Antimalarial activity of traditional Kampo medicine Coptis Rhizome extract and its major active compounds

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Abstract

Background: The herbal medicine has been a rich source of new drugs exemplified by quinine and artemisinin. In this study, examined a variety of Japanese traditional herbal medicine (Kampo) for their potential antimalarial activities.

Methods: We designed a comprehensive screening to identify novel antimalarial drugs from a library of Kampo herbal extracts (n = 120) and related compounds (n=96). The antimalarial 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 antimalarial activity.

Results: Out of 120 herbal extracts, Coptis Rhizome showed the highest antimalarial activity (IC 50 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 antimalarial activities with IC 50 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 antimalarial activity (IC 50 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 antimalarial activity in mice infected with P. 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 <.05) for Coptis Rhizome and from 81 to 89% (P <.01) for coptisine chloride.

Conclusion: Coptis Rhizome and its major active compound coptisine chloride showed promising antimalarial 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 antipathogenic agents.

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 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, 3, 4, 5]. Successful malaria control can be achieved through the treatment with efficient antimalarial drugs such as quinoline drugs like 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, 9, 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, 12, 13, 14].

Traditional medicine has been known for centuries and has been used to treat the myriads of ailment [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 antimalarial drug [19, 20] such as quinine which was isolated from *Cinchona bark* [21, 22, 23] and the use of *Artemisia annua* for isolation of artemisinin [24]. In Africa, herbal medicines are one of the most common traditional medicine and nearly 80% has been utilized as primary health care. Thus, safe and effective herbal medicine should be provided to expand the access to health care 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 attention into using herbs as a source of ideal candidates and resurgence 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, we screened 120 Kampo herbal extracts and 96 related compounds for their antimalarial activity *in vitro* by our system 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* antimalarial activity using *P. 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 Orengedokuto, and analyzed by Liquid chromatography–mass spectrometry (LC-MS).

Methods

In vitro culture of Plasmodium falciparum

P. falciparum CQ/mefloquine (MQ)-sensitive (3D7) and -resistant (Dd2) strains were originally obtained from Dr. Louis Miller, NIH, USA. The parasites were maintained with 2% hematocrit type O^+ red blood cells (RBCs) in RPMI-1640-based complete medium (CM) supplemented with 5% AB^+ human serum (prepared from plasma), 0.25% AlbuMax I (Gibco, Waltham, MA), 12.5 μg/mL gentamycin, and 200 mM hypoxanthine at 37°C under mixed gas (5% CO_2, 5% O_2, and 90% N_2) 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% CO_2. The primary cells for cytotoxicity assay were used after three passages.

Kampo crude drug extracts, compounds, and formula

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 formulated Orengedokuto (Kampo formula)) 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* assay, powder of Orengedokuto and Coptis Rhizome, as well as chloroquine, were dissolved in distilled water (DW) for oral administration.

In vitro antimalarial assay (first screening)

It was done by seeding the *P. falciparum* cultures (0.75% parasitemia and 2% hematocrit) 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 artesunate (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% CO_2) condition for 48 hrs. Each *in vitro* experiment was performed in duplicated wells and repeated twice. The inhibition was obtained by dividing the parasitemia of test samples by the average of the negative controls.

In vitro dose response assay

The dose-response assay was performed for samples that showed more than 50% inhibition in the first screening to obtain the 50% inhibitory concentration (IC_{50}; $10^{(LOG(A/B) \times (50 - C)/(D - C) + LOG(B)}$, where A represented the lowest concentration value at which the percentage inhibition showed greater than 50%, B was the highest concentration value at which the percentage inhibition showed less than 50%, C was the percentage inhibition value of the sample at concentration B, and D was the percentage inhibition value of the sample at concentration A). For the herbal extracts/Kampo formula, and for the compounds in the library, the anti-malarial activity was analyzed using a serial dilution of test samples at 500 μg/mL – 25.4 ng/mL or at 20 μM – 0.619 nM respectively. Artesunate (10 μM – 0.508 nM) for 3D7 and CQ (10 μM – 0.508 nM) for Dd2 were served as positive controls, while UPW (final 5%) or DMSO (final 0.5%) were assigned as negative controls. The final concentration of DMSO for all tested compounds, negative and positive controls were adjusted to 0.5%.

