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Author(s)	Murnigsih, Tri; Subeki; Matsuura, Hideyuki et al.
Citation	Journal of Veterinary Medical Science, 67(8), 829-831 https://doi.org/10.1292/jvms.67.829
Issue Date	2005-09-05
Doc URL	https://hdl.handle.net/2115/66408
Type	journal article
File Information	67_8_829-831.pdf



Evaluation of the Inhibitory Activities of the Extracts of Indonesian Traditional Medicinal Plants against *Plasmodium falciparum* and *Babesia gibsoni*

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(Received 18 October 2004/Accepted 22 April 2005)

ABSTRACT. Twenty-four kinds of water extracts derived from 22 plants that are traditionally used for the treatment of malaria on Java Island, Indonesia, were screened for their antibabesial and antimalarial activities. Among the extracts, 8 extracts displayed strong antimalarial activity, with an inhibition range from 89.6 to 100%, and 15 showed strong antibabesial activity, with an inhibition range from 84.2 to 98.1%. The extracts of *Achillea millefolium*, *Baeckea frutescens*, *Brucea javanica*, *Curcuma xanthorrhiza*, *Strychnos lucida* and *Swietenia macrophylla* showed both strong antibabesial and antimalarial activities. The antimalarial activities paralleled the antibabesial activities, but the converse was not true.

KEY WORDS: *Babesia gibsoni*, *Plasmodium falciparum*, traditional medicine.

J. Vet. Med. Sci. 67(8): 829–831, 2005

Babesia gibsoni is a canine intra-erythrocytic parasite that causes anemia through the physical destruction of erythrocytes by the parasite, nonspecific erythrocyte phagocytosis by activated macrophages, and the presence of a hemolytic substance in *B. gibsoni* infected serum [2, 4, 7]. Administration of the antiprotozoal drugs, such as diminazene diaceturate, pentamidine isethionate, and parvaquone decreases the mortality in dogs [5, 6]; however, they sometimes induce severe side effects [13]. One possible source of affordable treatment lies in the use of plant extracts and medicinal plants for the treatment of dogs infected with *B. gibsoni* [10].

Malaria is still the most important parasitic disease of humans in the world with an infection rate of 300–500 million persons and an annually fatal outcome of 1.5–2.7 million cases. It is estimated that about 40% of the world's population is at risk, with the fatality rate being extremely high among young children below 5 years of age [1, 8]. Malaria is a disease caused by *Plasmodium* infection transmitted via mosquitoes (*Anopheles* spp.). Typical symptoms of malaria include intervals of high fever, which are mainly induced by the lysis of erythrocytes. Thus, anemia is also induced upon infection by *Plasmodium* [12]. The mode of action and replication of malarial infection is similar to that of *B. gibsoni* [3]. Although *B. gibsoni* is transferred from ticks (*Haemaphysalis bispinosa*, *H. longicornis*) and restricted to dogs, *Plasmodium* and *B. gibsoni* have very

similar life cycles and show similar symptoms of disease [3, 12]. Therefore, it is proposed that an antibabesial component may be isolated from the extract of plants that are used for malarial treatment. The aim of this study was to identify the correlation between antimalarial and antibabesial activity of medicinal plants which are traditionally used in the treatment of malaria in Indonesia.

Sample specimens from 22 plant species reported to have antimalarial properties were collected from Surabaya (East Java) and Bogor (West Java), Indonesia (Table 1). The plant species belonged to thirteen families. Specimens were identified and deposited in The Research Center for Biology, the Indonesian Institute of Sciences. Dried plant material (10 g) from each specimen was extracted to obtain 24 extracts, by boiling the material twice for 30 min in 200 ml of water. The combined water extracts were concentrated under reduced pressure and then freeze-dried into a powder. An aliquot of the sample (1 mg) was used for the antimalarial and antibabesial activity tests according to the reported methods [9–11]. The final concentration of each extract was 1 mg/ml for both assays. Inhibitory rate was calculated according to the following numerical formula:

