Antimalarial activity and chemical studies of *Zingiber aromaticum* (Zingiberaceae) used as traditional medicine in Indonesia

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Abstract

Malaria is one of the major health threats in the world especially in developing countries. The high price of medicinal drugs has necessitated the optimization of traditional medicine especially from plants with antimalarial properties as a source for inexpensive treatment of this disease. *Zingiber aromaticum* is the folk medicinal plant commonly used in Indonesia for the treatment of malaria. However, a scientific validation of this utilization was not previously observed. The aim of these studies was to evaluate the potential efficacy of *Z. aromaticum* as a source of new antimalarial drug. The methanol extract from the rhizome of *Z. aromaticum* were fractionated by vacuum liquid chromatography (VLC) for further bioassay and chemical characterization. Fractionation and isolation of the methanolic extract by TLC followed by spectroscopic techniques IH-NMR, C-NMR and FTIR led to the identification of the active compound Zerumbone. The antimalarial activity against *Plasmodium falciparum* malaria parasites to assess the concentrations for 50% inhibition (IC_{50}) of zerumbon and extracts was screened *in vitro*. The results showed that activity of methanol and n-hexane extracts have a week antiplasmodial activity with IC_{50} 74.13 µg/ml and 53.45 µg/ml respectively; whereas Zerumbone was less active with IC_{50} 97.39 µg/ml.

Keywords: Antimalarial activity, *in vitro* study, *P. falciparum*, *Z. aromaticum*, Zerumbone,

Introduction

In the last few years, the re-emergence of malaria is influenced by several factors including the health care system, human migration, climate and the environmental changes, development of resistant strains of malaria parasite to anti-malarial drugs and the resistant strains of Anopheles to insecticide (1, 2). In Indonesia particularly in Sulawesi island, Malaria morbidity in 2006 was reported 15,17%, with the highest number of malaria incidences was in Mamuju. The analysis of 1,113 blood smears obtained from random individuals found 59 samples (5.3%) consisted of either *Plasmodium falciparum*, *P. vivax*, or in combination of both species (3, 4).

One of the efforts to overcome malaria is based on an appropriate treatment for the patients. However, due to limited availability of medicinal drugs in many remote areas of Indonesia, malaria is still endemic throughout the country. Moreover, the emergence of chloroquine-resistant strains of malaria parasites and the high price of other medicinal drugs increase the difficulty of eradicating malaria in community (2, 5). Therefore, the scientific researches for discovering the potential of new antimalarial drugs from nature especially from plants have increased substantially worldwide.

Some Zingiberaceae rhizome plants have historically been used as ethnomedical plants for malaria. Studies of *Zingiber officinale* and Zerumbone, a sesquiterpene, isolated from rhizome of *Zingiber zerumbet*, showed in vitro antimalarial activities against malaria paracites (5, 6, 7, 8). Another species of zingiberaceae which also has zerumbone compound and widespread found in Indonesia as traditional herbal remedies in the management of malaria is *Zingiber aromaticum*. Nevertheless, a scientific validation of this utilization was not previously observed. Therefore, this study was designed to examine the potential efficacy of *Z. aromaticum* as a source of new antimalarial drug against *Plasmodium falciparum* malaria parasites by assessing the concentrations of 50% inhibition (IC_{50}) of zerumbone and extracts of *Z. aromaticum*.

Materials And Methods

Sample preparation and Chemical Studies:

Rhizomes of *Zingiber aromaticum*, Vahl were collected from Maros, South Sulawesi, Indonesia. The plant was identified by a botanist from the Department

of Biological Sciences Hasanuddin University Indonesia. Dried and grinded rhizomes (500 g) were extracted with methanol and hexane as the solvents by modified maceration method at room temperature. Removal of solvent under reduced pressure using rotary evaporator then subsequently followed by evaporation to dryness using vacuum and freeze drier resulted crude extract of MeOH 24.54 g and Hexane extract 23.63 g. Four grams of MeOH extract was further fractionated using Vacuum Liquid Chromatography (VLC) by gradually increased polarity of elution solvents system (dichloromethane and MeOH). The fractions were collected separately and monitored by Thin Layer Chromatography (TLC) with a mobile phase combined solvents dichloromethane-methanol (8:2, v/v) and the stationary phase was MERCK silica gel 60 GF254 (0.200 mm). The fraction which showed similar spot to reference Zerumbone was subsequently subjected to chemical characterization by using spectroscopic techniques IH-NMR, C-NMR and FTIR.

Parasite Culture

P. falciparum used for the in vitro test was a chloroquine-resistant isolate. Parasites were maintained continuously using a simple candle jar method in RPMI 1640 medium containing 25 mM HEPES and supplemented with gentamycin, 0.23% sodium bicarbonate ($NaHCO_3$), and 10-15% human serum from blood type 'O'. Synchronization of the parasites to uniform the ring stages was achieved using 5% aqueous D-sorbitol. The parasite culture contents were centrifuged to pellet the cells. The supernatant media was discarded and packed cells were suspended in 10 times volume of 5% aqueous D-sorbitol and allowed to stand for 5-10 min. The cells were washed twice with RPMI medium without serum to remove sorbitol and appropriate volume of serum containing RPMI 1640 medium were added to obtain 4% hematocrit. The observation of parasitized ring stage erythrocytes and parasitaemia level were monitored by Giemsa staining method. The different stages of parasitized erythrocytes were microscopically observed using 10x100 magnification (9, 10, 11, 12).

