Anti-malarial activity of traditional Kampo medicine *Coptis* rhizome extract and its major active compounds

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Abstract

**Background:** Herbal medicine has been a rich source of new drugs exemplified by quinine and artemisinin. In this study, a variety of Japanese traditional herbal medicine ('Kampo') were examined for their potential anti-malarial activities.

**Methods:** A comprehensive screening methods were designed to identify novel anti-malarial drugs from a library of Kampo herbal extracts (n = 120) and related compounds (n = 96). The anti-malarial activity was initially evaluated in vitro against chloroquine/mefloquine-sensitive (3D7) and-resistant (Dd2) strains of *Plasmodium falciparum*. The cytotoxicity was also evaluated using primary adult mouse brain cells. After being selected through the first in vitro assay, positive extracts and compounds were examined for possible in vivo anti-malarial activity.

**Results:** Out of 120 herbal extracts, *Coptis* rhizome showed the highest anti-malarial activity (IC₅₀ 1.9 µg/mL of 3D7 and 4.85 µg/mL of Dd2) with a high selectivity index (SI) > 263 (3D7) and > 103 (Dd2). Three major chlorinated compounds (coptisine, berberine, and palmatine) related to *Coptis* rhizome also showed anti-malarial activities with IC₅₀ 1.1, 2.6, and 6.0 µM (against 3D7) and 3.1, 6.3, and 11.8 µM (against Dd2), respectively. Among them, coptisine chloride exhibited the highest anti-malarial activity (IC₅₀ 1.1 µM against 3D7 and 3.1 µM against Dd2) with SI of 37.8 and 13.2, respectively. Finally, the herbal extract of *Coptis* rhizome and its major active compound coptisine chloride exhibited significant anti-malarial activity in mice infected with *Plasmodium yoelii* 17X strain with respect to its activity on parasite suppression consistently from day 3 to day 7 post-challenge. The effect ranged from 50.38 to 72.13% (P < 0.05) for *Coptis* rhizome and from 81 to 89% (P < 0.01) for coptisine chloride.

**Conclusion:** *Coptis* rhizome and its major active compound coptisine chloride showed promising anti-malarial activity against chloroquine-sensitive (3D7) and -resistant (Dd2) strains in vitro as well as in vivo mouse malaria model. Thus, Kampo herbal medicine is a potential natural resource for novel anti-malarial agents.

**Keywords:** Herbal medicine, Kampo, Antimalarial

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**Background**

Malaria is still considered as a critical health problem in some areas of the world including tropical and subtropical parts. In 2018, 228 million cases of malaria resulted in 405,000 death, of which 93% of the cases and 94% of deaths were in the World Health Organization (WHO)
African region [1]. Although lots of efforts have been done, no effective vaccine is available to combat malaria, therefore, chemotherapy and vector control is still the main strategy to counter the parasite [2–5]. Successful malaria control can be achieved through the treatment with efficient anti-malarial drugs, such as quinine and chloroquine (CQ) [6, 7]. However, the inappropriate use of CQ led to the emergence and spread of CQ-resistant *Plasmodium falciparum* parasites which resulted in reducing CQ’s usage for the prophylaxis and treatment for malaria in the late 1970s [8–10]. As a result, artemisinin-based combination therapy (ACT) is highly recommended as a first-line therapy instead of CQ in treating uncomplicated falciparum malaria. However, *P. falciparum* has been recently reported to be resistant to artemisinin in Greater Mekong Sub-region [11–14].

