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 an attractive source of new antimalarial drugs exemplified by quinine and artemisinin, thus we 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 crude drug extracts (n = 120). 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. Subsequently, major active components of Kampo crude drug extracts showing high antimalarial activities and low cytotoxicity were further evaluated. Finally, the *in vivo* antimalarial activities of promising Kampo crude drug extract was investigated using *P. yoelii* infected mouse model in a seven-day suppressive test (treatment start two hours after challenge infection and continue for seven days).

Results: Out of 120 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 components in Coptis Rhizome also showed antimalarial activities with IC_{50} ranging from 1.1 to 6.0 μM (against 3D7) and from 3.1 to 11.8 μM (against Dd2). 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. Furthermore, Coptis Rhizome exhibited significant antimalarial activity in mice infected with *P. yoelii* 17X strain with respect to its activity on parasite suppression consistently throughout the entire test period ($P < 0.05$).

Conclusion: Coptis Rhizome showed a significant *in vivo* antimalarial activity in mice infected with *P. Yoelii*, thus it is a potential natural resource for antimalarials and its component coptisine chloride is a promising antimalarial lead compound.

Background

Malaria remains a critical health problem in tropical and subtropical parts of the world. In 2018, 228 million cases of malaria resulted in 405,000 death, 93% and 94% of which were in WHO African region, respectively [1]. Despite 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 reduce the burden [2, 3, 4, 5]. Successful malaria control has been dependent on the treatment with efficient antimalarial drugs such as quinoline drugs like quinine, and chloroquine (CQ) [6, 7]. However, the extensive use of CQ led to the emergence and spread of CQ-resistant *Plasmodium falciparum* parasites that reduced CQ's usage for the prophylaxis and treatment for malaria in the late 1970s [8, 9, 10]. In the recent decade, artemisinin-based combination therapy (ACT) is highly recommended as a first-line therapy for uncomplicated falciparum malaria globally, however, artemisinin-resistant *P. falciparum* has been recently reported in Greater Mekong Sub-region [11, 12, 13, 14, 15].

Traditional medicine has a long history and the therapeutic characteristics of plants have been known for centuries. It has been used to treat the myriads of ailment [16]. Some of the conventional medicines were derived from the plant-based herbal medicine, namely aspirin from willow bark [17], digoxin from foxglove [18], and morphine from the opium poppy [19]. Interestingly, it persists as a crucial source of drug discovery [16]. Furthermore, isolation of the natural product from herbal medicine has received increasing attention and a potential source of the conventional antimalarial drug [20] represented by the isolation of quinine from *Cinchona bark* [21, 22, 23] and artemisinin from *Artemisia annua* [24]. In Africa, herbal medicines are one of the most common traditional medicine and nearly 80% has been utilized as primary health care. Thus, providing safe and effective herbal medicine could contribute to expanding 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 curative modern therapy for several chronic diseases, the emergence of microbial resistance

and needs huge investment for modern drug development. On top of that, the pharmaceutical industries are interested in using herbs as a source of ideal candidates and resurgence their approach in favor of natural drug development [26].

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

Orengedokuto, a Kampo formula, is an aqueous extraction of four medicinal plants of Coptis Rhizome, Phellodendron Bark, Gardenia Fruit, and Scutellaria Root which were blended in the ratio of 3:2:2:2, respectively [33]. It has been clinically used for treating a gastrointestinal disorder, inflammation, cardiovascular disease and Alzheimer disease in China [34]. A recent report also demonstrated its effect reducing blood glucose and lipid level, hypertension, and anti-inflammatory [34, 35, 36]. Coptis Rhizome, one of the active components of Orengedokuto, have also extensive clinical potentiality predominantly as an anticancer [37] and combat aging [38]. Recently, the *in vitro* antimalarial activity of Coptis Rhizome and its major bioactive components were reported [39]. However, the antimalarial activity of Coptis Rhizome in a mouse model and *in vitro* assay of Kampo formula (Orengedokuto) which contains a high amount of Coptis Rhizome have not yet been investigated to date.

In this regards, investigation of the antimalarial activities in Kampo herbal medicines may result in discovering new herbs or formulas which had been well-tolerated to human use for thousands of years [40]. Therefore, we examined 120 Kampo crude drug extracts for their antimalarial activity *in vitro* using chloroquine/mefloquine-sensitive (3D7) and -resistant (Dd2) strains of *P. falciparum*. In addition, promising Kampo crude drug extract was further evaluated for their *in vivo* antimalarial activity against *P. yoelii* strain 17X in a mouse model.

