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Antioxidant, Antibacterial and Antiplasmodial Activities of Galactogogue Plant Extracts

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ABSTRACT

Galactogogue plants have been used traditionally worldwide to stimulate lactation. Research on antioxidant, antibacterial and antimalarial activities of the galactogogue plants are limited to a few of its species. Thus, this work aims to evaluate *in vitro* antioxidant (2,2-diphenyl-1-picrydydrazyl (DPPH) radical and 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation scavenging abilities, ferric reducing antioxidant power (FRAP), total phenolic content (TPC) and total flavonoid content (TFC)), and also antibacterial and antimalarial activities of sixteen galactogogue plants collected from northeastern Thailand. The antioxidant, antibacterial and antimalarial assays followed established procedures. Results indicated that the methanol and ethyl acetate extracts from the stem bark of *Caesalpinia sappan* (CS) and *Ochna integerrima* (OI) showed potent antioxidant capacity with the DPPH, ABTS and FRAP assays. These two particular plant extracts also possessed high TPC and moderate TFC. Both extracts of CS also exhibited good antibacterial activity, followed by extracts from OI which showed selective antibacterial activity toward three Gram-positive bacteria. The ethyl acetate extract from the stem bark of *Siphonodon celastrineus* (SCe), and the methanol and ethyl acetate extracts of *Micromelum minutum* (MM) also displayed strong antiplasmodial activity against *Plasmodium falciparum*. Our findings suggest that both CS and OI could be used as potential natural antioxidant and antibacterial (especially against Gram-positive bacteria) sources while MM and SCe could be promising alternative antimalarial plant for treating the *P. falciparum* parasites.

Keywords: Antioxidant activity, Antibacterial activity, Antimalarial activity, Galactogogue
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Introduction

Plants are important sources of bioactive compounds with a huge variety of different molecules and have long been used as natural medicine for centuries. The plant-derive compounds are commonly called "phytochemicals" or referred to "secondary metabolites". Although these compounds are not required for the growth and performance of basic life of functions, they show many of biological properties.[1,2] From searching of bioactive compounds from our local medicinal plants, we came across the galactogogue plants.

This type of plants has been traditionally used over the world to induce lactation.[3] In Thailand, especially in the Northeastern part, the galactogogue plants are still being used in traditional recipes for breastfeeding women to get sufficient breast milk.

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Plants are the best natural sources of antioxidant compounds. They are rich in antioxidant molecules with which they block the activity of free radicals which are linked to the pathogenesis of degenerative diseases like cancer, cardiovascular diseases, gout, diabetes, atherosclerosis, neurodegenerative, cataracts, Parkinson's diseases and ageing.[4] Therefore, antioxidants provide good health benefits. Phytochemicals including polyphenols are examples of natural antioxidant from plants that possess good antioxidant capacity.[5,6] In industry such as food, cosmetic and pharmacy, polyphenols are widely used nowadays.[7] Polyphenols have been used as natural food preservative based on their antibacterial activities.[8,9]

Malaria is an infectious disease caused by a parasite *Plasmodium falciparum*. It is the world's most important parasitic infectious disease and a big problem in the world in most region of the tropical and subtropical countries.[10,11] The infection of *P. falciparum* parasites, drug-resistant strain and virulent human parasite, cause most serious illnesses and deaths.[12] There are several evidences for artemisinin resistance and its spread but searching for new and effective drugs, which is important is also a challenge. Plant metabolites are good candidates since they are an important source of active compounds. Moreover, majority of antimalarial drugs are plant-derived natural molecules such as quinine or artemisinin.[13]

Although the galactogogue plants have been used and distributed widely in Northeastern Thailand, research studies of their biological properties are limited. Therefore, screening of the antioxidant,

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antibacterial (against foodborne pathogenic bacteria) and antiplasmodial (against *P. falciparum*) activities of this group of plants was studied. Due to the plant natural products are excellent sources of active molecules, study of unexplored activities of the plants are important to give us useful information for the utilization of our local resources.

Materials and Methods

Chemicals and instrument

All of these chemicals; 2,2-diphenyl-1-picrydydrazyl radical (DPPH$^{\bullet}$) (Sigma-Aldrich, Germany), 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma-Aldrich, China), ascorbic acid (UNILAB, Australia), potassium persulfate (KemAus, Australia), 2,4,6-tripyridyl-*S*-triazine (TPTZ), (Sisco Research Laboratories, India), iron (III) chloride hexahydrate solution (Global Chemie, India), iron (II) sulfate (Ajax FineChem Laboratory Chemicals, New Zealand), Folin-Ciocalteu (Carlo Erba Reagents, France), sodium carbonate (KemAus, Australia), gallic acid (Fluka, Spain), potassium acetate (KemAus, Australia), aluminium chloride (Global Chemie, India), quercetin (Sigma-Aldrich, China), Mueller Hinton Agar (MHA) (Difco, USA), Mueller Hinton Broth (MHB) (Difco, USA), dimethyl sulfoxide (DMSO) (Riedel-de Haën, Germany), tetracycline (Sigma, USA), artesunate (Sigma-Aldrich, St Louis, MO, USA), RPMI-1640 medium (HEPES, L-glutamine) (Gibco, Grand Island, NY, USA), Albumax I (Gibco, Grand Island, NY, USA), hypoxanthine (Sigma-Aldrich, St Louis, USA) and 3-(4,5-dimethylthiazole-2-yl)-2,5-diphyneltetrazolium bromide) (MTT) (Sigma-Aldrich, St Louis, MO, USA) were used during the experiments. The instrument used consists of rotary evaporator (Buchi, R120), UV-Vis spectrophotometer (Thermo Scientific GENESYS 20), EnVision Multi-Mode microplate reader (PerkinElmer, Waltham, MA, USA) and EZ Read 2000 microplate reader (Biochrom, MA, USA).