A SYBR Green based microfluorometric method was used to quantify parasite level as previously described [37]. Briefly, after 48 hrs of incubation with herbal extracts or compounds, we added 100 μL of lysis buffer to RBCs by using 20 mM Tris, 10 mM EDTA, 0.01% saponin (wt/vol), and then we added 0.1% Triton X-100 (vol/vol), in pH 7.5 as well as 1× the final concentration of SYBR Green - I (Lonza, Rockland, ME) into each well. The plates were then incubated at room temperature for one hr with gentle agitation. Finally, the relative fluorescence unit (RFU) per well was detected using a multilabel plate reader (ARVO 1430; Perkin Elmer, Waltham, MA) with 485 - 515 nm for 0.1 seconds per exposure.

Cytotoxicity assay

Cytotoxicity was initially screened at 500 μg/mL for herbal extracts and 20 μM for compounds. AMB cells (1×10^4 cell) were seeded in a 96-well plate (black plate with clear bottom) and incubated at 37°C in a CO_2 incubator for 48 hrs. Then, herbal extracts, compounds, or their negative controls were added, and the cells were further incubated for 48 hrs. 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 hrs. Then we measured the fluorescence intensity of each well using a multilabel plate reader at 590 nm for 0.1 seconds per exposure. The concentration of drug required to reduce cell viability by 50% (CC_{50}; $10^{(LOG(A/B) \times (50 - C)/(D - C) + LOG(B)}$, 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_{50} and CC_{50} values were used as an indicator of *in vitro* antimalarial 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_{50} value by IC_{50} value.

Assessment of antimalarial activity in mouse model

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

The *P. yoelii* 17X strain was provided by Dr. Tetsuo Yanagi, of National Bio-Resource Center (NBRC), NEKKEN, 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/6N mouse previously infected with *P. yoelii* and having parasitemia 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 1×10^4 *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 antimalarial effect of both Coptis Rhizome and Orengedokuto, we randomly distributed infected mice into four groups of five individuals per cage. Tested drug and CQ were dissolved in DW. Each groups received the drugs 2 hrs after infection with *P. yoelii* on day 0 and continued daily for 7 days. Animals in test groups were treated twice a day with 365mg/kg /day of Orengedokuto (Kampo formula) and 122mg/kg/day of Coptis Rhizome (Herbal extract) in 0.2 mL solution by oral administration. CQ groups, served as a positive control, received a dose of 10 mg/kg/day and DW groups as a negative control, received 0.2 mL. Amount of dosage is calculated according to the normal usage for humans. Moreover, blood was collected after 1hrs from mice treated with Coptis Rhizome and Orengedokuto to detect berberine, palmatine, and coptisine.

For the *in vivo* antimalarial evaluation of the coptisine chloride (Toronto Research Chemicals (TRC), North York, Canada), coptisine chloride, and CQ were dissolved in DW. Three groups of mice were injected intraperitoneally with 0.2ml of the test sample (30mg/kg /day Coptisine chloride), positive control (10 mg/kg/day CQ), and negative control (DW) 2hrs after infection with *P. yoelii*. The same dose of injection was performed once a day until day 6. The IP route of administration was used since the previous study revealed that coptisine has low oral bioavailability and poorly absorbed through gastrointestinal tracts [38, 39].

On day 3 (72 hrs post-infection), the parasitemia level were 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 2,000–4,000 erythrocytes when parasitemia was low (≤10 %) or up to 1000 erythrocytes when parasitemia was higher.

Results of the *in vivo* antimalarial activity were expressed as a mean ± standard deviation (SD) and the comparison of parasitemia was determined by using a Student's *t*-test in Microsoft Excel 2016 (Microsoft, USA). The Statistical significance level was set at $P < .05$ for all tests. The different field on each slide was examined to calculate the average parasitemia as shown below.

