# Antioxidant and Antiplasmodial Activities of *Curcuma Longa* and *Aegle Marmelos* on Malaria Infeced Mice (*In Vitro and In Vivo*)

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## ABSTRACT

The purpose of this study is to evaluate the antioxidant activity of Thai *Curcuma longa* and *Aegle marmelos*, and their parasite suppressive effects on malaria infection in mouse model. The polyphenol content and antioxidant activities (Oxygen radical absorbance capacity (ORAC) and Ferric Reducing Antioxidant Power (FRAP)) were measured in crude extracts of *Curcuma longa* and *Aegle marmelos*. Seven-week old female ICR mice were divided into 5 groups randomly. First two groups served as control and placebo, the other 3 served as experimental groups with various concentrations; 20, 40 and 60 mg/kg, respectively. On day 0, $10^6$ *Plasmodium yoelii* 17X (lethal) strain were inoculated to all mice. At day 1, placebo mice were given 30% ethanol. The latter 3 groups were treated with each concentration of each herb. Parasitemia was checked daily by tail snip bleed staining with Giemsa staining. Suppressive effects on day 4 were calculated. Student t-test was performed to display the difference among groups. Significantly different was justified at $p < 0.05$. The antioxidant activities (both ORAC and FRAP) and polyphenol content of *A. marmelos* were higher than those of *C. longa* significantly. The suppressive effect on parasite in infected mice, there showed no effect of *C. longa* treatment, oppositely, at the doses of 20 and 40 mg/kg body weigh of *A. marmelos* showed suppressive effect on infection by the parasite. In conclusion, a Thai traditional fruit, *A. marmelos* exhibited high antioxidant and antiplasmodial activities, this might be one of the candidates of the traditional plants for curing malaria.

**Keywords:** *Plasmodium yoelii*, Malaria, *Curcuma longa*, *Aegle marmelos*

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## INTRODUCTION

Malaria has continually been shown to be the major killers in developing countries. For instance, *Plasmodium falciparum* causes an estimated 1 million deaths annually.[1] World Health Organization (WHO) in 2008 reported that malaria is endemic in 109 countries, in subtropical and tropical countries, including Thailand. At present, one of the most 3 problems of malaria is the resistance of the malaria drug usage. To search for the novel drugs against malaria, traditional herbal medicines are approached. Vegetables and plant leaves, nowadays, are still used as medicinal plants for treatment of many diseases including malaria. There is much evidence that the consumption of plant foods, such as fruits, vegetables and spices, provides protection against various diseases.[2,3] This protection can be explained by the free-radical scavenging capacity of antioxidants in plant foods. Plant foods are a good source of polyphenols, which have been reported to decrease oxidative stress and inhibitors of lipid peroxidation.[4,5] In severe malaria, the oxidative stress is increased in the red blood cell membrane to induce the destruction of erythrocyte. There is suggestion that the compounds exhibiting both antioxidant and anti-plasmodial activities could be very interesting as leads for new anti-malarial drugs.[6-8] Thailand is the tropical country which has variety of herbal plants and many villagers are still depending on traditional drugs when they get ill. Thus, the use of traditional medicinal plant might be one of the alternative choices for curing malaria. Bael or "Matoom" (a Thai name) or *Aegle marmelos* Corr. (Scientific name) and Turmeric or "Kamin" (a Thai name) or *Curcuma longa* (Scientific name) are Thai traditional foods and also herbs for long time ago. Since, there is no information regarding to antiplasmodial activities, thus, it is interesting to evaluate the antiplasmodial and antioxidant activities in the both of Thai traditional plants, *Curcuma longa* and *Aegle marmelos*.

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## MATERIALS AND METHODS

### Plant materials

Rhizomes of *Curcuma longa* and fruits of *Aegle marmelos* were used and exposed them by drying under sunlight for 2 days, chopped into small pieces and grinded into powder.