Plant material and preparation

Sixteen plant species from six families were selected according to their traditional use as galactogogue plants in Northeastern Thailand and assigned codes (Table 1). The plant samples were collected with permission in 2018 from arboretum and affiliate garden, Walai Rukhavej Botanical Research Institute (WRBRI), Mahasarakham University (MSU) by K. Wongpakam, except for *Salacia verrucosa* (SV) which was collected from Amnat Charoen province. The samples were identified by S. Sedlak with the use of dichotomous keys. Voucher specimens (Wongpakam 19-XX) are deposited at the WRBRI, MSU. Fresh samples were washed, cut and sun dried. All dried samples were kept in zipped plastic bag and stored at room temperature until use.

Plant extraction

Each plant sample (50 g) was extracted by refluxing in distilled methanol (500 mL, 24 h). After filtration, the collected methanol layer was concentrated. A portion of the methanol extract was taken for experiments while the rest was suspended in deionized water (100 mL) and further partitioned with hexane and ethyl acetate (150 mL) respectively. The hexane and ethyl acetate layers were taken to evaporate to obtain hexane and ethyl acetate extracts respectively.

Antioxidant activity assay

DPPH$^{\bullet}$ scavenging activity

The DPPH$^{\bullet}$ scavenging activity was determined by modifying the method described by Seephonkai *et al.*[14] Percentage of radical scavenging activity (%RSA) of each concentration was calculated based on the absorbance of sample and control solutions. Graph plotted between the calculated %RSA and the used concentrations led to the calculation of 50% inhibitory concentration (IC_{50} value; µg/mL) value of each extract.

$$\%\text{RSA} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} * 100$$

ABTS$^{\bullet+}$ scavenging activity

The ABTS$^{\bullet+}$ scavenging activity was tested by using the procedure reported by Re *et al.*[15] %RSA was calculated based on the absorbance of sample and control solutions and then converted to IC_{50} value in the same manner as described for the DPPH assay.

$$\%\text{RSA} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \text{ X } 100$$

Ferric reducing activity power (FRAP)

FRAP assay was done following the method described by Benzie and Strain[16] with slight modification. Fresh working solutions of reference iron (II) sulfate concentrations (0.360 – 7.194 µM) were used for calibration curve in our study. FRAP was reported as µM Fe (II)/g extract.

$$\text{Fe (II) content} = \left[\frac{X\ (\text{mg Fe (II)/L}) \text{ x Dilution factor x } V}{\text{Sample weight (mg)}}\right] * \text{MW of Fe (II) sulfate} * 1{,}000$$

X was calculated from linear equation of Fe (II) sulfate. *V* was a total volume of the extract. MW stands for molecular weight.

Total phenolic content (TPC)

TPC was determine using Folin-Ciocalteu modify method reported by Seephonkai *et al.*[14] Results of the TPC were reported as mg of gallic acid equivalent per g of dry extract (mg GAE/ g extract).

$$\text{TPC} = \frac{X\ (\text{mg GAE/L}) \text{ x Dilution factor x } V}{\text{Sample weight (mg)}}$$

X was calculated from linear equation of gallic acid. *V* was a total volume of the extract.

Total flavonoid content (TFC)

TFC was measured following the procedure described by Chang *et al.*[17] with slight modification. Results of the TFC were expressed as mg of quercetin equivalent per g of dry extract (mg QE/ g extract).

$$\text{TFC} = \frac{X\ (\text{mg QE/L}) \text{ x Dilution factor x } V}{\text{Sample weight (mg)}}$$

X was calculated from linear equation of quercetin. *V* was a total volume of the extract.

Antibacterial activity assay

Bacterial strains and cultivation

The antibacterial activity of the extracts was assayed against eight strains of bacteria (three Gram-positive bacteria, methicillin-susceptible *Staphylococcus aureus* (MSSA) DMST 2933, methicillin-resistant *S. aureus* (MRSA) DMST 20651 and *Bacillus cereus* ATCC 11778 and five Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Salmonella enterica* serovar Typhi DMST 22482, *Vibrio cholerae* O 1DMST9700 , *Shigella fleneri* DMST 4423 and *S. dysenteriae* DMST15110). The selected strain was cultured on Mueller Hinton Agar (MHA) medium at 37 °C for 16 – 18 h.

Agar well diffusion method

The agar well diffusion method was performed to determine antibacterial activity of the extracts.[18] The extract solutions (50 mg/mL) were added to each well (0.1 mL per well). The zone of inhibition was measured after 16-18 h incubation of the plates at 37 °C. The negative controls of 10% (v/v) methanol and 10% dimethyl sulfoxide (DMSO) were used. A standard positive control was tetracycline (250 µg/mL).

MIC and MBC of active extract

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were investigated using the microdilution method.[18] MICs were determined by identifying the solutions that were clear after incubation compared with the control

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well. MBCs were determined by transferring bacterial suspensions from the MIC assay onto MHA plates. The MBC referred to the lowest concentration that showed no growth after 24 h incubation. A reference standard antibiotic tetracycline (250 µg/mL) was included in the study.