Traditional medicine has been known for centuries and has been used to treat the myriads of ailments [15]. Numerous traditional medicines were derived from the plant-based herbal medicine, namely aspirin from willow bark [16], digoxin from foxglove [17], and morphine from the opium poppy [18]. Interestingly, it persists as a crucial source of drug discovery [15]. Furthermore, the use of herbal medicine for isolation of the natural product from herbal medicine has received increasing attention. It also represented a potential source of the conventional anti-malarial drug [19, 20], such as quinine which was isolated from *Cinchona* bark [21–23] and the use of *Artemisia annua* for isolation of artemisinin [24]. In Africa, herbal medicines are one of the most common traditional medicines and nearly 80% has been utilized as primary healthcare. Thus, safe and effective herbal medicine should be provided to expand the access to healthcare service as one-third of the population lack access to essential medicine [25]. Recently, the use of herbal medicine attracts the scientist due to the minimal side effect, lack of modern curative therapy for several chronic diseases, the emergence of microbial resistance, and the needed huge investment for modern drug development. On top of that, the pharmaceutical industries have changed their approach in favor of current drug development [26].

Kampo is a Japanese traditional therapeutic system that originated from Chinese traditional medicine [27, 28]. In Japan, Kampo together with modern medicine are usually used in chronic diseases mainly [28]. Not only herbal medicine but also massage, moxibustion, acupuncture, and acupressure are included [29, 30]. Each formula among 148 Kampo formulas covered by the Japanese Health Insurance systems has a specific clinical indication for a specific disease and/or symptoms [31]. Moreover, Kampo medicine has been prescribed by over 80% of the physician in Japan and integrated into modern medicine due to high safety and quality [32].

It has been also well-tolerated to human use for thousands of years [33]. Therefore, in this study, 120 Kampo herbal extracts and 96 related compounds were screened for their anti-malarial activity in vitro by using chloroquine/mefloquine-sensitive (3D7) and -resistant (Dd2) strains of *P. falciparum*. After confirmation of *Coptis* rhizome extract, its major compound coptisine chloride, and related formulas having strong activity in vitro, those were further evaluated for their in vivo anti-malarial activity using *Plasmodium yoelii* strain 17X mouse malaria model. To check the presence of *Coptis* rhizome derived compounds in plasma, blood was collected from mice after oral administration of *Coptis* rhizome and Orenedokuto, and analysed by liquid chromatography–mass spectrometry (LC–MS).

**Methods**

**In vitro culture of Plasmodium falciparum**

*Plasmodium falciparum* CQ/mefloquine (MQ)-sensitive (3D7) and -resistant (strain Dd2) strains were originally obtained from Dr. Louis Miller, NIH, USA. The parasites were maintained with 2% haematocrit type O+ red blood cells (RBCs) in RPMI-1640-based complete medium (CM) supplemented with 5% AB+ human serum (prepared from plasma) and 15% AlbuMax I (Gibco, Waltham, MA), 12.5 µg/mL gentamycin, and 200 mM hypoxanthine at 37 °C under mixed gas (5% CO2, 5% O2, and 90% N2) condition basically as described [34]. Japanese Red Cross Society was responsible for supplying RBCs and human plasma (number: 28J0060).

**Isolation and culture of primary adult mouse brain cells**

Primary adult mouse brain (AMB) cells were isolated and established in NEKKEN Bio-Resource Center, Institute of Tropical Medicine, Nagasaki University as described [35]. The primary cells, which were passaged several times to be adapted to in vitro condition, were maintained in RPMI-1640 media supplemented with 10% fetal bovine serum, penicillin/streptomycin solution (100 units/mL penicillin G, 100 mg/mL streptomycin sulfate) (Wako Pure Chemicals Industrial Ltd, Osaka, Japan) and incubated at 37 °C under 5% CO2. The primary cells for cytotoxicity assay were used after three passages.