In vitro anti-malarial tests

The antiparasitic effects of methanol extract, hexane extract and Zerumbone compound of *Z. aromaticum* were measured by the percent inhibition of parasite growth in relation to the control (parasites cultivated in medium alone), as previously described (11). The extracts and zerumbone compound individually were diluted with their solvents in various concentrations of 0,1µg/mL, 1µg/mL, 10µg/mL, 100 µg/mL and 1000µg/mL. order to This serial dilutions and the control medium were remained to dry in 96 flat-bottom micro plates before adding the parasitized ring stage erythrocytes at 1% parasitemia level. The plates were agitated gently to ensure that the extracts homogenize in the blood mixture before incubation in a candle jar for either 48 h or 72 h at 37^{o}C. At different sequential intervals, the plates were removed and blood smears from the settled RBCs were made from each micro well. The Giemsa-stained smears were examined under the microscope (10x100) and the preschizonts (representing schizonts with at least 3 nuclei) were calculated for 200 asexual parasites in each smear. Each experiment was performed in triplicate and repeated three times. The blood smears were read in a double-blind manner.

Result And Discussion

In an attempt to search a novel blood schizonticides for treating malaria caused by chloroquine-resistant strains of *P. falciparum,* other studies showed that Zerumbone isolated from *Zingiber zerumbet* has a strong antimalarial activities with IC_{50} value of 11.8µM (sriphana). In this present study, Zerumbone were isolated from other species of Zingiberaceae i.e. *Zingiber aromaticum.* TLC identification showed that this compound has similar color of spot and Rf value (0.85) to the reference Zerumbone.

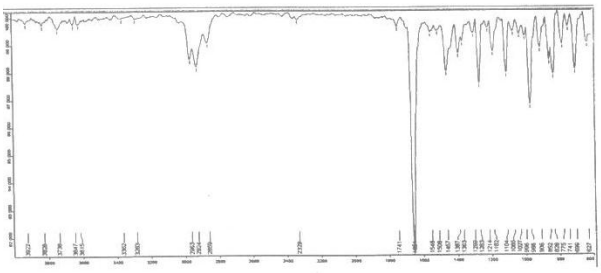

Fig. 1 Spectra of Zerumbone showed the absorption band of carbonyl group (1700 cm^{-1}), 1460, 1070 cm^{-1} identified by Spectrophotometry FTIR

Spectra of Zerumbone isolated from *Z.aromaticum* showed 15 carbon signals including the carbonyl carbons (δ 204.353) which similar to the molecular structure of Zerumbone **($C_{15}H_{23}O$).**

Table 1. Spectra data of Zerumbone in dichloromethane ($CDCl_3$) investigated by ^{1}H- and ^{13}C-NMR

Position	Zerumbone isolated from MeOH extract of *Z.aromaticum*			
	δ H	**Amount**	**δ C**	**Amount**
1, 1”	1,9203	2H-1	42,411	C1
2, 2”	2,3804	1H-2	124,995	C2
3, 3”	-	-	136,282	C3
4, 4”	2,5189	2H-4	24,402	C4
5, 5”	2,4725	2H-5	39,449	C5
6, 6”	5,8835	1H-6	148,829	C6
7, 7”	-	-	137,961	C7
8, 8”	-	-	204,353	C8
9, 9”	6,0318	1H-9	127,718	C9
10, 10”	5,9994	1H-10	160,742	C10
11, 11”	-	-	23,349	C11
12, 12”	1,6951	3H-12	29,700	C12
13, 13”	1,7982	3H-13	37,869	C13
14, 14”	1,0703	3H-14	15,220	C14
15, 15”	1,1856	3H-15	11,798	C15

Fractionation and isolation of *Z. aromaticum* methanol extract by TLC followed by investigation of the chemical structures using spectroscopic techniques IH-NMR, C-NMR and FTIR has led to identify the Zerumbone compound. For assessing the biological activity against *Plasmodium falciparum* malaria parasites, the crude methanol and hexane extracts as well as the Zerumbone compound was tested *in vitro.* The growth inhibition assays on the Chloroquine-resistant (W2) laboratory strain of P. falciparum are summarized in table 2.

Table 2. Percentage inhibition of synchronous W2 strain of *P. falciparum* by different concentrations of Zerumbone and *Z. aromaticum* extracts after 72 h incubation

Concentrations (µg/mL)	Average Percentage of Parasitemia Inhibition		
	Zerumbone	**MeOH extract**	**Hexane extract**
0.1	7.10	14.04	2.84
1	13.10	20.36	11.59
10	35.73	36.25	31.68
100	41.73	42.80	50.19
1000	73.87	77.95	84.52

The crude extracts and the Zerumbone compound isolated from *Z. aromaticum* rhizomes partially reduced the malaria parasitemia *in vitro*. The hexane extract was the most active which caused 50% and 84% reduction of parasitemia at doses 100 µg/mL and 1000 µg/mL respectively. Further calculation of its IC_{50} showed the lowest values (53.45 µg/mL), whereas methanol extract and Zerumbone compound exhibited lower parasitemia activity with IC_{50} values 74.13 µg/mL and 97.39 µg/mL respectively.

Table 3. Average IC_{50} of Zerumbone and *Z. aromaticum* extracts for *P. falciparum*

Substrates	**IC_{50} (µg/mL)**
Zerumbone	97.39
MeOH extract	74.13
Hexane extract	53.45

Although all of the compounds tested still can be categorized as an active antimalarial agent with weak potential activity, their antiplasmodial properties were lower than expected. The chemical transformation of Zerumbone compound isolated from *Z. aromaticum* rhizomes to optimize and enhance the antimalarial activity is worthy for further investigation.

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