Antiplasmodial activity assay
The Antiplasmodiall activity of the extracts as well as standard Antiplasmodiall drug, artesunate was carried out on chloroquine-resistant K1 strain of *P. falciparum* using a standardized fluorescent SYBR Green-based 96-microplate assay.[19,20] Parasites were cultured according to the procedure described by Trager and Jensen[21] with slight modification. The sample solution of 50 µL in DMSO was used. For Antiplasmodiall activity pre-screening, parasites were treated with 5 µg/mL extract solutions, 200 nM artesunate or with 0.1% DMSO alone (nonexposed, control) (37 ^{o}C, 72 h under a gas mixture of 5% CO_2, 5% O_2, 90% N_2). For dose-concentration response, parasites were exposed to 11-step 2-fold serial dilutions of the extract sample solutions ranged from 0.005 to 5 µg/mL. Parasite growth values were normalized to those for the DMSO solvent control. Values were plotted using a nonlinear regression analysis with a sigmoidal dose-response curve fit in GraphPad Prism (version 7.0e) software to generate drug curves and 50% effective concentration (EC_{50}) values.

Cell cytotoxicity assay
Vero cell toxicity was measured using colorimetric MTT test.[22] Percentage of cell viability compared to untreated controls was reported as the results.

Ethics approval
Human blood (Siriraj Hospital blood blank, Bangkok, Thailand) used in this study was performed under the human use protocol TMEC 18-004, approved by the Institute Ethical Review Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

Statistical analysis
The $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities, FRAP, TPC and TFC assays were tested in triplicates and mean ± standard deviation (SD) were reported. Data were analyzed for statistical significance ($p < 0.05$) by ANOVA post-hoc test (IBM SPSS statistics software, version 22, USA). Statistical significance was confirmed when the p values are less than 0.05.

Results and Discussion

Antioxidant activity
$DPPH^{\bullet}$ scavenging activity
The $DPPH^{\bullet}$ scavenging activity results are shown in Table 2. In the majority of cases, the ethyl acetate extracts of each plant showed significantly higher scavenging activity than their methanol extracts except for CS, SCh and OI. Both methanol and ethyl acetate extracts of CS exhibited similar scavenging capacity with the same potency level. Compared to the other extracts, their activity was also strongest. Only ascorbic acid showed similar ability (IC_{50} 5.39 ± 0.32 µg/mL). As results, the $DPPH^{\bullet}$ scavenging activity of methanol extracts was in the order of CS > OI > SCh while the activity of ethyl acetate extracts was CS > GP = MM = OI.

$ABTS^{\bullet+}$ scavenging activity
Results of the $ABTS^{\bullet+}$ scavenging activity are shown in Table 2. In most cases the $ABTS^{\bullet+}$ scavenging activities of the ethyl acetate extracts were significantly stronger than their methanol extracts by showing least number of the IC_{50} except for CS, SV and OI. This observation was similar to the DPPH results. Both of the methanol and ethyl acetate extracts of CS displayed the strongest scavenging properties in each group. These activities was slightly lower (~1.6 fold) than ascorbic acid (IC_{50} 18.78 ± 0.06 µg/mL). The $ABTS^{\bullet+}$ scavenging activity of the methanol extracts was CS > OI > SV while the activity of the ethyl acetate extracts was CS > CP = GP > CH.

Ferric reducing activity power (FRAP)
Fe (II) reducing activity power of the extracts is shown in Table 3. The high number of reduction in µM of Fe (II) express strong reductive power of Fe (III) to Fe (II). Similar results were observed for majority of the ethyl acetate extracts of each plant with most of them having stronger reducing activity power than those of their methanol extracts except for PD, SV and OI. The methanol and ethyl acetate extracts of CS showed the strongest reducing activity power in each group. For the group of the methanol extracts the activity was CS > OI > SV while the activity of the ethyl acetate extracts was CS > OI > CH = GP. The reducing activity of CS was less active (~1.3 fold) when compared to ascorbic acid (3,567.16 ± 1.87 µM Fe (II)/g extract).

Total phenolic content (TPC) and Total flavonoid content (TFC)
Results of TPC and TFC of all the extracts are shown in Table 4. In most cases, the TPC and TFC values of the ethyl acetate extracts of each plant were higher than their methanol extracts. The highest TPC were found in CS extracts for both of the methanol and ethyl acetate groups. The TPC values were found in the following order of CS > OI > SCh for the methanol and CS > OI > CP for the ethyl acetate extracts. The highest TFC were found in the methanol and ethyl acetate extracts from the stem bark of PD. The TFC of the methanol extracts decreased in the series of PD > CS > PE and PD > OI > PE for the ethyl acetate extracts. The results of the experiments indicate that there were strong and remarkable antioxidant properties from both the methanol and ethyl acetate extracts of the CS. These results are similar with results from previous studies of CS antioxidant activities.[23-24] Not only CS but also OI extracts showed strong antioxidant activity results in this work but research study on antioxidant property of OI is limited. These two plant extracts showing strong antioxidant activities based on three methods (DPPH, ABTS and FRAP assays) used were also possessed high TPC and moderate TFC. Therefore, the antioxidant ability of the extracts was correlated well with the TPC. Polyphenols are believed to be the main active compounds responsible to the antioxidant capacities of the plants.[25,26] Interestingly, the highest TFC in the methanol and acetyl acetate extract groups were the extracts from the stem bark of PD. There is no report yet of flavonoids having being isolated from the stem bark of this plant.