**Kampo crude drug extracts, compounds, and formula (extracted from Coptis japonica)**

A Kampo library containing 120 herbal extracts (10 mg/mL), 96 Kampo-related active compounds including three compounds (coptisine chloride, berberine chloride, and palmatine chloride), and powder of experimental Orenedokuto (a Kampo formula containing an
aqueous extraction of four medicinal plants, including *Coptis* rhizome, *Phellodendron* bark, *Scutellaria* root, and *Gardenia* fruit, which were blended in the ratio of 3:2:2:2, respectively) were provided by the Institute of Natural Medicine (WAKANKEN), at the University of Toyama as described [36] and stored at −80 °C. All the herbal extracts were dissolved in ultra-pure water (UPW) generated by Milli-Q (Merck KGaA, Darmstadt, Germany). Compounds were preserved at a concentration of 2 mM dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemicals Industrial Ltd) solution, the most common solvent for chemicals. For in vivo assays, powder of Orengedokuto and *Coptis* rhizome, as well as chlo-roquine, were dissolved in distilled water (DW) for oral administration.

In vitro anti-malarial assay (first screening)
It was done by seeding the *P. falciparum* cultures (0.75% parasitaemia and 2% haematocrit) on 96-well clear flat-bottom plates (Thermo Fisher Scientific, Rochester, NY) and exposed it to Kampo herbal extracts (final concentration of 500 µg/mL). The final UPW solution was less than or equal to 5% of the culture volume, which had no inhibitory effect on parasite growth. CQ (Sigma-Aldrich, St. Louise, MO) and artemisin (Shin Poong Pharm Co, Seoul, South Korea) were used as positive controls (10 µM–0.508 nM), while 5% UPW was used as negative control. The culture plates were kept at 37 °C under mixed gas (90% nitrogen, 5% oxygen and 5% CO2) condition for 48 h. Each in vitro experiment was performed in duplicated wells and repeated twice. The inhibition was obtained by dividing the parasitaemia of test samples by the average of the negative controls.

Cytotoxicity assay
Cytotoxicity was initially screened at 500 µg/mL for herbal extracts and 20 µM for compounds. AMB cells (1 × 10⁴ cell) were seeded in a 96-well plate (black plate with clear bottom) and incubated at 37 °C in a CO₂ incubator for 48 h. Then, herbal extracts, compounds, or their negative controls were added, and the cells were further incubated for 48 h. To examine the cell viability (%), 10 µL of Alamar Blue solution (10%, Funakoshi Co., Tokyo, Japan) were added into each well and the cells were incubated for 2 h. Then the fluorescence intensity of each well was measured using a multilabel plate reader at 590 nm for 0.1 s per exposure. The concentration of drug required to reduce cell viability by 50% (CC<sub>50</sub>):

\[
10^{(\log(A/B) \times (50-C)/(D-C) + \log(B))}
\]

where A represented the lowest concentration value at which the percentage viable cell showed greater than 50%, B was the highest concentration value at which the percentage viable cell showed less than 50%, C was the percentage viable cell value of the sample at concentration B, and D was the percentage viable cell value of the sample at concentration A) was determined for samples that showed less than 50% viability in the initial screening. The assays of duplicated well were performed twice independently. IC<sub>50</sub> and CC<sub>50</sub> values were used as an indicator of in vitro anti-malarial activity and an indicator of cytotoxicity in AMB cells. The curve was plotted using GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA). Selectivity index (SI) was calculated by dividing CC<sub>50</sub> value by IC<sub>50</sub> value.

Assessment of anti-malarial activity in mouse model
A Kampo herbal extract (*Coptis* rhizome) and formula (Orengedokuto) exhibited in vitro anti-malarial activity were tested for in vivo activity against *P. yoelii* strain 17X in a mouse model. Female of 6–7 weeks C57BL/6 N mice (SLC Japan), weighing 18–20 g, were
used. The mice were kept in a clean room under conventional conditions then were acclimatized for 1 week before the experiments.

The *P. yoelii* 17X strain was provided by Dr. Tetsuo Yanagi, of National Bio-Resource Center (NBRC), NEKEN, Nagasaki University, Nagasaki, Japan. and maintained by successive serial passage in mice of study. The parasite was maintained frozen at −80 °C. For each individual assay, an aliquot was injected intraperitoneally (IP) in a mice, and infected donor mice were produced after three in vivo passage. A female C57BL/6 N mouse previously infected with *P. yoelii* and having parasitaemia levels of 20 to 30% were used as a parasite donor. At day 0, mice were injected IP with 0.2 mL of infected blood suspension containing $1 \times 10^4$ *P. yoelii* parasitized red blood cells obtained from the tail vein of *P. yoelii* infected donor mouse. The *P. yoelii* infected blood was diluted in physiological saline and injected via syringes.