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 [41]. RBCs and human plasma were supplied by Japanese Red Cross Society (number: 28J0060).

Isolation and culture of primary Adult Mouse Brain cells

Primary Adult Mouse Brain (AMB) cells used in this study were isolated and established in NEKKEN Bio-Resource Center, Institute of Tropical Medicine, Nagasaki University as described somewhere else [42]. 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.

Kampo crude drug extracts, compounds, and formula

A Kampo library containing 120 crude drug extracts (10 mg/mL), three chlorinated chemical compounds (coptisine chloride, berberine chloride, and palmatine chloride), and powder of formulated Orengedokuto (Kampo formula) was established in the Institute of Natural Medicine (WAKANKEN), at the University of Toyama as previously described [33] and stored at -80°C. All the crude drug extracts were dissolved in ultra-pure water generated by Milli-Q (Merck KGaA, Darmstadt, Germany). Three

chlorinated chemical compounds (coptisine chloride, berberine chloride, and palmatine chloride), were used in this study to evaluate the activity of the bioactive components of Coptis Rhizome (coptisine, berberine, and palmatine, respectively). Chemical compounds were received at a concentration of 10 mM, however, the stock solution was dissolved in dimethyl sulfoxide (DMSO; Wako Pure Chemicals Industrial Ltd.) solution a concentration of 2 mM. For *in vivo* assay, powder of Orengedokuto and Coptis Rhizome were dissolved in ultra-pure water.

In vitro antimalarial assay (first screening)

The *P. falciparum* cultures (0.75% parasitemia and 2% hematocrit) were seeded on 96-well clear flat bottom plates (Thermo Fisher Scientific, Rochester, NY) and exposed to Kampo crude drug extracts (final concentration of 500 μg/mL). The final ultra-pure water 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% ultra-pure water 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. All extracts were independently examined twice and each experiment was performed in duplicated wells. The percent of 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). Moreover, parasite-infected RBCs (0.75% parasitemia and 2% hematocrit) were treated with titrated crude drug extracts/Kampo formula (500 μg/mL – 25.4 ng/mL). Furthermore, titrated bioactive component (compounds in the library) of selected crude drug extracts (20 μM – 0.619 nM) were also examined. Artesunate (10 μM – 0.508 nM) for 3D7 and CQ (10 μM – 0.508 nM) for Dd2 were served as positive controls, while ultra-pure water (final 5% for crude drug extracts) or DMSO (final 0.5% for compounds) were assigned as negative controls.

A SYBR Green based microfluorometric method was used to quantify parasite level as previously described [43]. Briefly, after 48 hrs of incubation with crude drug extracts or compounds, RBCs were lysed by adding 100 μL of lysis buffer (20 mM Tris, 10 mM EDTA, 0.01% saponin (wt/vol), and 0.1% Triton X-100 (vol/vol), pH 7.5) and 1× 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 crude drug 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, Kampo crude drug 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. The fluorescence intensity of each well was measured 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. All assays were performed twice independently in duplicated wells. 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 the GraphPad Prism 6 (GraphPad Software, Inc., San Diego, CA). Selectivity index (SI) was obtained by dividing CC_{50} value by IC_{50} value.

Assessment of antimalarial activity in mouse model

A Kampo crude drug extract (Coptis Rhizome) and formula (Orengedokuto) exhibited *in vitro* antimalarial activity were tested for oral activity against *P. yoelii* strain 17X in a mouse model [44].

Female of 6-7 weeks C57BL/6N mice, weighing 18–20 g (supply by the SLC Japan), experimental and control mice free from pathogen were used in the study. The mice were kept in a well-ventilated room at a constant ambient temperature (23 ± 3°C) and air humidity (55 ± 15%) with a light-dark cycle situation, and unlimited food and tap water supplies. All mice were acclimatized for one week prior to the experiments.

The *P. yoelii* 17X strain was kindly donated by Dr. Tetsuo Yanagi, of National Bio-Resource Center (NBRC), NEKKEN, Nagasaki University, Nagasaki, Japan. and maintained by successive serial passage in mice were used for the study. The parasite was maintained frozen at -80°C. For each individual assay, an aliquot was injected intraperitoneally in a mice and donor infected 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 donor. At day 0 mice were injected intraperitoneally with 0.2 mL of infected blood suspension containing 1×10^4 *P. yoelii* parasitized red blood cells (RBCs) obtain from the tail vein of *P. yoelii* infected donor mouse. The *P. yoelii* infected blood was diluted in physiological saline and injected via syringes.

