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Evaluation of the antimalarial activity of *Curcuma longa* Linn., singly and in combination with *Eupatorium odoratum* Linn.

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ABSTRACT

Following the observation of in vitro anti-malarial activity of the ethanolic extract of *Curcuma longa* Linn., the active compound was isolated from its rhizome and detected *in vitro*, the results showed that schizont suppression of 62.63% even at the lowest concentration 0.625 µg/ml on human malaria with IC_{50} values of <0.625ug/ml. Another test sample extract of *Eupatorium odoratum* Linn, revealed that the significant antimalarial activity with IC_{50} value of 0.8581 ug/ml. The combination of *C. longa* and *E. odoratum* was found to be IC_{50} of <0.625 µg/ml showing their probable synergistic effect on the human malaria parasite. The extract and isolated compound of *C. longa* was further tested for their antimalarial activity using *Plasmodium berghei* mouse model. The highest *in vivo* parasite suppression was found to be 67.9% and 72.97% at the dosage of 50 mg/kg body weight for *C. longa* extract and isolated compound respectively. Moreover, brine shrimp toxicity test and in vivo toxicity test in mice showed no lethal effect of both *C. longa* and *E. odoratum* at the tested concentration which is moderately high. The results showed that both plant samples are potentially safe and possess prospective anti-malarial activity. It can be used as a single or combination.

Keywords: Extracts, *Curcuma longa* Linn, *Eupatorium odoratum* Linn, *Plasmodium falcipium*, *Plasmodium berghei*.

INTRODUCTION

P*lasmodium falciparum*, the most dominant and pathogenic of the four human plasmodia, is responsible for almost all malaria mortality and morbidity in tropical and subtropical countries.[1] Although an effective vaccine is the best longterm control option for malaria, current work on vaccine development largely remains at a preclinical stage, and considering the different phases of vaccine development, it is predicted that a reliable malaria vaccine may be several years away. Thus, the current management strategy mainly depends on chemotherapy. However, multiple antimalarial drug-resistant *P. falciparum* is causing not only the spread of malaria to new areas but also to its reemergence in areas where it had previously been eradicated. Many antimalarial drugs in current usage are chemically related and hence the development of resistance to one drug can facilitate the development of resistance to others.

Currently, malaria chemotherapy is targeting the use of drug combinations, providing a possible means of enhancing the anti-parasitic activity as well as circumventing or delaying the induction of drug resistance.[2] Hence, the present investigation is to find out which have antimalarial activity of Myanmar medicinal plants by using *in vitro* and *in vivo* methods.

MATERIALS AND METHODS

Preparation of Crude Plant Extract

The rhizome powder of *Curcuma longa* Linn. and *Eupatorium odoratum* Linn of 500 g were macerated with 95% ethanol for one month with regular stirring and then filtered. The filtrate was concentrated in a rotatory evaporator and allowed to dryness.

Isolation of Compound from Rhizome of *Curcuma longa* Linn. By using Solvent Separation Method

Extracted ethanol residues were triturated with petroleum ether or special boiling point till complete extraction was affected. Therefore two portions were obtained as SBP soluble and SBP insoluble fraction. SBP insoluble fraction was triturated with chloroform as in the previous method to obtain chloroform

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soluble and chloroform insoluble fraction. that of authentic compound.

Chloroform insoluble fraction was dissolved in methanol, which was all soluble in methanol. The isolated compound was purified and identified by UV, IR sectrum with

***In Vitro* Antimalarial Test**

Plant extract of *Curcuma longa* and *Eupatorium odoratum* and Isolated compound, curcumin were assessed for *in vitro* activity against *Plasmodium falciparium* using a method modifying the microdilution technique of Desjardins *et al.,*[3] and Le Bras *et al*.,[4]. *Plasmodium falciparum* infected blood samples from malaria patients attended the Malaria Control Clinic (Department of Health), Gyogone, Yangon were used for the experiments. Informed connect was obtained from each selected patients. Serial double dilutions of the extracts were made in duplicate in sterile 96-well flat botton microtitre plates.

All the wells containing drugs, extracts and compound were dosed with 100 µl of malaria infected blood to each well and non-infected blood for erythrocyte control wells. Each concentraction of each sample100 µl in volume was tested in duplicated manner. The test plate was placed in a candle jar and incubated at 37ºC for 24 to 40 hours in an incubator.