$$(\text{Inhibitory rate}) = [(A-B)/A] \times 100 (\%)$$

A: percentage of parasitemia in control, B: percentage of parasitemia in sample

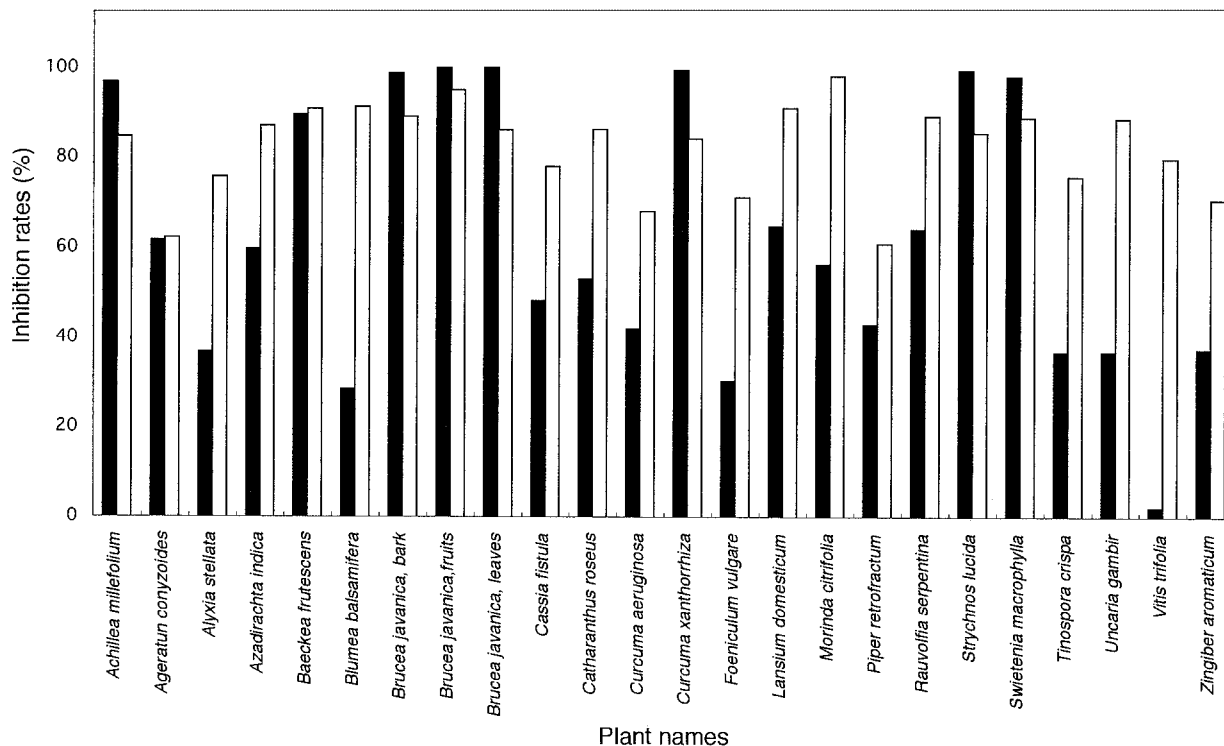
The percentage of parasitemia of *B. gibsoni* was determined by counting the number of parasitized cells in 1,000 erythrocytes, and that of *P. falciparum* was determined by counting the number of schizonts in 200 parasitized cells. Inhibitory activities of the plant extracts against *P. falciparum* and *B. gibsoni* were classified as follows; at an inhi-

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Table 1. Plant species screened for antimalarial and antibabesial activities