For the antimalarial evaluation of the Coptis Rhizome and Orengedokuto, infected mice were distributed randomly into four groups of five individuals per cage. Tested drug and CQ were dissolved in ultra-pure water. Each group of mice received the first dose of tested and control drug 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 (crude drug 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 ultra-pure water groups as a negative control, received 0.2 mL. The oral route of administration was used as the product have already available as oral dosage forms. Amount of dosage is calculated according to the dosage and administration presented for the human. Then we used 10× the human dose. Moreover, blood was collected after 1, 4 and 8 hrs from mice treated with Coptis Rhizome and Orengedokuto to detect the plasma concentration of berberine, palmatine, and coptisine.

On day 3 (72 hrs post infection), the parasitemia level of individual mouse were determined by examination of Giemsa-staining from tail 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 $p < 0.05$ for all tests. The different field on each slide was examined to calculate the average parasitemia as shown below.

$$\text{Percentage parasitemia} = \frac{\text{Number of parasitized RBC}}{\text{Total number of parasites}} \times 100$$

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 x100

Mean parasitemia of negative control group

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 crude drug extracts and Kampo formula

We initially tested the *in vitro* antimalarial activity of Kampo crude drug extracts (Additional file: Table S1) and their major bioactive compounds against CQ/MQ-sensitive (3D7) strain of *P. falciparum.* Of 120 crude drug 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). Due to 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 S2) 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 was further investigated for its three major bioactive components (coptisine, berberine, and palmatine) 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 Table 2. The parasite density of Coptis Rhizome revealed low as compared with the negative control (MQ) and its parasite suppression were ranging from 50.38 to 72.13% from day 3 to day 7 post-challenge (Table 2). Coptis Rhizome treated mice showed significant antimalarial activity consistently throughout the entire test period to that of negative control (p-value < 0.05 on day 4 and < 0.0l on day 3, 5, 6 and 7) (Figure 1, Table 2). Mice treated with CQ significantly suppress the parasitemia and showed the most potent antimalarial activity (0% parasitemia and 100% suppression). Even if Orengedokuto demonstrated some activity on day 4, it's not significant. In addition, after oral administration, the major chemical components were detected in mice plasma treated with Orengedokuto and Coptis Rhizome, namely berberine, palmatine, and coptisine. Among the active components, berberine was detected high plasma concentration than the others (Figure 2, Additional file: Table S3).

Discussion

Since *P. falciparum* has quickly acquired resistance against currently available all antimalarials [45, 46, 47], it is urgently required to develop novel antimalarial drugs. Here we evaluated the antimalarial activity of 120 Kampo drugs and found that Coptis Rhizome showed low IC_{50} and high SI. We also found that three chemical compounds (coptisine chloride, berberine chloride, and palmatine chloride) representing major bioactive components of Coptis Rhizome exhibited antimalarial activity with IC_{50} less than 12 μM. All three Coptis Rhizome components (coptisine, berberine, and palmatine) tested in this study belong to the berberine family and have a similar chemical structure containing isoquinoline alkaloids, which was an active part the antimalarial drug quinine. The structural similarity to quinine may play an important role for antimalarial activity [48]. The *in vivo* test showed a significant parasite suppression by Coptis Rhizome throughout the entire test periods.

Coptis Rhizome has been used to formulate Orengedokuto to treat inflammatory disease [34] and berberine is responsible for its anti-inflammatory activity [35, 36]. In our study, Coptis Rhizome showed good antimalarial activity with IC_{50} 1.9 μg/mL against 3D7. The antimalarial activity of Orengedokuto using *in vitro* assay demonstrated good activity and reported for the first time with IC_{50} 3.11 μg/mL against 3D7 this could be resulted from due to the contents of Coptis Rhizome (33.3%) [33].

Coptis Rhizome, obtained from dried Rhizome of the medicinal plant from family Ranunculaceae are well known herbal medicine plant in China [38, 49], comprises five constitutes of alkaloidal compounds, namely berberine (6.88 to 13.64 %), palmatine (1.28 to 2.12%), jatrorrhizine (0.77 to 1.32%), coptisine (0.42 to 0.85%, and epiberberine (0.42 to 0.92%), with berberine which is a major bioactive composition [38]. Berberine and its derivatives have broad clinical potentiality particularly as an anti-inflammatory, cancer [50] and also showed antimalarial activity [51]. Consistent with this report, we also detected the antimalarial activity of berberine chloride in this study. In addition, we also found that coptisine chloride had a similar level of antimalarial activity (IC_{50} = 1.1 or 3.1 μM against 3D7 or Dd2 strains, respectively) to berberine chloride (IC_{50} = 2.6 and 6.3 μM, respectively). Because of the higher SI values (37.8 and 13.2, respectively) than those of berberine chloride (3.3 and 1.3, respectively), coptisine chloride may serve as a better leading compound for further antimalarial development.