### *In vitro* antioxidant activity assays

Each sample was extracted with 95% ethanol on a mechanical shaker at 400 rpm for 1 hour. Afterwards, the mixture was centrifuged at 4,400 g for 15 min. Finally, the supernatant was collected and analyzed for total antioxidant activity by ORAC and FRAP assays.

### Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay measures the ability of antioxidant compounds in test materials to protect against oxidation induced by the peroxyl radical generator AAPH. The antioxidant activity of the sample extract was measured according to the method described by Huang, *et al.*[9] at the excitation and emission wavelengths of 493 and 515 nm, respectively. Trolox, 6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid (Aldrich # 238813), was used as a standard. The results were expressed as micromole Trolox equivalent per gram of sample (µmol TE/g).

### Ferric reducing antioxidant power (FRAP) assay

FRAP was evaluated according to the method of Benzie and Strain[10] at 593 nm by spectrophotometer (Shimadzu UV-1601 UV-VIS). Trolox was used as a standard. Test solution absorbance was measured and compared with that of the Trolox standard solution. The results were expressed as micromole Trolox equivalent per gram of sample (µmol TE/g).

### Determination of polyphenol content

Polyphenol content in *Curcuma longa* and *Aegle marmelos* was determined according to the Folin-Ciocalteu method of Brenna and Pagliarini.[11] The absorbance of product was measured at 750 nm by a microplate reader (TECAN sunrise microplate reader, Austria). Gallic acid was used as a standard. The polyphenol content was expressed as milligram gallic acid equivalents per gram sample (mg GAE/ g).

### *In vivo* antiplasmodial activity assays

### Animals

Pathogen-free forty 7-week-old female ICR mice weighing approximately 20 g obtained from National Laboratory Animal Center, Mahidol University. Procedures of animal experiment were approved by Ethical committees on Animal Experimentation of Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand.

### Parasite

*Plasmodium yoelii* 17X (lethal) strain-parasitized erythrocytes were obtained from donors infected ICR mice.

### Preparation of crude extracts

The powder of Rhizomes of *Curcuma longa* and fruits of *Aegle marmelos* were used in this experiment. Two, four and six hundred milligrams of each herbal powder, respectively, went for extraction with 1 ml of 95% ethanol. Later, ethanol extracted soluble part was diluted with distilled water to 30% ethanol and adjusted to the final concentration. All were prepared fresh before using.

### Procedures

All female ICR mice were randomly divided into 5 groups; each group contained 5 mice. First group was served as control which mice of this group were given food pellets and drinking water only. Second group was served as vehicle (placebo group) which were given 30% ethanol and third, fourth and fifth groups were served as experimental groups which mice were injected intraperitoneally in the amounts of 20, 40 and 60 mg/kg of *C. longa* and *A. marmelos*, respectively. On day 0, $1x10^6$ *P. yoelii* 17X (lethal) strain-parasitized erythrocytes were injected to all mice. Starting from day 1, 30% ethanol, 20, 40 and 60 mg/kg of each herb were injected into placebo, experimental groups 1, 2 and 3, respectively. Percent parasitemia from tail snip bleeds was checked by Giemsa staining daily and examined under light microscope.

### Statistics analysis

The statistical analysis was performed using the statistical package for the social sciences (SPSS), version 17 for Windows 98, SPSS Inc. Differences in polyphenol content and antioxidant activity of Curcuma longa and Aegle marmelos were considered statistically significant at $p$ less than 0.05 and 0.01.

Percent parasitemia of all mice were analyzed by using *t test* and plot as a graph. All values were expressed as mean ± standard deviation. Differences in parasite infectivity were considered statistically significant at $p$ less than 0.05. At day 4, percent parasitemia in each group of treatments were used to calculate the suppressive effect by using the following formula:

Average percent suppression is equal:

$$\frac{\text{average \% parasitemia in control} - \text{average \% parasitemia in treatment}}{\text{average \% parasitemia in control}} \times 100$$

## RESULTS

### *In vitro* antioxidant activity

The antioxidant activities of *C. longa* and *A. marmelos* determined by ORAC and FRAP assays are shown in Table 1. The antioxidant activities of *A. marmelos* was significantly higher than *C. longa* at p <0.01 and 0.05 for ORAC and FRAP, respectively.

