Abstract of Master's Thesis

No.1

Course	Health Innovation	Name	Awet Alem Teklemichael
Thesis Title	The Antimalarial Activity of Japanese Traditional (Kampo) Medicine		

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Background:

Malaria is a critical global health issue, especially in tropical and subtropical countries. The emergence of resistance to the available antimalarial drugs and the lack of an effective vaccine require the urgent development of a new medicine with new mechanisms of action. The herbal medicine is still an attractive source of a new antimalarial drug since quinine and artemisinin are well-known antimalarial drugs originated from herbs. Kampo is a known Japanese traditional medicine that has been used for long periods of time as a treatment as well as a supplement. However, surprisingly there is no systematic study searching for antimalarials from Kampo medicine. Therefore, we designed a comprehensive Kampo extracts and their major active compounds screening to identify a new antimalarial drug in collaboration with WAKANKEN, Institute of Natural Medicine at University of Toyama.

Objective:

To find antimalarials from a well refined set of Kampo compounds, crude drug extracts and formula using *in vitro* and *in vivo* screening.

Method :

The antimalarial activity of Kampo library (96 compounds and 120 extracts), which were provided by University of Toyama (Japan) were evaluated using *in vitro* antimalarial assay against chloroquine – mefloquine sensitive (3D7A) and chloroquine – mefloquine resistant (Dd2) strains of *Plasmodium falciparum* in the erythrocytic cycle. After the drug treatment, the infected red blood cells (iRBCs) were stained with SYBR Green to detect the parasite. A dose response antimalarial assay was carried out in order to determine the minimum inhibition concentration for 50% of the pathogen (IC_{50}).

＊ The abstract, containing the objective, method, result and conclusion should not exceed c.1000 words (300-500words/page, double sided on A4 paper)

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In addition to this, Kampo compounds, crude drug extracts, and formulas were evaluated for the toxicity against primary Adult Mouse Brain (AMB) cells to measure cytotoxicity concentration to cause a death to 50% of the viable cell (CC_{50}) and estimate the selective index (SI). Moreover, active Kampo crude drug extract and formula which contains the crude extract were further examined against *P. yoelii* in mice model. *In vivo* parasite growth suppression effect of crude drug extract and formula were evaluated in mice inoculated with *P. yoelii* (1×10^4 parasite/mouse). This *in vivo* antimalarial suppressive effect was performed in a 5-day suppressive test (drug treatment starts 48 hr before challenge and continue until 48 hr after challenge infection).

Result：

Our results of first screening yielded a total of 12 compounds and 27 crude drug extracts possessing antimalarial activity against chloroquine-mefloquine sensitive strain (3D7A) of *P. falciparum* with IC_{50} ranging from 1.1 to 16.5 μM and 2.5 to 482.8 μg/mL, respectively. The cytotoxicity assay with compounds and crude drug extracts that showed antimalarial activity on first screening revealed three compounds exhibiting toxicity with CC_{50} ranging from 5.1 to 8.5 μM and one crude drug extract exhibiting toxicity with CC_{50} at 23.5 μg/mL. Coptisine chloride showed the highest antimalarial activity (IC_{50} 1.1 μM) among the compounds and it was safe enough (SI 37.8). In addition, coptis rhizome had the highest antimalarial activity (IC_{50} 2.5 μg/mL) among crude drug extracts without a detectable cytotoxicity, and it contains active compounds like coptisine chloride. In parallel with the compound based drug development strategy, we sought a way of drug discovery based on already approved Kampo formula which contains the effective Kampo crude extracts because it is proven to be very safe. Thus, we focused on two already approved Kampo formula, Sanohshashinto and Ohrengedokuto, containing coptis rhizome, and those exhibited good antimalarial activities (IC_{50} 36 and 40 μg/mL, respectively). Moreover, both the formula (Ohrengedokuto) and crude drug extract (coptis rhizome) showed a suppression of parasitemia even on preliminary experiment with mice model.

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Conclusion :

A high antimalarial activity with low cytotoxicity was observed from Kampo compounds, crude drug extracts, and Kampo formula, suggesting Kampo formula as a potential antimalarial drug. This study is an important input for the development of an antimalarial drug from a Japanese traditional (Kampo) medicine.

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