The solvent control containing the same concentration of the solvent as that used in the test wells (≤ 0.05%) was also included. The extracts and compound concentration ranged from 0.625 µg/ml to 40 µg/ml. Chloroquine diphosphate was included as a positive control.

After incubation, the supernatant in each test well was removed by using an eppendorf pipette with clean tip and the red blood cells deposited on the flat bottom of the wells were transferred on to clean microscopic slides to prepare thick blood film. The resulted blood films were air dried for 24 hours. Then the blood films were stained with 10 % Giemsa stain for 20 minutes.

***In Vivo* Drug Testing**

This experiment was conducted to find out the effect of crude extract and isolated compound of *Curcuma longa* Linn. on *Plasmodium berghei* infection in mice by using suppressive test. Supprerssive test was carried out as described by Peters, 1965, and Kiseko Kamei *et al*., 2000 with some modifications.

Mus musculus mice about 4 to 5 weeks old were inoculated intraperitoneally with 1x 10^7 *P. berghei* infected erythrocytes on day 0. The mice were then randomly divided into 6 groups of with 4 mice in each group. One group was treated with PBS as negative control group, another group as treated with chloroquine diphosphate standard dose-10 mg/kg/day as positive control and the others were treated orally with different dose levels of test samples daily for 5 days starting 1-2 hours (day 0) after inoculation of the parasites. Doses of 50,100, 200 mh/kg twice a day of both samples were given to individual groups by oral administration. The blood films were taken daily until day 10 after initiation of infection and the mean erythrocyte infection rate of each group of mice was measured. From these data, the 50% effective dosage (ED_{50}) was worked out. Percent suppression of parasitaemia in terms of control was also calculated. The drugs having a 25 percent suppression of parasitaemia or more in the test group compared to that of the control was taken as having an antimalarial activity.

RESULTS AND DISCUSSIONS

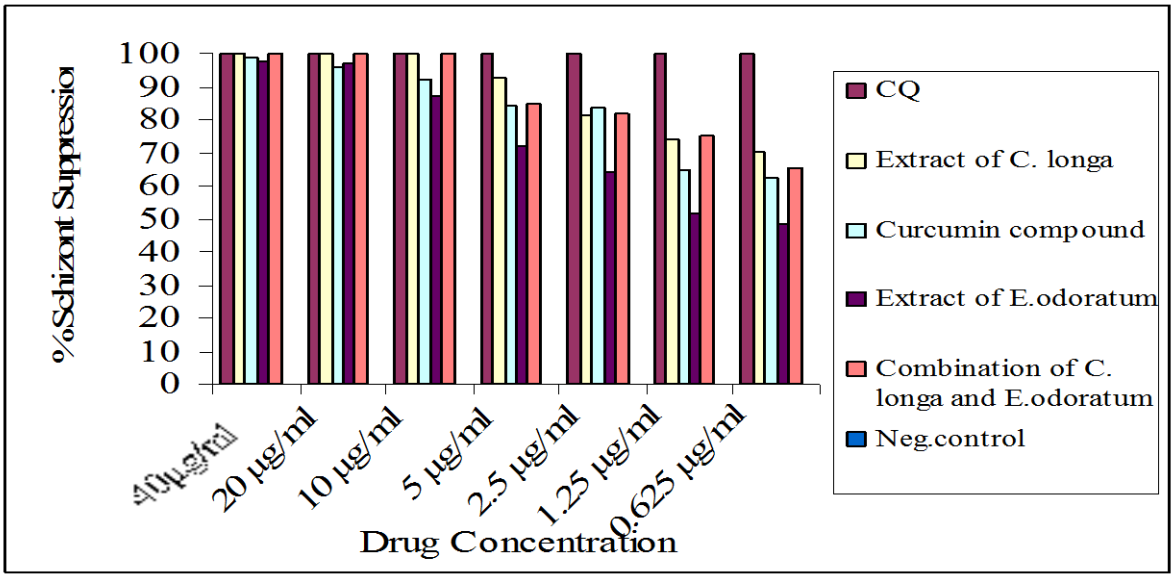
The active compound was isolated from its rhizome and identified by UV-IR spectrum following the observation of the *in vitro* anti-malarial activity of the ethanolic extract of Curcuma longa Linn. Isolated compound was found to be Curcumin (Table 1).