### Polyphenol content

The result of polyphenol content analysis of two plant materials was shown in Table 1. The polyphenol

**TABLE 1.** Antioxidant activities and polyphenol content of *C. longa* and *A. marmelos.*

| Plant materials | Antioxidant activity[1] | | Polyphenol content[1] |
|---|---|---|---|
| | ORAC[2] | FRAP[3] | (mg GAE[4] /g) |
| *C. longa* | 815.4 ± 10.7** | 35.2 ± 1.5* | 61.8 ± 3.1* |
| *A. marmelos* | 1474.6 ± 12.3 | 43.8 ± 1.1 | 84.4 ± 4.5 |

[1]Mean ± SD from 3 replicates analysis

[2]Oxygen radical absorbance capacity (ORAC), expressed as micromole trolox equivalents per gram

[3]Ferric reducing antioxidant power (FRAP), expressed as micromole trolox equivalents per gram

[4]Gallic acid equivalent.

*, ** Significant differences of polyphenol content or antioxidant activity values in the same column (* $p$ <0.05, ** $p$ <0.01)

amount of *A. marmelos* was significantly higher than *C. longa* at p <0.05.

### *In vivo* antiplasmodial activity

#### 1. Percent parasitemia

***C. longa* treatment**

For control, one mouse started to die on day 17 and all mice died on day 21 whereas in placebo group, one mouse started to die on day 12 but the rest of mice died gradually until day 21 (Table 2). Percent parasitemia time of dead mice of these 2 groups ranged from 45.1-78.7% and 56.9-85.2%, respectively. For 20, 40 and 60 mg/kg *C. longa* treated mice, mice started to die on day 15-19, 4-21 and 3-17, respectively. Percent parasitemia at the time of dead mice ranged from 15.6-52.4%, 14.0-40.0% and 2.7-55.5% in 20, 40 and 60 mg/kg *C. longa* treated mice, respectively (Fig 1).

***A. marmelos* treatment**

For control, one mouse started to die on day 17 and all mice died on day 21 whereas in placebo group, one mouse started to die on day 12 but the rest of mice died gradually until day 19 (Table 3). Percent parasitemia time of dead mice of these 2 groups ranged from 45.1-78.7 % and 56.9-85.2%, respectively. For 20, 40 and 60 mg/kg *C. longa* treated mice, mice started to die on day 9-22, 8-19 and 7-19, respectively. Percent parasitemia at the time of dead mice ranged from 12.8-38.8%, 10.7-73.5 % and 14-55% in 20, 40 and 60 mg/kg *A. marmelos* treated mice, respectively (Fig 2).

**TABLE 2.** The days of each groups of mice that died after *P. yoelli* inoculation and treatment with *C. longa*

| n=5 | First day of death | Last day of death |
|---|---|---|
| Control | 17 | 21 |
| Placebo | 12 | 21 |
| 20 mg/kg | 15 | 19 |
| 40 mg/kg | 4 | 21 |
| 60 mg/kg | 3 | 17 |

**TABLE 3.** The days of each groups of mice that died after *P. yoelli* inoculation and treatment with *A. marmelos*

| n=5 | First day of death | Last day of death |
|---|---|---|
| Control | 17 | 19 |
| Placebo | 12 | 19 |
| 20 mg/kg | 9 | 22 |
| 40 mg/kg | 8 | 19 |
| 60 mg/kg | 7 | 19 |

#### 2. Percent suppressive index

***C. longa* treatment**

On day 4 at 20, 40 and 60 mg/kg, percent suppressive index showed at -12.8, -26.5 and -174.4, respectively.

