sitosterol and linolenic acid. Regarding squalene, a linear unsaturated triterpenoid isolated from many plant species, including the marine sponges *Spongia* sp. and *Ircinia* sp., has been reported to possess antioxidant and antitumor activities [43]. It has also been reported to exhibit antiplasmodial effects against *P. falciparum* [44]. β-Sitosterol was reported to show high activity against a chloroquine-sensitive (3D7) strain with an IC_{50} value of 5.51 μM [45]. For phytol, this compound was proven to possess anxiolytic, metabolism-modulating, cytotoxic, antioxidant, antinociceptive, anti-inflammatory, immune-modulating, antimicrobial and antiplasmodial activities [46, 47, 48]. Therefore, these compounds may be the active compounds that possess antiplasmodial activity in ethanolic leaf extracts of *M. erythrophylla* and *M. philippica Dona Luz x M. flava*.

Regarding *B. balsamifera*, a perennial herb or subshrub of the Asteraceae family whose ethanolic leaf extract exhibited high antiplasmodial activity (IC_{50} = 9.66 μg/ml), the results of our study are in accordance with a previous study in which a methanolic extract of the roots and stems of this plant exerted antiplasmodial effects against a CQ-sensitive *P. falciparum* (D10) strain with IC_{50} values of 26.25 and 7.75 μg/ml, respectively [49]. The *B. balsamifera* leaf extract contained high concentrations of terpenoids and moderate concentrations of flavonoids and tannins, which may be responsible for its antiplasmodial properties.

B. balsamifera has been reported to exhibit various biological activities, such as hepatoprotective, antioxidant, antitumor, anti-inflammatory, antityrosinase, antiobesity, antimicrobial, antiplasmodial, and wound healing activities [50]. The most abundant compounds in this extract were oleamide, α-amyrin, a pentacyclic triterpenoid and β-eudesmol, including flavonoids such as sakuranin, quercetin, pilloin, 5,7-dihydroxy,3′,4′,5′-trimethoxyflavone, retusin and 7,3′-dimethylquercetin. The antiplasmodial activity of *B. balsamifera* may be produced by the synergistic effect of these compounds.

Several plant species of the Asteraceae family have been reported as potential sources for antiplasmodial drugs [51, 52, 53]. The most famous plant is *A. annua* L., a Chinese herb (Quinghaosu). Its active compound is artemisinin, an endoperoxide sesquiterpene lactone that is a potent antimalarial agent used in regions where the parasite has developed resistance to other antimalarial agents [29, 54, 55]. Among plant secondary metabolites, alkaloids are potential candidates for antiplasmodial drug development, and various classes have been reported to possess promising activities [56]. Quinine, an example of an alkaloid, was the first antiplasmodial drug to be discovered and was isolated from the bark of *Cinchona succirubra* (Rubiaceae) [29]. In addition, other cinchona alkaloids, including quinidine, cinchonine and cinchonidine, are all effective against malaria [8]. Flavonoids have also been reported to exhibit promising antiplasmodial activities [57]. Some flavonoids have been shown to inhibit the influx of L-glutamine and myoinositol into *P. falciparum*-infected erythrocytes [58]. Terpenoids have been suggested to possibly interfere with polyisoprenoid biosynthesis in the parasite, which occurs during the asexual intraerythrocytic developmental cycle as well as during gametocytogenesis [59, 60].

5. Conclusion

Evaluation of the antiplasmodial activity of plants in the Asteraceae and Rubiaceae families provided varying results. The highest yield was

found for the aqueous flower extract of *H. annuus*. The ethanolic leaf extract of *M. erythrophylla,* leaf extract of *M. philippica Dona Luz* x *M. flava* and leaf extract of *B. balsamifera* possessed high antiplasmodial properties with minimal toxicity. Our findings indicated that these plants contain active antiplasmodial substances that are potential candidates for the development of new antiplasmodial agents. Further investigations using bioassay-guided isolation of the active compounds from these extracts are necessary for the development of novel antiplasmodial drugs.

Declarations

Author contribution statement

Prapaporn Chaniad; Chuchard Punsawad: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Arisara Phuwajaroanpong; Tachpon Techarang: Performed the experiments; Analyzed and interpreted the data.

Parnpen Viriyavejakul; Arnon Chukaew: Performed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

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