

IN VITRO RESPONSE OF *Plasmodium falciparum* TO CHEMICAL CONSTITUENTS ISOLATED FROM THAI MEDICINAL PLANTS

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OBJECTIVE : To identify and chemically isolate components of Thai Medicinal Plants exhibiting an inhibitory effect on *in vitro* growth of *Plasmodium falciparum*.

BACKGROUND : Numbers of Thai Medicinal Plants are specified for treatment of malaria infection in Folk Medicine. Thai villagers residing in various endemic areas throughout Thailand are familiar with these plants and use them for alleviating symptoms of malaria. During World War II, due to the shortage of synthetic antimalarials, Thai Medicinal Plants played an important role in self treatment of malaria.

In 1950, clinical trials (1) on treatment of *P. vivax* and *P. falciparum* infected patients with preparations of various Thai Medicinal Plants confirmed the schizontocidal effect of a number of these plants. To date, there have been no further reports published concerning the antimalarial activity of the components or the chemical characterization of their active substances.

METHODS : The *in vitro* cultivation system described previously (2) is used for comparison of the parasitic growth. Water and alcoholic extracts prepared from dry powder of either the stem bark, whole plant or root of five different local Medicinal Plants are included in the culture system comparison with standard antimalarials. Evaluations of the schizontocidal effect are determined by parasite count made on Giemsa stained thin smears taken at beginning and the end of the experiment.

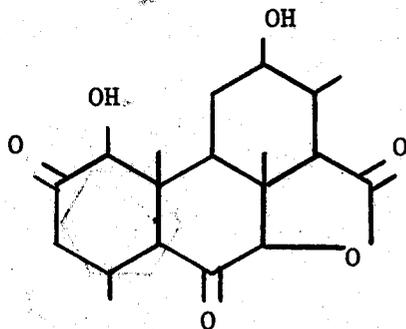
RESULTS : Thai Medicinal Plants selected for this study were :

1. Whole root of *Eurycoma longiflora* : SIMAROUBACEAE
2. Stem bark of *Oroxylum indicum* : BIGNONIACEAE
3. Whole plant of *Tinospora rumphii* : MENISPERMACEAE
4. Whole root of *Morinda coreia* : RUBIACEAE
5. Stem bark of *Cinchona succirubra* : RUBIACEAE

An investigation was carried out to examine the chemical extracts of these plants on the *in vitro* development of *P. falciparum* isolated from naturally infected patients. Different concentrations of crude alcoholic and water extracts were added to the culture medium. A control with ethanol at the concentration introduced with the plant preparations was included in each experiment. The antimalarial effect of test substances were compared with

that of chloroquine and quinine. The results showed that ethanol at the concentration introduced in the culture exerted no effect on normal development of the parasites. An inhibitory effect on the parasite development was clearly demonstrable by both alcoholic and water extracts of *Cinchona succirubra* and *Eurycoma longiflora*. The other three plants, this effect can be detected only at a very high concentration of the alcoholic extract (Table 1).

Results obtained from crude plant extracts indicated a high content of a compound exhibiting antimalarial activity in the root of *Eurycoma longiflora*. Thus an intensive chemical analysis was conducted on this plant. Five different fractions (A,B,C,D,E) of various quantities were obtained from a combined solvent extraction and chromatographic technique. Only two fractions (B,C) were obtained with sufficient quantity for further studies. The *in vitro* inhibitory effect on *P. falciparum* development was confirmed on preparation of fraction B. Further purification was conducted on both fractions (B and C). Four different compounds (a,b,c,d) were isolated from fraction B, while only one compound was isolated from fraction C. The major compound (a) isolated from fraction B was identified as lactone with the structural formula as shown below :



An *in vitro* study on the effect of these isolated compounds on *P. falciparum* development was conducted (Table 2). Results obtained indicated that the antimalarial activity of *Eurycoma longiflora* is confined to the Eurycomalactone which can be isolated as pure compound in a fair yield.

REFERENCES :

1. Ketusingh, O. : Report on Experimental Antimalarial Therapy of Thai Medicinal Plants. Proceedings of the Siriraj 60th Anniversary Meeting April, 275-281, 1950.
2. Iber, K.P., et al. : Evaluation of In vitro Drug Sensitivity of Human *Plasmodium falciparum* by Incorporation of Radioactive Isoleucine. J. Med. Ass. Thailand 58:559-566, 1975.

Table 1. *In vitro* Effect of Medicinal Plant Preparation on *P. falciparum*

		Conc. of total inhibition ($\mu\text{g/ml}$ blood suspension)
1.	Control	-
2.	Alcohol	-
3.	Chloroquine diphosphate	0.8521
4.	Quinine sulphate	1.4063
5.	<i>Cinchona succirubra</i> Alc. Ext.	3.05
6.	<i>Cinchona succirubra</i> Water Ext.	19.05
7.	<i>Eurycoma longiflora</i> Alc. Ext.	6.72
8.	<i>Eurycoma longiflora</i> Water Ext.	13.37
9.	<i>Oroxylum indicum</i> Alc. Ext.	627.56
10.	<i>Oroxylum indicum</i> Water Ext.	no effect (672.75)
11.	<i>Tinospora rumphii</i> Alc. Ext.	720.5
12.	<i>Tinospora rumphii</i> Water Ext.	no effect (715.75)
13.	<i>Morinda coreia</i> Alc. Ext.	63.5
14.	<i>Morinda coreia</i> Water Ext.	170.13

Table 2. Effect of Fractions and Compounds Isolated from Root of *Eurycoma longiflora* on *in vitro* Growth of *P. falciparum*

	Conc. of total inhibition ($\mu\text{g/ml}$ blood suspension)
1. Fraction B	1.5653
2. Fraction C	12.6440
3. Compound a	0.3945
4. Compound b	no effect (3.00)
5. Compound c	no effect (3.175)
6. Compound e	no effect (3.125)

Extraction and Purification of *Eurycoma longiflora*