Table 1: Yield percentage of Extract and Isolated Compound of Plant samples

Samples	Sample Weight	Crude Weight	% Yield
Extract (*C. longa* Linn.)	500g	89.0g	17.8
Extract (*E*. *odoratum* Linn.)	500g	62.5g	12.1
Isolated compound	500g	41.0g	8.2

Table 2: Percent Schizont Suppression of Crude Extracts and Isolated Compounds of *Curcuma long* Lin*n.* and *Eupatorium odoratum* Linn

Samples	40 µg/ml	20 µg/ml	10 µg/ml	5 µg/ml	2.5 µg/ml	1.25 µg/ml	0.625 µg/ml
Chloroquine diphosphate	100	100	100	100	100	100	100
Extract of *C. longa*	100	100	100	93	81	74	70.1
Curcumin compound	98.5	95.64	92.23	84.03	83.63	64.74	62.63
Extract of *E.odoratum*	97.73	96.77	87.39	72.22	63.97	51.65	48.61
Combination of *C. longa and E.odoratum*	100	100	100	84.84	82.08	75.15	65.29



Neg. control (NC) = Infected blood sample without drug; CQ = Chloroquine diphosphate

Figure 1: Comparison of Schizont Suppression of Extracts and isolated compound from Various Plant Samples Compared to that of Control

Table 3: Parasitemia Suppressive Test of Ethanol Extract and Isolated Curcumin Compound of *Curcuma longa* Linn. against *P. berghei* in mice

Test substances	Dose mg/kg	% parasitemia Day- 7	% suppression
Extract of Curcuma longa Linn.	200	1.82	38.51
	100	1.60	45.95
	50	0.95	67.90
Isolated curcumin compound	200	1.50	49.32
	100	1.00	66.21
	50	0.80	72.97
PBS (Negative control)	1ml/mouse	2.96	0.00
Chloroquine (Positive control)	10	0.73	75.35

Each results is a mean of 4 mice

In *in vitro* anti-malarial activity test, the crude extract and isolated compound of *C. longa* Linn. have significant antimalarial activity 70.1% and 62.63% at the lowest concentration (0.625 µg/ml) with IC_{50} values of <0.625 µg/ml respectively on human malaria(Table 2 and Fig 1). The higher percent schizont suppression of crude extract compared to isolated compound could be due to the presence of active constituents included in the crude extract that could be remained after isolation of the compound. The exact mechanism(s) of curcumin's antimalarial action is not clear. The inhibition of parasite growth in culture suggests a direct mechanism of action involving parasite biochemical processes. One of the orthologue of mammalian sarcoplasmic–endoplasmic reticulum Ca2+-ATPase (SERCA). possible targets for curcumin action could be PfATP6, the parasite Artemisinin has recently been shown to inhibit PfATP6. It is therefore possible that curcumin and artemisinin act through similar mechanisms.[5]

Another test plant extract of *Eupatorium odoratum* Linn. showed moderate schizont suppression of 48.61% with IC_{50} values 0.8581 µg/ml, however, the schizont suppression activity (65.29%) became higher when it combined with extract of *C. Longa* Linn at the same concentration showing their probable synergistic effect on the malaria parasite.

The results of *in vivo* study indicated that, ethanol extract and isolated compound of *C. Longa* Linn. displayed a significant activity against *P. berghi*. The 200 mg/kg of ethanol extract and isolated compound showed significant difference on day 7parasitemia level compared to the negative control. The 100 mg/kg also showed a significant difference on day 7 parasitemia level. A high level of suppression (67.09% and 72.97%) inhibition at 50 mg/kg dosage of extract and isolated compound of *C. longa* Linn. respectively (Table 3). Hence, the *in vivo* test results also in agreement with that shown in *in vitro* test result. Reddy *et al.*[5] were discovered Curcumin treatment resulted in an overall survival rate of 29% compared to 0% in vehicle-fed animals by day 21 post-infection. Curcumin was administered 48 h after infection (3–5% parasitemia), once daily for 5 days at a dose of 100 mg/kg body weight. Nandakumar DN, *et al* also found that 3 oral doses of curcumin following a single injection of α,β-arteether to *Plasmodium berghei* infected mice were able to prevent recrudescence due to α,β-arteether monotherapy and ensure almost 100% survival of the animals.

CONCLUSION

In vitro and *in vivo* results showed that both of tested samples have significant antimalarial activity. It was observed that there was no lethal effect on experimental mice model. Therefore, *Curcuma longa*

Linn. and *Eupatorium odoratum* Linn. are potentially safe and could be used as traditional healer in treatment of malaria disease.

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