Botanical name	Family	Local names	Collection area	Part used	Voucher code
<i>Achillea millefolium</i> L.	Asteraceae	Daun seribu	Surabaya	Whole plant	04-SB-12
<i>Ageratum conyzoides</i> L.	Asteraceae	Babandotan	Surabaya	Whole plant	04-SB-20
<i>Alyxia stellata</i> Blume.	Apocynaceae	Pulosari	Surabaya	Bark	04-SB-02
<i>Azadirachta indica</i> Juss.	Meliaceae	Mimba	Surabaya	Leaves	04-SB-19
<i>Baeckea frutescens</i> L.	Myrtaceae	Jungrahay	Surabaya	Leaves	04-SB-07
<i>Blumea balsamifera</i> L.DC.	Asteraceae	Sembung	Surabaya	Leaves	04-SB-15
<i>Brucea javanica</i> (L.) Merr.	Simaroubaceae	Brucea	Bogor	Bark	04-BG-02
<i>Brucea javanica</i> (L.) Merr.	Simaroubaceae	Brucea	Bogor	Fruits	04-BG-03
<i>Brucea javanica</i> (L.) Merr.	Simaroubaceae	Brucea	Bogor	Leaves	04-BG-04
<i>Cassia fistula</i> L.	Fabaceae	Trengguli	Surabaya	Pods	04-SB-16
<i>Catharanthus roseus</i> L.	Apocynaceae	Tapak dara	Surabaya	Aerial part	04-SB-05
<i>Curcuma aeruginosa</i> Roxb.	Zingiberaceae	Temu hitam	Surabaya	Rhizomes	04-SB-04
<i>Curcuma xanthorrhiza</i> Roxb.	Zingiberaceae	Temu lawak	Surabaya	Rhizomes	04-SB-11
<i>Foeniculum vulgare</i> Mill.	Umbelliferae	Adas	Surabaya	Seeds	04-SB-01
<i>Lansium domesticum</i> Corr.	Meliaceae	Duku	Bogor	Bark	04-BG-06
<i>Morinda citrifolia</i> L.	Rubiaceae	Mengkudu	Surabaya	Fruit	04-SB-06
<i>Piper retrofractum</i> Vahl.	Piperaceae	Cabe	Surabaya	Fruit	04-SB-09
<i>Rouvolfia serpentina</i> (L.) Benth.	Apocynaceae	Pule pandak	Bogor	Bark	04-BG-07
<i>Strychnos lucida</i> R.Br.	Loganiaceae	Bidara laut	Bogor	Wood	04-BG-05
<i>Swietenia macrophylla</i> King.	Meliaceae	Mahoni	Surabaya	Seeds	04-SB-08
<i>Tinospora crispa</i> Miers.	Menispermaceae	Brotowali	Surabaya	Stem	04-SB-03
<i>Uncaria gambir</i> Roxb.	Rubiaceae	Gambir	Bogor	Leaves	04-BG-01
<i>Vitis trifolia</i> L.	Vitaceae	Galing	Surabaya	Leaves	04-SB-10
<i>Zingiber aromaticum</i> Valetton.	Zingiberaceae	Lempuyang	Surabaya	Rhizomes	04-SB-17

Fig. 1. Inhibition rates of plant extracts against *Babesia gibsoni* and *Plasmodium falciparum*. ■: *Plasmodium falciparum*, □: *Babesia gibsoni*.

bition rate of parasites less than 50%, the extract was considered to be inactive, from 50%–80%, it was considered to have moderate activity, and over 80%, it was considered to have strong activity. The *in vitro* antimalarial and antibabesial activities of these twenty-four extracts are summarized in Fig. 1. Almost all of the tested extracts inhibited the growth of *B. gibsoni*, while they demonstrated various levels of growth inhibition on *P. falciparum* (Fig. 1). Eight extracts displayed strong antimalarial activity, with an inhibition range from 89.6% to 100%, and fifteen extracts displayed strong antibabesial activity, with an inhibition range from 84.2% to 98.1%. The extracts of *Achillea millefolium*, *Baeckea frutescens*, *Brucea javanica*, *Curcuma xanthorrhiza*, *Strychnos lucida*, and *Swietenia macrophylla* showed strong activity against both parasites. Some of the plants collected in this study did not show any antibabesial and antimalarial activities. It is likely that the effects of these plants are due to other activities. Although we could not provide statistical analyzes for the data in Fig. 1 due to preliminary experiment (n=1), we concluded that strong antimalarial activities paralleled strong antibabesial activities, but not vis versa. These findings suggested the possibility to find antibabesial ingredients from sources possessing antimalarial activity. Deferent degrees of efficacies of the plant extracts against *B. gibsoni* and *P. falciparum* are still unknown.

ACKNOWLEDGEMENT. This study was supported by

Japan Society for the Promotion of Science (JSPS). The authors wish to thank the Herbarium Bogoriense of Indonesia for helping with the determination of voucher herbarium specimens.

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