***A. marmelos* treatment**

On day 4 at 20, 40 and 60 mg/kg, percent suppressive index showed at 29.9, 17.7 and -146.9, respectively.

## DISCUSSION

Several assays have been developed to estimate the total antioxidant capacity in foods and plant materials. The test methods of antioxidant capacity are basically divided into two groups of reaction mechanisms: the hydrogen atom transfer reaction and the single electron transfer reaction.[12, 13] It was suggested that each evaluation on antioxidant activity should be done with various methods based on different mechanisms and measurement techniques.[14] Thus, our present study, we selected ORAC and FRAP assays, which are the widely used in evaluating antioxidant activity of various food samples based on different mechanisms of the hydrogen atom transfer and the single electron transfer reaction, respectively.[15,16] The crude extract of both plant materials showed high antioxidant activities when compare with Trolox, a hydrophilic derivative of vitamin E (815.4-1476.6 and 35.2-43.8 µmole Trolox equivalents/g for ORAC and FRAP, respectively). The ORAC value of *A. marmelos* was much larger than that of *C. longa* (significant difference, *P* <0.01). The principle of ORAC method is measuring the ability of antioxidant compounds in test materials to protect against oxidation induced by generated peroxyl radical which very similar to biological mechanism of body.[9] while FRAP method measured the ferric reducing ability of test materials.[10] Thus, we estimated that the ability of crude extract of *A. marmelos* could be better *C. longa* for helping inhibit oxidative stress in mice's red blood cells by scavenging the peroxyl radical induced by hydrogen peroxide during malaria infection.

There was one report from Reddy C, *et al* in 2004.[17] showing that *P. berghei*-infected Swiss mice after orally administrated with 100 mg/kg of *C. longa* showed the reduc-

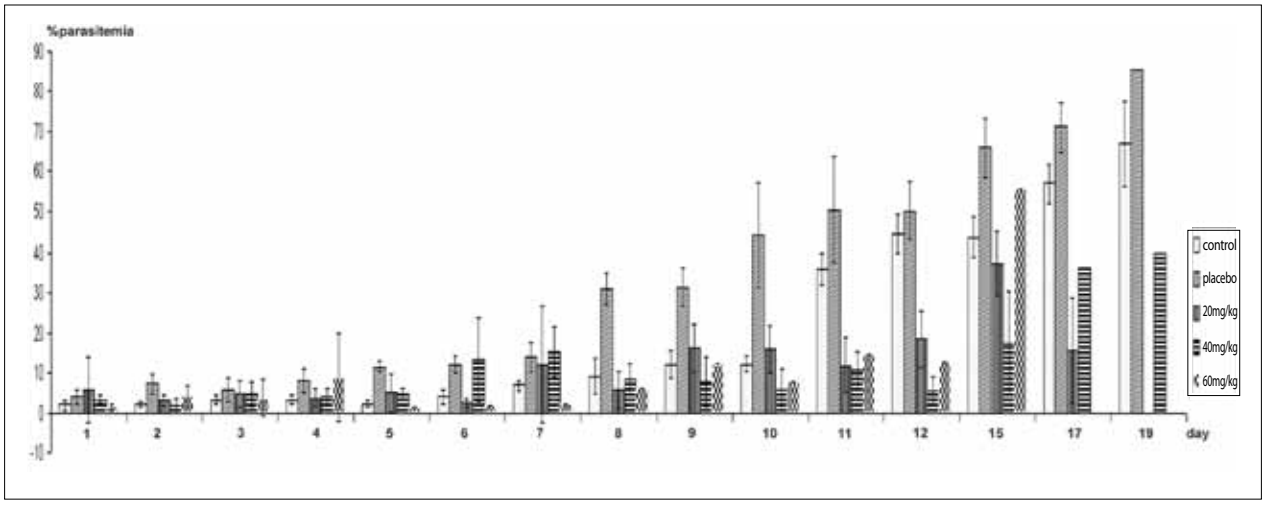


**Fig 1.** Percent parasitemia of each group of mice (Control, Placebo and various concentrations of *C. longa* treatments) from the first day until the last day (the day of mice died).