Percentage parasitemia = (Number of parasitized RBC/ Total number of parasites) x100

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

Percentage suppression = (Mean parasitemia of negative control – Mean parasitemia of treated group)/ (Mean parasitemia of negative control group) x100

Preparation of plasma samples

Healthy 6 weeks old female mice that were subjected to overnight fasting were used for this study. To analyze berberine, coptisine, and palmatine after oral administration of Coptis Rhizome and Orengedokuto, doses of 122mg/kg and 365mg/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 1000x*g* 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 14000 rpm for 15 min. The supernatant was transferred into an Amicon Ultra filter (molecular weight cutoff of 10 kDa, Millipore Corporation), and centrifuged at 14000 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 (0-2min), 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 msec.; 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 antimalarial activity and cytotoxicity of 120 Kampo herbal extracts, related compounds, and Kampo formula

We initially tested the *in vitro* antimalarial activity of Kampo herbal extracts and their related compounds (Additional file: Table S1, Table S2) against CQ/MQ-sensitive (3D7) strain of *P. falciparum.* Of 120 herbal extracts, Coptis Rhizome demonstrated good antimalarial activity against *P. falciparum* 3D7 (IC_{50} =1.9 µg/mL) with the minimal toxicity (CC_{50} > 500 µg/mL, SI > 263) (Additional file: 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, we selected the Kampo formula Orengedokuto that contains high amount of Coptis Rhizome by percentage weight 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: Table S4) as the content of active ingredient is differentthe 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 antimalarial activity and cytotoxicity of three major bioactive components of Coptis Rhizome

Because of its highest antimalarial 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 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).

In vivo antimalarial 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: 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: Table S5) . Coptis Rhizome treated mice showed significant antimalarial activity consistently throughout the entire test period to that of negative control (*P <.05* on day 4 and *P <.0l* on day 3, 5, 6 and 7) (Figure 1, Additional file: Table S5). Mice treated with CQ significantly suppress the parasitemia and showed the most potent antimalarial activity (0% parasitemia and 100% suppression).

In addition, to analyze the presence of major components derived from Coptis Rhizome, mice plasma treated with Coptis Rhizome and Orengedokuto were analyzed 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_R) 14.0 min. The signals of palmatine (*m/z* 352.1, t_R 13.8 min) and coptisine (*m/z* 320.1, t_R 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: Table S6, Figure S1).

Figure 2 clearly showed that coptisine chloride suppressed the parasitemia, which displayed a mean percentage suppression throughout the entire test period (*P <0.1* on days 3 and 4, and *P <.001*, on days 5 and 6) (Figure 2, Additional file: Table S7). The parasitemia suppression (%) of mice treated with coptisine chloride was 89 %, 87 %, 82 %, and 81 % at days 3, 4, 5, and 6, respectively.

Discussion

Since *P. falciparum* has quickly acquired resistance against currently available all antimalarials [40, 41, 42], it is urgently required to develop novel antimalarial drugs. Here we found that Coptis Rhizome showed 1.9 µg/ml and 4.9 µg/ml of IC_{50} and >263 and >103 SI for Chloroquine sensitive and resistant *P. falciparum* strains, respectively. We also found that three chemical compounds (coptisine, berberine, and palmatine), which are related to Coptis Rhizome exhibited antimalarial 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 antimalarial drug quinine. This structural similarity to quinine is an important indicator for their antimalarial 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 our study, we have confirmed the *in vivo* anti-malarial activity of Coptis Rhizome, but the Orengedokuto, whose 33.3% content [36] is Coptis Rhizome did not show a comparable effect on the reduction of parasitemia. 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.

Our *in vitro* finding of Coptis Rhizome and its bioactive compounds strongly supports a previous report [46]. However, their antimalarial 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 hrs but we used 48 hrs. The IC_{50} difference between the 48 hr and 72 hr may result from time of action. The former method detects the merozoite invasion and subsequent

parasite growth from 32 to 46 hrs and, the latter one detects the mature trophozoites and schizonts, respectively [47]. In addition, when we saw the cytotoxicity, 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..91 μg/mL) [49], and 8.75 μg/mL [50].

The *in vivo* results of coptisine chloride remarkably suppress the parasitemia of greater than 80 %, and the density of parasitemia was significantly lower than the negative control (*P <.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 antidiabetic [53]. However, this is the first report of the coptisine chloride to have an *in vivo* antimalarial 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, our 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, 56, 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* antimalarial activity of Coptis Rhizome and coptisine chloride. Our finding suggests that Coptis Rhizome is a potential natural resource for antimalarial, promising drug re-purposing for malaria, and its active compound coptisine chloride could be a potential antimalarial lead candidate.