To evaluate the anti-malarial effect of both *Coptis* rhizome and Orengedokuto, infected mice were randomly distributed into four groups of five individuals per cage. Tested drug and CQ were dissolved in DW. Each groups received the drugs 2 h after infection with *P. yoelii* on day 0 and continued daily for 7 days. Animals in test groups were treated twice a day with 365 mg/kg/day of *Coptis* rhizome and Orengedokuto (Kampo formula) and 122 mg/kg/day of *Coptis* rhizome (Herbal extract) in 0.2 mL solution by oral administration. CQ groups, served as a positive control, received 0.2 mL of infected blood suspension containing 1 mL of *P. yoelii* parasitized red blood cells obtained from the tail vein of *P. yoelii* infected donor mouse. The *P. yoelii* infected blood was diluted in physiological saline and injected via syringes.

On day 3 (72 h post-infection), the parasitaemia level was determined by Giemsa-staining of the tail vein blood smears that was characterized by random counting of the number of parasitized erythrocytes on randomly selected fields of the slide under microscopy of 2000–4000 erythrocytes when parasitaemia was low (≤10%) or up to 1000 erythrocytes when parasitaemia was higher.

The average percentage of parasite growth suppression was calculated by comparing percentage parasitaemia suppression of the test group with respect to the control according to the equation:

$$\text{Percentage suppression} = \frac{(\text{Mean parasitaemia of negative control} - \text{Mean parasitaemia of treated group})}{\text{Mean parasitaemia of negative control group}} \times 100$$

### Preparation of plasma samples

Healthy 6 weeks old female mice that were subjected to overnight fasting were used for this study. To analyse berberine, coptisine, and palmatine after oral administration of *Coptis* rhizome and Orengedokuto, doses of 122 mg/kg and 365 mg/kg were used for each group, respectively. Five mice per cage were used for each tested drugs. One hour after administration, the blood samples were collected from the tail vein with heparin and centrifuged at 1000xg for 20 min to yield plasma sample. Plasma samples were stored at −80 °C. Before analysis, thawed plasma samples were mixed with methanol with 0.05% (vol/vol) formic acid for 15 min and centrifuged at 14,000 rpm for 15 min. The supernatant was transferred into an Amicon Ultra filter (molecular weight cut-off of 10 kDa, Millipore Corporation), and centrifuged at 14,000 rpm for 60 min at 4 °C. The filtrate was evaporated and redissolved with 50 µL of 30% (vol/vol) MeOH in water to prepare LC–MS sample. LC–MS analyses were conducted with ODS Atlantis T3 (3 µm, 2.1 × 150 mm) column and Shimadzu LCMS system (Shimadzu, Tokyo, Japan) consisting DGU-20A5 on-line degasser, LC-20AD pumps (2 units), SIL-20A autosampler, CTO-20A column oven, SPD-M20A PDA detector, and hybrid ion trap time-of-flight (IT-TOF) mass spectrometer equipped with an ESI (electrospray ionization)
interface and chromatogram data were acquired and processed by LCMS Solution (ver. 3.81, Shimadzu). Gradient elution of two solvent mixture consisting of 0.1% (vol/vol) formic acid in water (mobile phase A) and 0.1% (vol/vol) formic acid in acetonitrile (mobile phase B) was run at a flow rate of 0.2 mL/min under the following gradient program: 10% B (0–2 min), 10–100% B (2–20 min), 100% B (20–25 min), 100–10% B (25–26 min), and 10% B (26–36 min). TOF Analyzer was calibrated by sodium trifluoroacetate solution. Data was acquired using the following parameters: detector voltage, 1.80 kV; probe voltage, +4.5 kV (positive mode) or −3.5 kV (negative mode); nebulizing gas flow, 1.5 L/min.; drying gas pressure, 100 kPa; temperature for CDL (curved desolvation line) and heat block, 200 °C; ion accumulation time, 30 ms.; scanning range, m/z 100–2000. The temperature of the column oven was set at 40 °C and the injection volume was 5 μL.