Antibacterial activity
The results from agar well diffusion studies showed that all the extracts exhibited greater antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. The MIC and MBC values of extracts were determined and results are shown in Table 5. Among the extracts, the methanol extract of CS exhibited strong antibacterial activity against all the tested pathogenic bacteria especially the Gram-positive bacteria *B. cereus* ATCC11778 and the Gram-negative *S. dysenteriae* DMST15110 . Other methanol extracts of HC, PD, PE, UR, MM, DE and OI also showed some antibacterial activities against the Gram-positive bacteria *S. aureus* (MSSA) DMST 2933 and *S. aureus* (MRSA) DMST 20651. The MIC and MBC values they gave were in the range of 1.56 – 12.50 mg/mL and 1.56 – 12.50 mg/mL. Based on the activity results of the methanol extracts, six of the ethyl acetate extracts from CS, OI, PD, PE, SCh and DE were taken to screen for their antibacterial activities. The results indicated that the antibacterial property of these ethyl acetate extracts decreased when compared to their methanol extracts. Among them, the extract of CS remained its activity against Gram-positive and Gram-negative pathogenic bacteria. The methanol and ethyl acetate extracts of CS and the methanol extracts of OI exhibited good activity toward Gram-positive pathogenic bacteria. For the Gram-negative, the interaction of the extracts to cytoplasmic membrane may cause membrane permeability and membrane leakage.[27] Gram-positive, the extracts may also involves disruption of peptidoglycan biosynthesis of bacterial cell wall.[28] The antibacterial property of CS[24,29,30] has been studied extensively due to its traditional use of diarrhea and dysentery treatments[31] while the antibacterial activity of OI has never been documented. Phenolic compounds and flavonoids are reported to be a major group of constituents of OI.[32-34] This antibacterial action may be attributed extracts that could inhibit bacterial cells.

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The antibacterial activity of flavonoids in both direct and synergistic effects is reported.[35]

Antiplasmodial activity

For primary screening, all of the ethyl acetate extract solution at a concentration 5 µg/mL exhibited stronger antiplasmodial activity than their methanol extracts. From the results, a methanol extract of MM and the ethyl acetate extracts of SV, SCe, CH, MM and DE were selected from all of the tested samples. These extracts inhibited growth of *P. falciparum* parasites by ≥ 75% (Figure 1A). Artesunate at 200 nM showing 100% parasite growth inhibition was used as a positive control in this study (EC_{50}: 2.63 ± 1.64 nM). The six active extracts as mentioned above were used for the determination of accurate 50% effective concentration (EC_{50}) values against *P. falciparum* parasites, while other extracts demonstrating low activity were de-prioritized and not taken further for EC_{50} testing. Promising antiplasmodial activity was observed in both methanol and ethyl acetate extracts of MM and an ethyl acetate extract of SCe (Figure 1B and Table 6). The remaining three active extracts based on primary screening appeared to be less active (EC_{50} > 5 µg/mL) (Table 6). None of the active extracts showed cytotoxic activity against Vero (an African green monkey kidney cell line) cells at the maximum test concentration (5 µg/mL) used. The methanol and ethyl acetate extracts from the stem bark of MM and ethyl acetate extract from the stem bark of SCe showed strong antiplasmodial activity. The antiplasmodial activity of these two plants has never been reported. Sesquiterpenes, sesquiterpene alkaloids, triterpenes and sterols have been isolated from the fruit, root bark and stem of SCe.[36-39] Coumarins and triterpenes have been reported in the extracts of the leaf and bark of MM.[40,41] Since the antiplasmodial property and active compounds of SCe and MM plants have not previously been investigated and extensive study is required for the separation, purification and characterization of its active compounds, so that in-deep studies for the promising antiplasmodiall molecules from the stem bark of MM and SCe could be conducted.