List Of 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

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

Availability of data and material

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

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Authors' contributions

S.M., K.T., K.K., J.K., N.T.H., and K.H. conceived and designed the experiments. A.A.T., S.M., K.T., and F.M. performed the experiments. A.A.T, S.M., K.T., F.M., M.G.K., O.K., K.K., J.K., N.T.H., and K.H. analyzed and interpreted the data. A.A.T., S.M., M.G.K., O.K., N.T.H., and K.H. wrote the manuscript. All authors read and approved the final manuscript.

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Table

Table 1. *In vitro* antimalarial 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

IC_{50}: 50% inhibitory concentration.

CC_{50}: 50% cytotoxic concentration using adult mouse brain cells

SI: Selectivity Index

Values are the mean from two independent experiments performed in duplicate.

Figures

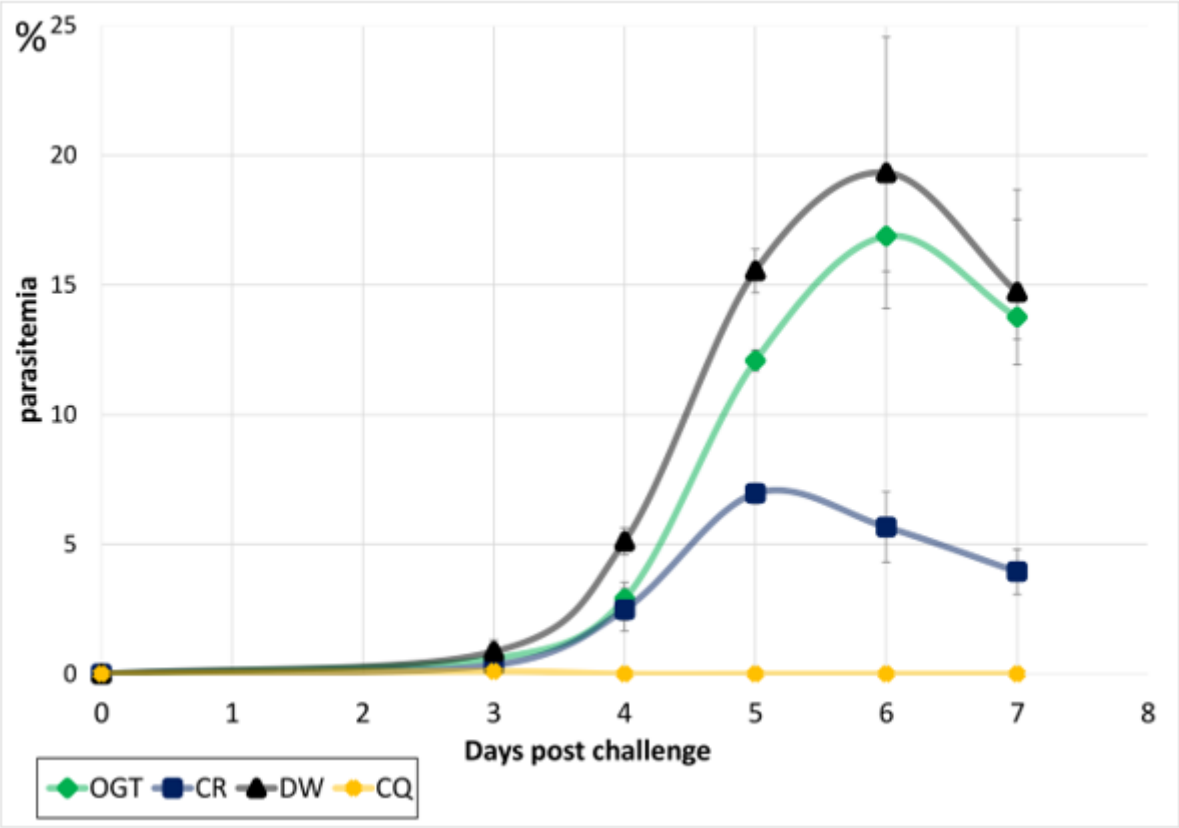

Figure 1

Kinetics of parasitemia with or without administration of test samples. The above figure indicates the average group parasitemia of Coptis Rhizome (CR) and Orengedokuto (OGT) compared with negative control (DW) and positive control (CQ). On day 0, all mice were injected 1x104 P. yoelii 17X strain intraperitoneally. Two hrs post-challenge, two tested drugs, negative and positive control were administered orally. On day 3 (72 post-challenge) parasitemia was determined. The x-axis is days after parasite infection while the y-axis shows the percentage of iRBCs.