This *in vitro* finding of Coptis Rhizome and its bioactive compounds underpins the previous report that showed good antimalarial activity, but their IC_{50} were lower than the present study [39]. This difference may be due to using a longer culture duration (96 hrs) and low parasitemia (0.3%). However, the present study used a shorter culture time and higher percentage parasitemia (48 hrs and 0.75%) respectively, this suggests that the parasite was exposed for a short time with a higher parasitemia as compared to the other study. Thus, this IC_{50} difference is more likely resulted from the different culture time as well as % parasitemia used. In addition, berberine showed very low CC_{50} (CC_{50} 8.3 μM) than the previous study [39]. However, other study reported cytotoxicity of berberine using murine macrophage (CC_{50} 27.3 μg/mL) [52], induce cell death in MCF-7 cell (CC_{50} 36..91 μg/mL) [53], and 8.75 μg/mL [54].

Based on the result of the *in vivo* antimalarial assay, Coptis Rhizome at the dose of 122mg/kg body weight demonstrated a significant antimalarial activity relative to the negative control with regard to parasite reduction (P-value < 0.05) and it suppresses the parasite > 50% from day 3 to 7, suggesting a good antimalarial activity and reported the first time. However, Orengedokuto revealed a disappointing result and this perhaps due to the composition of different extract that might interact with each other and the presence of non-active ingredients used to formulate may reduce its activity. This *in vivo* assay confirms the potential antimalarial activity of coptisine chloride, one of the major bioactive components of Coptis Rhizome [38]. The oral administration of Coptis Rhizome and Orengedokuto, plasma concentration and tissue distribution of coptisine was detected much lower than the berberine and palmatine suggesting that the poor oral absorption and bioavailability of coptisine and fast elimination rate [55, 56].

Recently, re-purposing of the existing drugs for use in different disease attract the researcher and a promising area for the discovery of drugs due to cost-effectiveness, shorter development timeline which speeds up the new indication of available

drug and safety profile [57, 58, 59]. 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 suppression of Coptis Rhizome and *in vitro* efficacy of Orengedokuto. Furthermore, this study confirms the previous *in vitro* antimalarial activity finding of Coptis Rhizome and its components. Our finding suggests that Coptis Rhizome is a potential natural product resource for antimalarial, promising drug re-purposing to use for malaria and its active component coptisine chloride could be a potential antimalarial lead candidate.

Declarations

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.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

Abbreviations

ACT: artemisinin-base combination therapy

AMB: Adult Mouse Brain

CC_{50}: 50% cytotoxic concentration

CM: complete media

CQ: chloroquine

IC_{50}: 50% inhibitory concentration

iRBC: infected RBC

MQ: mefloquine

SI: selectivity index

WHO: World Health Organization

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Tables

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
Crude 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.

Table 2. The average percentage parasitemia and suppression profile

Treatment	Dose in mg/kg/day	% parasitemia					% suppression				
		Day 3	Day 4	Day 5	Day 6	Day 7	Day 3	Day 4	Day 5	Day 6	Day 7
Orengedokuto	365	0.60 ± 0.20	2.89 ± 0.63	12.07 ± 0.42	16.87 ± 2.65	13.75 ± 4.91	23.18	42.48	22.13	11.25	8.07
Coptis Rhizome	121.5	**0.34 ± 0.16	*2.48 ± 0.82	**6.96 ± 0.39	**5.66 ± 1.35	**3.94 ± 0.87	59.25	50.38	55.21	70.57	72.13
NC (MQ)	0.2 mL	0.85 ± 0.46	5.11 ± 0.52	15.54 ± 0.84	19.32 ± 5.23	14.72 ± 2.80	0	0	0	0	0
PC (CQ)	10	0.1 ± 0.022	0.0005 ± 0.001	0	0	0	100	100	100	100	100

Data are presented mean + SD (five mice for each group). The average percentage parasitemia and suppression activity of CR and OGT treated C57BL/6N mice infected with *P. yoelii*. CR: Coptis Rhizome, OGT: Orengedokuto, NC (MQ): (Negative control, MilliQ), PC (CQ): (Positive control, Chloroquine)
* P value < 0.05
** P value < 0.0