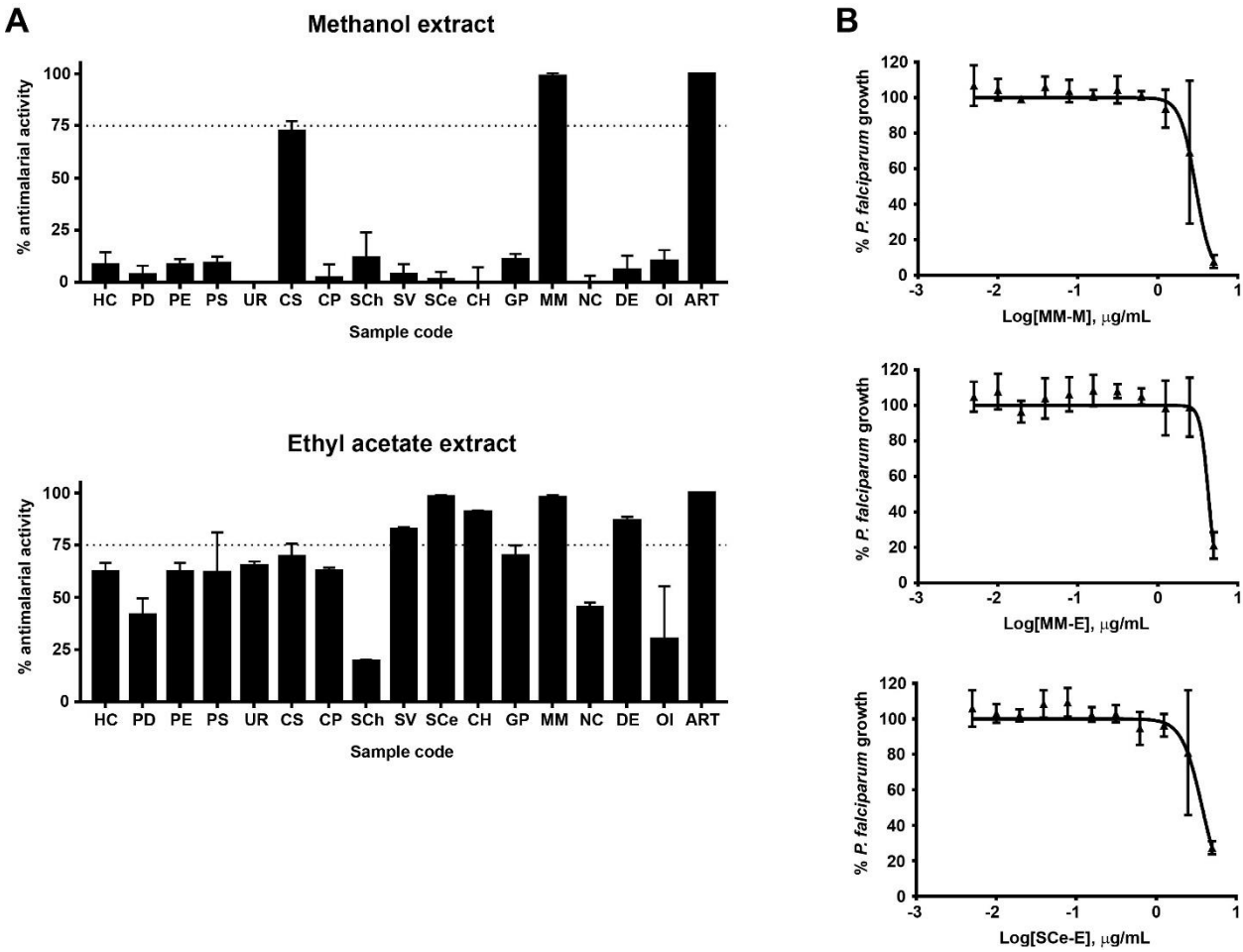

Figure 1: Antiplasmodiall activity against *P. falciparum* K1 parasites of the methanol and ethyl acetate extracts in the screening experiment **(A)** and determination of EC_{50} value of the active extracts **(B).**

A standardized fluorescent SYBR Green-based 96 microplate assay was used to examine Antiplasmodiall activity against *P. falciparum* K1 (chloroquine-resistant strain). (A) Plant extracts were tested at 5 µg/mL. Artesunate (ART), a known Antiplasmodiall drug control, was tested at 200 nM. Antiplasmodiall activity of the extracts was measured from 3 independent experiments and each carried out in duplicate wells. (B) Dose response curves of three active plant extracts plotted by GraphPad Prism Software. Each point represents the mean ± SD from 3 independent experiments.

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Table 1: Information of sixteen galactogogue plant materials.

Plant species (Code)	Local Thai name (in English)	Family	Used part	Collecting date	Voucher no.
Hubera cerasoides (Roxb.) Chaowasku (HC)	กะเจียน (Ka chian)	Annonaceae	Stem bark	15 Jul, 2018	Wongpakam 19-08
Polyalthia debilis (Pierre) Finet & Gagnep. (PD)	กล้วยเต่า (Kluai Tao)	Annonaceae	Stem bark	27 Jul, 2018	Wongpakam 19-09
Polyalthia evecta (Pierre) Finet & Gagnep. var. *evecta* (PE)	นมน้อย (Nom Noi)	Annonaceae	Stem bark and root	27 Jul, 2018	Wongpakam 19-11
Polyalthia suberosa[a] (Roxb.) Thwaites (PS)	กลึงกล่อม (Klueng Klom)	Annonaceae	Stem bark	6 Sep, 2018	Wongpakam 19-14
Uvaria rufa Blume (UR)	นมควาย (Nom Khwai)	Annonaceae	Stem bark	19 Jul, 2018	Wongpakam 19-07
Caesalpinia sappan[b] L. (CS)	ฝาง (Fang)	Fabaceae	Stem bark	29 May, 2018	Wongpakam 19-01
Celastrus paniculatus Willd. (CP)	กระทงลาย (Kra Thong Lai)	Celastraceae	Stem bark	19 Jul, 2018	Wongpakam 19-10
Salacia chinensis L. (SCh)	กำแพงเจ็ดชั้น (Kam phaeng Chet Chan)	Celastraceae	Stem bark	16 Jun, 2018	Wongpakam 19-04
Salacia verrucosa Wight (SV)	กำแพงเก้าชั้น (Kam phaeng Kao Chan)	Celastraceae	Stem bark	20 Jun, 2018	Wongpakam19-03
Siphonodon celastrineus Griff. (SCe)	มะดูก (Ma Duk)	Celastraceae	Stem bark	16 Jun, 2018	Wongpakam 19-13
Clausena harmandiana[a] (Pierre) Pierre ex Guillaumin (CH)	ส่องฟ้าดง (Song Fa Dong)	Rutaceae	Root	6 Sep, 2018	Wongpakam 19-15
Glycosmis pentaphylla[a] (Retz.) DC. (GP)	เขยตาย (Khoei Tai)	Rutaceae	Stem bark	2 Aug, 2018	Wongpakam 19-05
Micromelum minutum (Forst.f.) Wight & Arn. (MM)	หัสคุณ (Hatsa Khun)	Rutaceae	Stem bark	27 Jul, 2018	Wongpakam 19-16
Naringi crenulata (Roxb.) Nicolson (NC)	ตุมตัง (Tum Tang)	Rutaceae	Stem bark	15 Jul, 2018	Wongpakam 19-12
Diospyros ehretioides Wall. ex G.Don (DE)	ลินกวาง (Lin Kwang)	Ebenaceae	Stem bark	29 May, 2018	Wongpakam 19-06
Ochna integerrima (Lour.) Merr. (OI)	ช้างน้าว (Chang Nao)	Ochnaceae	Stem bark	29 May, 2018	Wongpakam 19-02