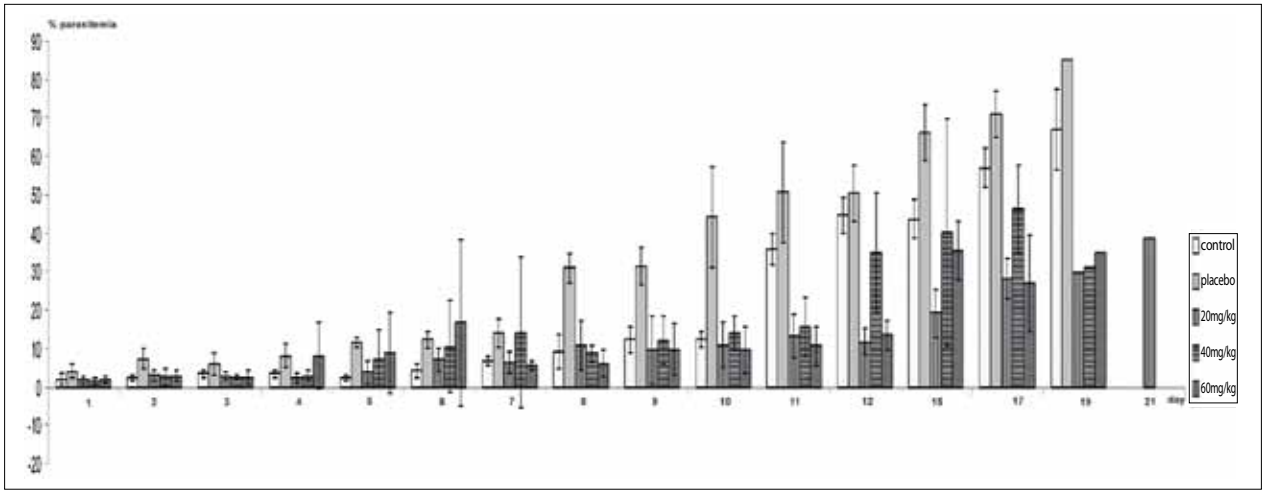


**Fig 2.** Percent parasitemia of each group of mice (control, placebo and various concentrations of *A. marmelos* treatments) from the first day until the last day (the day of mice died).

tion on percent parasitemia by 80-90 percent that enhance mice survival. The combination of curcuma and artemisinis in Plasmodium berghei followed by the single injection of alpha and beta arteeether showed the preventation almost 100% survival.[18] In 2007, there was the experiment using *C. longa* with artimisinin in *P.yoelii* infection mice at the doses of 10, 30 and 60 mg/kg that effected to the longevity of mice.[19] The comibination of this combination also showed the delay of percent parasitemia in *P.chabaudi* infected mice.[20] Furthermore, *P.beghei*-infected mice also showed the suppression of parasitemia up to 40 percent.[21] All mentioned results showed different results from ours. We found no suppressive effect of *C. longa* to *P. yoelii* infected mice. It might be explained that we used only crude extract by not combining to any other drugs or herbs. Moreover, the concentrations we used in this experiment might not enough and it could be increases if possible in order to see its effect. However, *C. longa* seem not effective to malaria if using alone. Anyhow, further study is needed to be generated.

So far, there is only one report from Bora, *et al* in 2007 that they had given *A. marmelos* to the patients in the Northeast of india as the traditional medicinal plant and this *A. marmelos* showed the good effect to patients.[22] Our result showed similar even we performed in malaria mouse model. Furthermore, there shows the suppressive effect on malaria parasite. Thus, *A. marmelos* should be considered and further study for malaria treatment in term of using the crude extract, purify potential substances or combine to any other drugs or herbs.

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