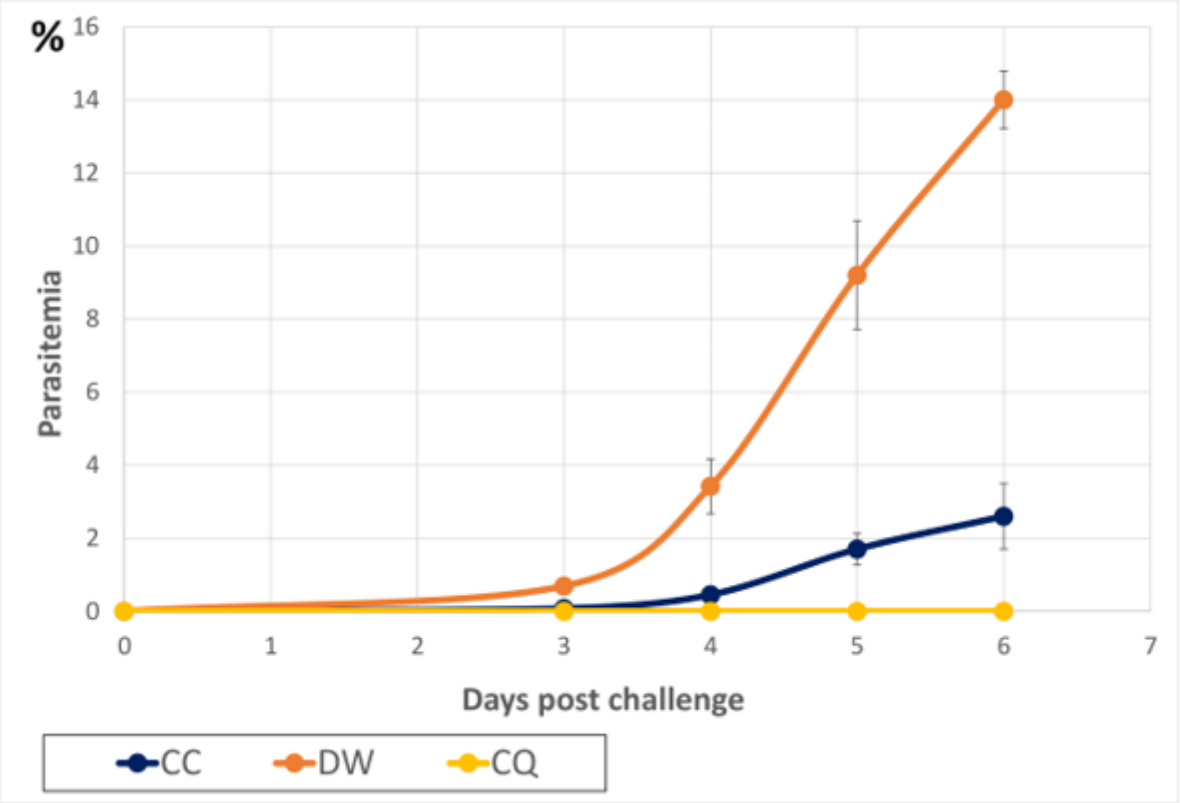

Figure 2

Kinetic of parasitemia with or without administration of tested samples. The above figure indicates the average group parasitemia of coptisine chloride (CC) compared with negative control (DW) and positive control (CQ). On day 0, all mice were injected 1x104 P. yoelii 17X strain intraperitoneally. Two hrs post-challenge, tested drug, negative and positive control were administered via intraperitoneally. On day 3 (72 hrs post-challenge) parasitemia was determined. The x-axis is days after parasite infection while the y-axis shows the percentage of iRBCs.

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