Ethics statement
Human RBCs and plasma were obtained and used after the approval (Number: 15 12 03 146-2) by the institutional ethical review board of Institute of Tropical Medicine, Nagasaki University. The animals in this study were handled according to the international guidelines and institutional guideline of Nagasaki University for the use and maintenance of experimental animals and used after approval (number 1710061412) by the institutional ethical review board of Institute of Tropical Medicine, Nagasaki University.

Results
Initial in vitro screening of anti-malarial activity and cytotoxicity of 120 Kampo herbal extracts, related compounds, and Kampo formula
Initially the in vitro anti-malarial activity of Kampo herbal extracts and their related compounds (Additional file 1: Table S1, Table S2) were tested against CQ/MQ-sensitive (3D7) strain of *P. falciparum*. Of 120 herbal extracts, *Coptis* rhizome demonstrated good anti-malarial activity against *P. falciparum* 3D7 (IC$_{50}$ = 1.9 μg/mL) with the minimal toxicity (CC$_{50}$ > 500 μg/mL, SI > 263) (Additional file 1: Table S3). Due to the lowest IC$_{50}$ and high SI, *Coptis* rhizome was further evaluated against *P. falciparum* Dd2 strain and IC$_{50}$ and SI were determined to be 4.85 μg/mL and >103, respectively (Table 1). Furthermore, the Kampo formula Orengedokuto that contains a high amount of *Coptis* rhizome by percentage weight was selected and evaluated against CQ/MQ-sensitive (3D7) and resistant (Dd2) strain of *P. falciparum*. This formula was received from Institute of natural medicine (WAKANKEN) at the University of Toyama and Tsumura Company (Additional file 1: Table S4) as the content of active ingredient is different. As a result, the IC$_{50}$ of the former was identified to be 3.1 and 6.34 μg/mL against 3D7 and Dd2, respectively. While sample from Tsumura Company showed 36 and 104 μg/mL against 3D7 and Dd2, respectively.

In vitro anti-malarial activity and cytotoxicity of three major bioactive components of *Coptis rhizome*
Because of its highest anti-malarial activity and SI, *Coptis* rhizome, as well as its related compounds, are shown in the supplemental table (coptisine, berberine, and palmatine) these three compounds of the test samples were further investigated against 3D7 and Dd2 strains of *P. falciparum*. IC$_{50}$ values for these components were 1.1, 2.6, and 6.0 μM against 3D7 strain and 3.1, 6.3, and 11.8 μM against Dd2, respectively (Table 1). CC$_{50}$ values were 41.1 μM, 8.64 μM, and >100 μM, respectively. Thus, the SI of these components were 37.8, >3.3, and >16.7 against 3D7 strain and 13.2, 1.3, and 8.5 against Dd2 strain, respectively (Table 1).

Table 1 *In vitro* anti-malarial activities and the cytotoxicities of *Coptis* rhizome and three chlorinated compounds representing its major bioactive components

| Name                      | IC$_{50}$        |           | CC$_{50}$       | SI          |           |
|---------------------------|------------------|-----------|----------------|-------------|-----------|
|                           | 3D7              | Dd2       | 3D7            | Dd2         |
| Crud drug extract (μg/mL) |                  |           |                |             |           |
| *Coptis* rhizome          | 1.9±0.84         | 4.85±2.33 | >500           | >263        | >103      |
| Compounds (μM)            |                  |           |                |             |           |
| Coptisine chloride        | 1.1±0.05         | 3.1±0.07  | 41.1           | 37.8        | 13.2      |
| Berberine chloride        | 2.6±1.22         | 6.3±0.47  | 8.6            | 3.3         | 1.3       |
| Palmatine chloride        | 6.0±3.4          | 11.8±1.62 | >100           | >16.7       | >8.5      |