Figures

Figure 1. The average group parasitemia profile

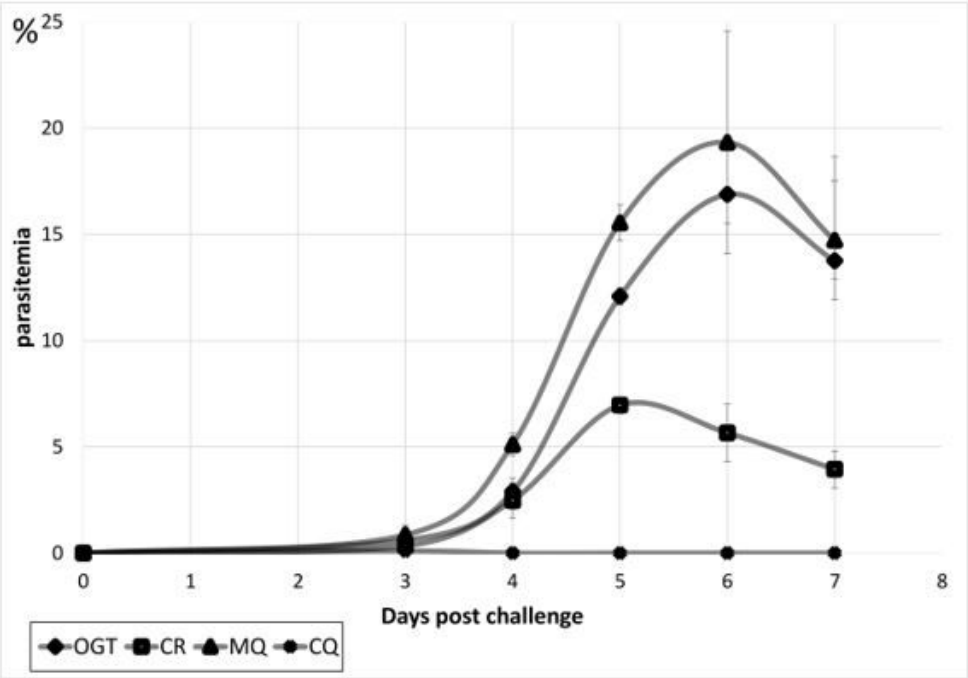
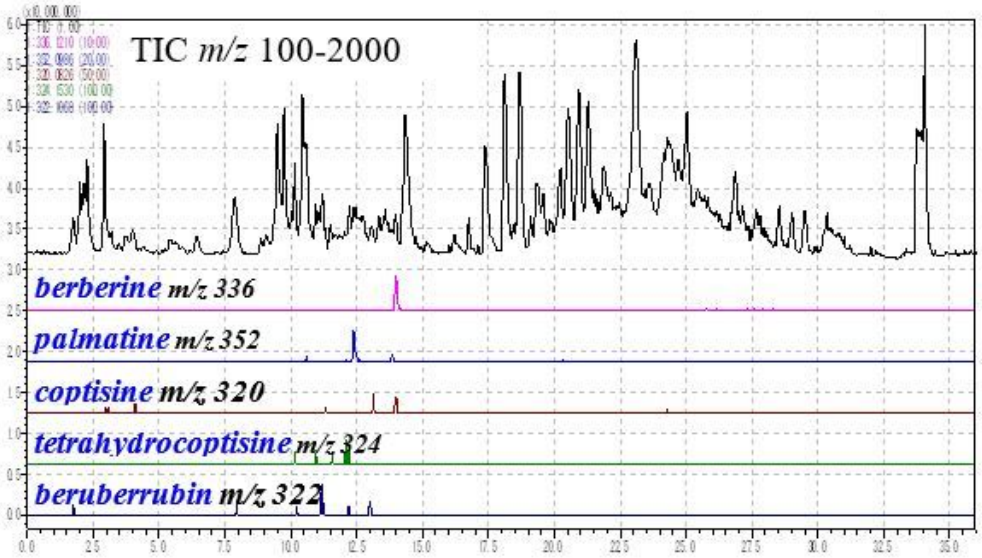


Figure 1

The average group of parasitemia profile. The above figure indicates parasitemia suppression of Coptis Rhizome (CR) and Orengedokuto (OGT) compared with negative control (MQ) 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. LCMS profile of Coptis Rhizome treated mice plasma



LCMS condition (Shimadzu LCMS-IT-TOF)
Column: ODS atlantis T3 3 μm (2.1×150 mm)
Solvent A: water (+0.1% formic acid)
Solvent B: acetonitrile (+0.1% formic acid)
Gradient: 10%B (0-2 min), 10-100%B (2-20 min), 100%B (20-25 min),
100-10%B (25-26 min), 10%B (26-36 min),

Figure 2

Supplementary Files

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