[a] Samples were received as dried plant materials from Thai folk healers in Maha Sarakham province. [b] *Caesalpinia sappan* L. was recently renamed to *Biancaea sappan* (L.) Tod. based on taxonomic revision and International Plant Names Index (IPNI) produced by the Royal Botanic Gardens, Ke

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Table 2: $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging activities of the methanol and ethyl acetate extracts

Plant species (Code)	$DPPH^{\bullet}$ (IC_{50}; µg/mL)		$ABTS^{\bullet+}$ (IC_{50}; µg/mL)	
	Methanol extract	**Ethyl acetate extract**	**Methanol extract**	**Ethyl acetate extract**
Hubera cerasoides (HC)	$45.81 \pm 0.15^{d,G}$	$25.67 \pm 0.23^{e,E}$	$323.16 \pm 0.70^{k,U}$	$70.33 \pm 0.99^{d,F}$
Polyalthia debilis (PD)	$103.10 \pm 1.10^{j,P}$	$55.83 \pm 0.82^{h,H}$	$226.76 \pm 5.58^{f,P}$	$191.83 \pm 4.97^{m,O}$
Polyalthia evacta (PE)	$61.80 \pm 0.29^{f,J}$	$59.54 \pm 0.58^{i,I}$	$353.42 \pm 1.68^{m,W}$	$162.31 \pm 3.00^{k,M}$
Polyalthia suberosa (PS)	$110.45 \pm 0.98^{k,Q}$	$61.79 \pm 0.15^{j,J}$	$228.87 \pm 1.17^{f,P}$	$76.48 \pm 0.36^{e,G}$
Uvaria rufa (UR)	$94.73 \pm 1.55^{i,O}$	$61.63 \pm 0.29^{j,J}$	$275.63 \pm 2.38^{h,R}$	$114.57 \pm 1.84^{i,K}$
Caesalpinia sappan (CS)	$6.70 \pm 0.02^{a,A}$	$5.62 \pm 0.04^{a,A}$	$30.10 \pm 0.29^{a,A}$	$28.61 \pm 0.04^{a,A}$
Celastrus paniculatus (CP)	$61.06 \pm 0.77^{f,J}$	$25.66 \pm 0.29^{e,E}$	$162.38 \pm 4.28^{d,M}$	$50.09 \pm 1.00^{b,C}$
Salacia chinensis (SCh)	$19.23 \pm 0.11^{c,C}$	$24.61 \pm 0.32^{d,E}$	$171.39 \pm 1.75^{e,N}$	$68.87 \pm 2.17^{d,F}$
Salacia verrucosa (SV)	$56.74 \pm 0.15^{e,H}$	$45.98 \pm 0.99^{g,G}$	$53.58 \pm 0.53^{c,D}$	$90.82 \pm 1.04^{g,I}$
Siphonodon celastrineus (SCe)	$193.71 \pm 0.43^{n,T}$	$67.65 \pm 0.74^{k,K}$	$443.42 \pm 3.43^{n,X}$	$80.29 \pm 0.44^{f,H}$
Clausena harmandiana (CH)	$203.84 \pm 1.31^{o,U}$	$39.76 \pm 0.24^{f,F}$	$259.91 \pm 1.78^{g,Q}$	$61.12 \pm 0.24^{c,E}$
Glycosmis pentaphylla (GP)	$75.71 \pm 0.16^{g,M}$	$21.49 \pm 0.14^{b,D}$	$173.44 \pm 0.80^{e,N}$	$50.00 \pm 0.55^{b,C}$
Micromelum minutum (MM)	$83.70 \pm 0.05^{h,N}$	$22.11 \pm 0.17^{b,c,D}$	$338.03 \pm 2.34^{l,V}$	$173.69 \pm 0.52^{l,N}$
Naringi crenulata (NC)	$112.38 \pm 0.36^{l,R}$	$40.13 \pm 0.68^{f,F}$	$305.73 \pm 3.96^{i,S}$	$103.78 \pm 1.60^{h,J}$
Diospyros ehretioides (DE)	$120.89 \pm 3.43^{m,S}$	$71.36 \pm 0.86^{l,L}$	$318.53 \pm 2.20^{j,T}$	$121.26 \pm 1.07^{j,L}$
Ochna integerrima (OI)	$16.86 \pm 0.27^{b,B}$	$22.47 \pm 0.46^{c,D}$	$46.35 \pm 0.18^{b,B}$	$173.69 \pm 0.52^{l,N}$

The different superscript letters in lower and upper cases indicate statistically significant difference within the same column and between two columns of each test respectively (ANOVA, post-hoc; $p < 0.05$). The IC_{50} of ascorbic acid was 5.39 ± 0.32 and 18.78 ± 0.06 µg/mL