Values are the mean from two independent experiments performed in duplicate
IC$_{50}$, 50% inhibitory concentration
CC$_{50}$, 50% cytotoxic concentration using adult mouse brain cells
SI, selectivity index
**In vivo anti-malarial activity**

Results of the in vivo malarial suppression test of *Coptis* rhizome and Orengedokuto in mice infected *P. yoelii* 17X strain are summarized in the supplementary file (Additional file 1: Table S5). The parasite density of *Coptis* rhizome revealed low as compared with the negative control (DW) and its parasite suppression were observed from 50.38 at day 3 to 72.13% at day 7 post-challenge (Additional file 1: Table S5). *Coptis* rhizome-treated mice showed significant anti-malarial activity consistently throughout the entire test period to that of negative control (*P* < 0.05 on day 4 and *P* < 0.01 on day 3, 5, 6 and 7) (Fig. 1, Additional file 1: Table S5). Mice treated with CQ significantly suppress the parasitaemia and showed the most potent anti-malarial activity (0% parasitaemia and 100% suppression).

In addition, to analyse the presence of major components derived from *Coptis* rhizome, mice plasma treated with *Coptis* rhizome and Orengedokuto were analysed by LC-MS. Five mice were used in each treatment group. After oral administration of *Coptis* rhizome, the signal of berberine was observed in the plasma from four mice at m/z 336.1 and retention time (t<sub>R</sub>) 14.0 min. The signals of palmatine (m/z 352.1, t<sub>R</sub> 13.8 min) and coptisine (m/z 320.1, t<sub>R</sub> 13.0 min) were observed in three plasma samples. In the same way, after oral administration of Orengedokuto, the signal of berberine, palmatine, and coptisine were detected in five, three, and one mice, respectively. Comparing the signal intensities of these three components, the signal of berberine was relatively stronger than the other two compounds (Additional file 1: Table S6, Figure S1).

**Discussion**

Since *P. falciparum* has quickly acquired resistance against currently available all anti-malarials [40–42], it is urgently required to develop novel anti-malarial drugs. Here it is found that *Coptis* rhizome showed 1.9 µg/ml and 4.9 µg/ml of IC<sub>50</sub> and >263 and >103 SI for Chloroquine sensitive and resistant *P. falciparum* strains, respectively. Furthermore, three chemical compounds...
(coptisine, berberine, and palmatine), which are related to *Coptis* rhizome exhibited anti-malarial activity with IC$_{50}$ less than 12 µM. These compounds belong to the berberine alkaloidal family and share the same isoquinoline skeletons, which is similar to quinoline skeleton found in anti-malarial drug quinine. This structural similarity to quinine is an important indicator for their anti-malarial activity.

*Coptis* rhizome is one of the components of a formula, Orengedokuto, which has been used to treat inflammatory disease [43], and berberine is strongly suggested to be responsible for its anti-inflammatory effect [44, 45]. In this study, it has confirmed the in vivo anti-malarial activity of *Coptis* rhizome, but the Orengedokuto, whose content [36] is 33.3% *Coptis* rhizome did not show a comparable effect on the reduction of parasitaemia. Because of those mice who got Orengedokuto showed damage in general condition, this formula might contain some interfering components on anti-malarial efficacy. Although this formula is available over the counter and is not necessary to check its safety issue, it is difficult to bring it directly to human trials.

