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In vitro antiplasmodial activity of *Dysoxylum caulostachyum* (Miq) and *Garcinia celebica* (L) leaf extracts against *Plasmodium falciparum*

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Abstract

Aims:
Malaria is known as acute febrile illness in endemic areas and the most infectious diseases in the world. Malaria in humans is caused by five species of parasites belonging to the genus *Plasmodium*. The search for new remedies from medicinal plant species is an alternative choice for the treatment of malaria. In this study, we investigated the *in vitro* antiplasmodial activity of ethanol extracts from *Dysoxylum caulostachyum* (Miq) and *Garcinia celebica* (L) leaves against *Plasmodium falciparum* 3D7.

Methods:
The in vitro antiplasmodial activity test was conducted by determining the parasitemia for each sample concentration by manual counting on thin Giemsa smears after a 48-hour incubation with the extracts in order to determine the IC_{50} values.

Results:
The testing revealed that the ethanol extracts of *D. caulostachyum* (Miq) and *G. celebica* (L) leaves exhibited antiplasmodial activity against *P. falciparum* with IC_{50} values of 3.75 µg/mL and 9.13 µg/mL, respectively.

Conclusion:
In conclusion, the ethanol extract of *D. caulostachum* (Miq) leaf exhibited the moderate to high antiplasmodial activity (1.1-10 µg/mL), while the ethanol extract of *G. celebica* (L) leaf exhibited the weak antiplasmodial activity (11-25 µg/mL). This study may be reasonably considered for *D. caulostachyum* (Miq) leaf extract to continue further work for isolation of antiplasmodial compounds through a bioassay-guided fractionation method.

Keywords: Antiplasmodial activity, *Dysoxylum caulostachyum* (Miq), *Garcinia celebica* (L), *in vitro*, *Plasmodium falciparum* 3D7

1. INTRODUCTION

Malaria is known as acute febrile illness in endemic areas and the most infectious diseases in the world [1]. Malaria in humans is caused by five species of parasites belonging to the genus *Plasmodium*, The four of which are *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale* which are human malaria species that are spread from one person to another via the bite of female mosquitoes of the genus *Anopheles*. *P. falciparum* is the most prevalent on the African continent, and is responsible for most deaths from malaria [2]. People affected by malaria will suffer from symptoms of fever, chills, sweating, headache, nausea or vomiting. In Indonesia, Papua and West Papua have the highest Annual Parasite Incidence (API) number in 2015 that represents the number of malaria positive cases per 1,000 population in a year [3].

The search for new remedies from medicinal plant species used as an alternative choice for the treatment of malaria depends on the accurate and specific ethnobotanical and ethnopharmacological information obtained from local healers [4]. The plants are usually used to treat the malaria disease, and the people commonly use the extract of this plants for this purpose [5].

The area of nature reserves in Indonesia, especially in Pangandaran, is an area that has geological elements where local people are invited to participate in protecting and improving the function of natural heritage, including the archaeological, ecological and cultural values in it. In this area, there is a natural potential that can be used as a source of malaria treatment.

In previous study, Subarnas *et al.* (2012) have tested 42 primates-consumed plants from Pangandaran Beach Conservation area for their antiproliferative activity against cell lines of human breast adenocarcinoma (MCF-7). The results showed that four extracts of *Dysoxylum caulostachyum*, *Eugenia aquea*, *Garcinia celebica*, and *Psychotria valentonic* leaves strongly inhibited the MCF-7 cell proliferation with IC_{50} values of 12, 58, 87, and 87 µg/mL, respectively [6].

In the series of our investigations, we have currently evaluated two species of Indonesian primate-consumed plants for their in vitro antiplasmodial activity against *Plasmodium falciparum* 3D7 in the search for a new natural antimalarial agent. The plants used for the study were *D. caulostachyum* (Miq) and *G. celebica* (L). The results showed that the extracts of *D. caulostachyum* (Miq) and *G. celebica* (L) leaves exhibited moderate to high antiplasmodial activity (1.1-10 µg/mL) against *P. falciparum* [7]. The phytochemical screening of the extracts was also performed to detect the presence of groups of secondary metabolite compounds contained in the extracts as a preliminary step to search compounds that potentially have antiplasmodial activity. Further research can be developed further in the search of new antiplasmodial compounds that have the potential to be developed as a herbal medicine for malaria treatment.

2. MATERIALS AND METHODS

2.1 Plant material

Leaves of *D. caulostachyum* (Miq) and *G. celebica* (L) were collected from Pangandaran Beach Conservation area, Ciamis, Central Java, Indonesia. The plant materials were authenticated and determined (number 426/HB/08/2017 and 427/HB/08/2017) in herbarium by a senior taxonomist scientist at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematic and Natural Science, Universitas Padjadjaran, Indonesia.

2.2 Extraction

Fresh leaves of *D. caulostachyum* (Miq) and *G. celebica* (L) were air dried in the laboratory at room temperature (30 ± 2°C) for 10

days, cut into small pieces using blender and the sample obtained were stored until further use. 250 g of the sample from *D. caulostachyum* (Miq) were extracted with ethanol (70%) of 1.75 L for 24 hours. The extraction process was repeated with ethanol (70%) of 1.0 L for 24 hours, and then 0.75 L for another 24 hours. Then, 250 g of the sample from *G. celebica* (L) were extracted with ethanol (70%) of 2.25 L for 24 hours. The extraction process was repeated with ethanol (70%) of 2.25 L for 24 hours, and then 2.25 L for another 24 hours. The mixtures were filtered using Whatman paper No.1. The filtrates measured were evaporated using IKA® RV 10 rotary evaporator at a temperature of 40-50°C to produce viscous extracts with constant weight.