Table 3: Ferric reducing antioxidant power (FRAP) of the methanol and ethyl acetate extracts

Plant species (Code)	FRAP assay (µM Fe (II)/g extract)	
	Methanol extract	**Ethyl acetate extract**
Hubera cerasoides (HC)	$280.98 \pm 0.71^{f,P}$	$566.46 \pm 6.47^{d,G}$
Polyalthia debilis (PD)	$363.36 \pm 2.45^{e,M}$	$279.76 \pm 7.77^{l,P}$
Polyalthia evacta (PE)	$151.30 \pm 4.94^{i,j,T}$	$283.84 \pm 3.24^{l,P}$
Polyalthia suberosa (PS)	$150.08 \pm 2.55^{i,\ j,\ T}$	$424.54 \pm 7.64^{g,J}$
Uvaria rufa (UR)	$154.56 \pm 1.87^{i,T}$	$342.97 \pm 2.55^{j,N}$
Caesalpinia sappan (CS)	$2{,}695.66 \pm 3.08^{a,B}$	$2{,}812.70 \pm 1.22^{a,A}$
Celastrus paniculatus (CP)	$390.28 \pm 4.41^{d,L}$	$539.13 \pm 4.30^{e,H}$
Salacia chinensis (SCh)	$366.63 \pm 2.55^{e,M}$	$456.34 \pm 4.41^{f,I}$
Salacia verrucosa (SV)	$714.90 \pm 1.87^{c,E}$	$303.41 \pm 2.12^{k,O}$
Siphonodon celastrineus (SCe)	$70.55 \pm 1.87^{l,V}$	$408.22 \pm 3.08^{h,K}$
Clausena harmandiana (CH)	$97.47 \pm 1.87^{k,U}$	$627.63 \pm 7.44^{c,F}$
Glycosmis pentaphylla (GP)	$215.33 \pm 2.45^{g,R}$	$632.52 \pm 3.24^{c,F}$
Micromelum minutum (MM)	$195.75 \pm 2.45^{h,S}$	$409.85 \pm 3.24^{h,K}$
Naringi crenulata (NC)	$148.44 \pm 1.41^{j,T}$	$258.96 \pm 5.52^{m,Q}$
Diospyros ehretioides (DE)	$191.27 \pm 6.04^{h,S}$	$369.89 \pm 7.77^{i,M}$
Ochna integerrima (OI)	$1{,}387.79 \pm 6.74^{b,C}$	$950.21 \pm 4.94^{b,D}$

The different superscript letters in lower and upper cases indicate statistically significant difference within the same column and between two columns respectively (ANOVA, post-hoc; $p < 0.05$). The Fe^{3+} reducing power capacity of ascorbic acid was $3{,}567.16 \pm 1.87$ µM Fe (II)/g extract (Fe^{2+} standard $r^2 = 0.9982$).

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Table 4: TPC and TFC of the methanol and ethyl acetate extracts

Plant species (Code)	TPC (mg GAE/g extract)		TFC (mg QE/g extract)	
	Methanol extract	Ethyl acetate extract	Methanol Extract	Ethyl acetate extract
Hubera cerasoides (HC)	$106.79 \pm 0.00^{i,S}$	$231.32 \pm 2.52^{e,H}$	$102.77 \pm 2.18^{k,S}$	$122.89 \pm 4.36^{j,N}$
Polyalthia debilis (PD)	$95.47 \pm 2.18^{k,U}$	$126.08 \pm 0.73^{m,P,Q}$	$207.17 \pm 0.00^{a,B}$	$280.13 \pm 4.36^{a,A}$
Polyalthia evacta (PE)	$146.62 \pm 2.62^{e,N}$	$145.37 \pm 3.17^{k,N}$	$168.18 \pm 2.18^{c,F}$	$192.08 \pm 3.77^{c,D}$
Polyalthia suberosa (PS)	$51.45 \pm 2.18^{o,X}$	$179.33 \pm 5.67^{h,K}$	$153.08 \pm 2.18^{d,H}$	$134.21 \pm 2.18^{g,h,K,L}$
Uvaria rufa (UR)	$101.76 \pm 0.00^{j,T}$	$175.97 \pm 1.26^{h,K}$	$116.60 \pm 3.77^{i,j,P,Q,R}$	$168.18 \pm 2.18^{e,F}$
Caesalpinia sappan (CS)	$840.96 \pm 1.92^{a,B}$	$979.33 \pm 6.93^{a,A}$	$193.33 \pm 2.18^{b,D}$	$179.50 \pm 2.18^{d,E}$
Celastrus paniculatus (CP)	$112.66 \pm 0.73^{h,R}$	$270.73 \pm 0.73^{c,F}$	$119.12 \pm 2.18^{i,N,O,P}$	$21.64 \pm 2.18^{j,N,O}$
Salacia chinensis (SCh)	$290.02 \pm 1.45^{c,E}$	$204.91 \pm 2.18^{g,J}$	$131.70 \pm 3.77^{g,h,L,M}$	$161.89 \pm 0.00^{f,G}$
Salacia verrucosa (SV)	$126.92 \pm 0.00^{g,P}$	$122.73 \pm 0.73^{m,Q}$	$154.34 \pm 0.00^{d,H}$	$129.18 \pm 2.18^{i,M}$
Siphonodon celastrineus (SCe)	$13.71 \pm 0.00^{p,M}$	$165.49 \pm 0.73^{i,L}$	$114.09 \pm 2.18^{j,Q,R}$	$131.70 \pm 3.77^{h,i,L,M}$
Clausena harmandiana (CH)	$91.70 \pm 2.18^{l,U}$	$214.97 \pm 1.26^{f,I}$	$117.86 \pm 2.18^{i,O,P,Q}$	$182.01 \pm 2.18^{d,E}$
Glycosmis pentaphylla (GP)	$139.50 \pm 3.33^{f,O}$	$242.22 \pm 1.92^{d,G}$	$135.47 \pm 3.77^{f,g,J,K,L}$	$165.66 \pm 0.00^{e,f,F,G}$
Micromelum minutum (MM)	$159.20 \pm 1.92^{d,M}$	$136.56 \pm 0.73^{l,O}$	$148.05 \pm 2.18^{e,I}$	$178.24 \pm 2.18^{d,E}$
Naringi crenulata (NC)	$71.15 \pm 0.73^{m,V}$	$137.40 \pm 4.76^{l,O}$	$112.83 \pm 0.00^{j,R}$	$132.96 \pm 2.18^{g,h,i,K,L,M}$
Diospyros ehretioides (DE)	$61.51 \pm 1.26^{n,W}$	$160.04 \pm 0.73^{j,M}$	$129.18 \pm 2.18^{h,M}$	$136.73 \pm 2.18^{g,J,K}$
Ochna integerrima (OI)	$463.61 \pm 0.73^{b,D}$	$496.73 \pm 4.54^{b,C}$	$139.25 \pm 0.00^{f,J}$	$198.36 \pm 2.18^{b,C}$