This in vitro finding of *Coptis* rhizome and its bioactive compounds strongly supports a previous report [46]. However, their anti-malarial activity showed much lower IC$_{50}$ using exactly a similar lot of extract and compounds provided from the same KAMPO library of Toyama University. One of the differences between the two institutions is an incubation time of co-culture in vitro before the estimation of parasite number. The previous report used 72 h, but the present study used 48 h. The IC$_{50}$ difference between the 48 h and 72 h may result from time of action. The former method detects the merozoite invasion and subsequent parasite growth from 32 to 46 h and, the latter one detects the mature trophozoites and schizonts, respectively [47].

In addition, the cytotoxicity of berberine showed much lower CC$_{50}$ (CC$_{50}$ 8.3 µM) than the previous one [46]. Other study also reported high levels of cytotoxicity of berberine using murine macrophage (CC$_{50}$ 27.3 µg/mL) [48], or MCF-7 cell (CC$_{50}$ 36.0.91 µg/mL) [49], and 8.75 µg/mL [50].

The in vivo results of coptisine chloride remarkably suppress the parasitaemia of greater than 80%, and the density of parasitaemia was significantly lower than the negative control ($P<0.01$). As previously reported, coptisine had wide verities of activities such as inducing apoptosis in human colon cancer [51], inhibiting inflammatory response of mast cell [52], and anti-diabetic [53]. However, this is the first report of the coptisine chloride

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**Fig. 2**  Kinetic of parasitaemia with or without administration of tested samples. The above figure indicates the average group parasitaemia of coptisine chloride (CC) compared with negative control (DW) and positive control (CQ). On day 0, all mice were injected $1 \times 10^4$ *P. yoelii* 17X strain intraperitoneally. Two hrs post-challenge, tested drug, negative and positive control were administered via intraperitoneally. On day 3 (72 h post-challenge) parasitaemia was determined. The x-axis is days after parasite infection while the y-axis shows the percentage of iRBCs.
to have an in vivo anti-malarial activity. After the oral administration of *Coptis* rhizome and Orengedokuto, coptisine were detected in 1/5 and 3/5 of mice, respectively, and the signal of coptisine in plasma samples were relatively lower than that of berberine. The content of coptisine is approximately 1/16 of coptisine in *Coptis* rhizome [54]. Therefore, this results reflect the contents of these alkaloids in *Coptis* rhizome, which suggest that the poor oral absorption and bioavailability, and fast elimination rate of coptisine [38, 39].

Recently, re-purposing of the existing drugs for use in different disease attract the researcher because of cost-effectiveness [55–57]. Thus, it is noteworthy that the activity of *Coptis* rhizome in this study could be a promising re-purposing of Kampo medicine to formulate the treatment of malaria.

**Conclusions**

In summary, this is the first study demonstrating the in vivo anti-malarial activity of *Coptis* rhizome and coptisine chloride. This finding suggests that *Coptis* rhizome is a potential natural resource for anti-malarial, promising drug re-purposing for malaria, and its active compound coptisine chloride could be a potential anti-malarial lead candidate.

**Supplementary information**

**Supplementary information** accompanies this paper at https://doi.org/10.1186/s12936-020-03273-x.

**Abbreviations**

ACT: Artemisinin-base combination therapy; AMB: Adult Mouse Brain; CC~50~: 50% cytotoxic concentration; CDL: Curved desolvation line; CM: Complete media; CQ: Chloroquine; DW: Distilled water; ESI: Electrospray ionization; IC~50~: 50% inhibitory concentration; IP: Intraperitoneal; iRBC: Infected RBC; IT-TOF: Ion trap time-of-flight; LC-MS: Liquid chromatography–mass spectrometry; RFU: Relative fluorescence unit; SI: Selectivity index; UPW: Ultra-pure water; WHO: World Health Organization.

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**Authors’ contributions**

SM, KT, KK, JK, NTH, and KH conceived and designed the experiments. AAT, SM, KT, and FM performed the experiments. AAT, SM, KT, FM, MGK, OK, KK, JK, NTH, and KH analysed and interpreted the data. AAT, SM, MGK, OK, NTH, and KH wrote the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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