2.3 Phytochemical screening of extracts

The phytochemical screening of the extracts was performed to detect the presence of groups of secondary metabolite compounds contained in the extracts, including groups of alkaloid, polyphenol, tannin, quinone, saponin, flavonoid, monoterpene and sesquiterpene, steroid and triterpenoid. Phytochemical screening was performed using standard procedures [8].

2.4 *In vitro* antiplasmodial testing

The ethanol extracts of *D. caulostachyum* (Miq) and *G. celebica* (L) leaves were tested against *P. falciparum* strain 3D7 (MRA-102, MR4, ATCC, Manassas, Va, USA). *P. falciparum* were cultured in RPMI medium 1640 (Sigma Aldrich, St. Louis, Mo, USA) supplemented with 10% human serum, 0.005% hypoxanthine, 0.21% $NaHCO_3$, 0.596% HEPES, 0.25% gentamicin and 3% human erythrocytes. Cultures were stored as thin layers in 25 mm^3 flasks in a candle jar at 37°C. Parasitemia was determined by manual counting on thin Giemsa smears. Parasite cultures at 2% parasitemia were placed in a 96-well plate (180 µL per well) and incubated with various concentrations of extracts, ranging from 0.01-10,000 µg/mL achieved with ten-fold serial dilutions. After a 48-hour incubation with the drug, thin Giemsa smears were made and parasitemia for each sample concentration was determined, parasitemia versus extract concentration was plotted and the IC_{50} was determined [9].

3. Results And Discussion

3.1 Phytochemical screening of extracts

The phytochemical screening test of the ethanol extract of *D. caulostachyum* (Miq) leaves showed the presence of polyphenol, quinone, flavonoid, monoterpene and sesquiterpene, and no alkaloid, tannin, saponin, steroid and triterpenoid were identified (Table 1). In the ethanol extract of *G. celebica* (L) leaves, all secondary metabolites were present, except steroid and triterpenoid were not identified (Table 1). The phytochemical screening was performed as a preliminary step to search for compounds that potentially have antiplasmodial activity. It is worthwhile testing crude extractives from plants and also compounds isolated on the basis of bioguided fractionation for antimalarial [5].

Ultimately, the goal in searching plants for biologically active or medicinally useful compounds should be to isolate the one or more constituents responsible for a particular activity. Hence, with the selection of plant for phytochemical investigation, either on the basis of one or more approaches set forth under phytopharmacological approaches, or through some other avenue, phytochemical screening techniques can be a valuable aid [8].

3.2 *In vitro* antimalarial testing

The results from the *in vitro* antiplasmodial testing of ethanol extracts form from *D. caulostachyum* (Miq) and *G. celebica* (L) leaves revealed that the extracts exhibited antiplasmodial activity against *P. falciparum* 3D7 with an IC_{50} values of 5.10 µg/mL and 11.34 µg/mL, respectively (Table 2). Based on the IC_{50} values, *D. caulostachyum* (Miq) leaf extract showed the highest antiplasmodial activity than *G. celebica* (L) leaf extract. To discriminate active extracts, Rasoanaivo (2004) previously ranked the levels of antiplasmodial activities [7][10] (Table 3).

Table 1: Phytochemical constituents of ethanol extracts of *D. caulostachyum* (Miq) and *G. celebica* (L) leaves

Phytochemical screening test	Extracts	
	D. caulostachyum (Miq)	*G. celebica* (L)
Alkaloid	-	+
Polyphenol	+	+
Tannin	-	+
Quinone	+	+
Saponin	-	+
Flavonoid	+	+
Monoterpene and sesquiterpene	+	+
Steroid and triterpenoid	-	-

Note: (+) Detected, (-) Undetected

Tabel 2: *In vitro* antiplasmodial activity of *D. caulostachum* (Miq) and *G. celebica* (L) leaf extracts

Botanical names	Plant part	IC_{50} (µg/mL)	Level of antiplasmodial activity
D. caulostachyum (Miq)	Leaf	5.10 ± 1.08	Good to moderate
G. celebica (L)	Leaf	11.34 ± 1.10	Weak

Table 3: Proposed thresholds for *in vitro* activity of antiplasmodial extracts [7][10]

IC_{50} (µg/mL)	Level of activity
< 0.1	Very good
0.1–1.0	Good This is the interval of concentrations which is generally considered as active in screening programmes for antimalarial activity, warranting bioassay-guided fractionation [11].
1.1–10	Good to moderate This interval may be reasonably considered for bioassay-guided fractionation.
11– 25	Weak
26–50	Very weak
>100	Inactive
0.12	IC50 of chloroquine against FCM29 strain
0.062	IC50 of chloroquine against FcB1 strain

As the results, the ethanol extract of *D. caulostachyum* (Miq) leaf exhibited the moderate to high antiplasmodial activity (1.1-10 µg/mL), while the ethanol extract of *G. celebica* (L) leaf exhibited the weak antiplasmodial activity (11-25 µg/mL). For the *D. caulostachyum* (Miq) leaf extract, it may be reasonably considered for bioassay-guided fractionation [7][10]. Afterwards, the research in searching plants for biologically active should be to isolate the one or more constituents that responsible for a antiplasmodial activity.

CONCLUSIONS

In conclusion, the ethanol extract of *D. caulostachyum* (Miq) leaf exhibited the moderate to high antiplasmodial activity (1.1-10 µg/mL) with an IC_{50} values of 5.10 µg/mL, while the ethanol extract of *G. celebica* (L) leaf exhibited the weak antiplasmodial activity (11-25 µg/mL) with an IC_{50} values of 11.34 µg/mL, and this work is reasonable to be continued for the isolation of antiplasmodial compounds from *D. caulostachyum* (Miq) plant.

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