The different superscript letters in lower and upper cases indicate statistically significant difference within the same column and between two columns of each test respectively (ANOVA, post-hoc; $p < 0.05$). The TPC and TFC of the extracts were calculated based on gallic acid ($r^2 = 0.9989$) and quercetin ($r^2 = 0.9985$) standard linear regressions.

Table 5: MIC/MBC values of the antimicrobial activity of the methanol and ethyl acetate extracts

Sample code	MIC/MBC value (mg/mL)						
	Gram-Positive		Gram-Negative				
	S. aureus	*B. cereus*	*S. typhi*	*V. cholera*	*E. coli*	*S. flexneri*	*S. dysenteriae*
	MSRA20651	ATCC11778	DMST24822	DMST9700	ATCC25922	DMST4423	DMST15110
Methanol extract							
HC	3.12/3.12	12.50/12.50	-	-	-	-	-
PD	1.56/1.56	> 50/> 50	-	3.12/3.12	-	-	-
PE	1.56/1.56	> 50/> 50	-	6.25/3.12	-	-	-
PS	-	> 50/> 50	-	-	-	-	-
UR	1.56/1.56	> 50/> 50	-	3.12/1.56	-	-	-
CS	1.56/3.12	0.78/0.78	3.12/6.25	3.12/3.12	1.56/3.12	1.56/1.56	0.78/0.78
CP	6.25/6.25	-	-	-	-	-	-
SCh	1.56/3.12	-	-	-	-	-	-
SV	-	-	-	-	-	-	-
SCe	12.500/12.50	-	-	-	-	-	-
CH	-	> 50/> 50	-	-	-	-	-
GP	-	-	-	-	-	-	-
MM	12.50/12.50	> 50/> 50	-	-	-	-	-
NC	6.25/6.25	-	-	-	-	-	-
DE	1.56/1.56	-	-	-	-	-	-
OI	3.12/3.12	3.12/12.50	-	>25.00/>25.00	-	-	-
Ethyl acetate extract							

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PD	6.25/25.0	6.25/6.25	-	25.0/25.0	-	-	-
PE	6.25/50.0	12.50/12.50	-	12.50/12.50	-	-	-
CS	1.56/6.25	3.125/3.12	6.25/6.25	6.25/6.25	6.25/6.25	3.125/3.12	1.56/1.56
SCh	25.0/25.0	50.0/50.0	-	-	-	-	-
DE	6.25/50.0	12.50/12.50	-	12.50/12.50	-	-	-
OI	6.25/25.0	6.25/12.50	-	-	25.0/25.0	-	-

Table 6: Antiplasmodial activity of some active plant extracts against *P. falciparum*

Plant species (Code)	Sample	Antiplasmodial activity (EC_{50}; µg/mL)
Salacia verrucosa (SV)	Ethyl acetate extract	> 5
Siphonodon celastrineus (SCe)	Ethyl acetate extract	3.85 ± 1.25
Clausena harmandiana (CH)	Ethyl acetate extract	> 5
Micromelum minutum (MM)	Methanol extract	3.20 ± 1.33
Micromelum minutum (MM)	Ethyl acetate extract	4.14 ± 0.64
Diospyros ehretioides (DE)	Ethyl acetate extract	> 5

Any extracts having EC_{50} at < 1.56 µg/mL are considered strongly active and samples with EC_{50} at < 12.5 and > 1.56 µg/mL are considered active while extract showing EC_{50} at > 12.5 µg/mL is considered inactive (WHO, TDR guidelines).

Conclusion

The antioxidant, antibacterial and antiplasmodial activities of the sixteen galactogogue plants collected from Northeastern Thailand were investigated. Among them, the extracts of CS and OI presented potent antioxidant activity and exhibited antibacterial property while the ethyl acetate extract of SCe and methanol and ethyl acetate extracts of MM showed strong antiplasmodial activity against *P. falciparum*

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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