Original Research Article

Antimalarial activity of extract and fractions of Castanopsis costata (Blume) A.DC

Maulana Yusuf Alkandahri 1,†, Afiat Berbudi 2,†,* Novi Vicahyani Utami 3, Anas Subarnas 1

1Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Padjadjaran University, Jatinangor, West Java, Indonesia.
2Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia.
3Department of Biomedical Sciences, Pharmacology Division, Faculty of Medicine, Padjadjaran University, Bandung, West Java, Indonesia.

Article history:
Received: Jan 27, 2019
Received in revised form: Apr 17, 2019
Accepted: Apr 23, 2019
Vol. 9, No. 5, Sep-Oct 2019, 474-481.

† Equal first author
* Corresponding Author:
Tel: +62 82297033616
a.berbudi@unpad.ac.id

Keywords:
Malaria
Castanopsis costata
Antimalarial drugs
Plasmodium berghei

Abstract

Objective: One of the biggest health problems in the world, which occurs in more than 90 countries, is the spread of malaria. Cep-cepan leaves (Castanopsis costata), was empirically used as an antimalarial herb in North Sumatra. Since its use has not been scientifically studied, we investigated the antimalarial activity of extract and fractions of C. costata against Plasmodium berghei ANKA (PbA) in a mouse model.

Materials and Methods: This experimental study was conducted using 32 male Balb/C mice. PbA inoculation was performed intraperitoneally with 10^6 parasites/mouse. Immediately after parasitemia reach >2% (day 0), the mice were treated orally with daily artesunate (36.4 mg/kg/day) (positive control), ethanolic extract (100, 200, and 400 mg/kg/day), and the fractions of water, ethyl acetate and n-hexane (108 mg/kg/day each) for 5 consecutive days (from day 0 to 4). Parasitemia inhibition was observed to determine the antimalarial activity of each type of C. costata extract and fractions.

Results: The administration of C. costata leaves ethanolic extract (100, 200, and 400 mg/kg) significantly inhibited the growth of PbA in Balb/C mice (42.66%, 66.2 1% and 80.99 % inhibition, respectively) (p<0.05). Similarly, all C. costata fractions also produced antimalarial activity against PbA with administration of the ethyl acetate fraction presenting the highest activity (79.85 % inhibition).

Conclusion: The C. costata leaves showed antimalarial activity against PbA. However, further studies are necessary to elucidate the underlying mechanisms of this effect and the active compounds involved. Our current study revealed that C.costata could be a potential candidate to be used as a new antimalarial drug.

Introduction

Malaria remains the main cause of death in developing countries with tropical climates. It was estimated that around 350-500 million people in the world were infected with this disease and more than 1.5
Inhibitory effect of *Castanopsis costata* extract on *Plasmodium berghei*

Million people die each year due to malaria. Rapid population growth and overpopulation, migration, and poor sanitation, have increased the spread of the disease. Malaria becomes a serious threat since many studies reported parasitic resistance upon the first-line antimalarial drugs used in the treatment and prevention of malaria (Burke et al., 2003; Hayati et al., 2012). Given that no effective antimalarial drugs have been found so far, the efforts to introduce new antimalarial drugs, both synthetic drug and plant-based medicine, are still on going, especially in exploration of plants that were traditionally used to treat malaria in various endemic regions of the world (Mustofa, 2003). Natural products particularly those used in the plant-based medicine, contain a great variety of chemical structures. Many studies screened the antiplasmodial activities of some plants as potential sources of new antimalarial drugs. Ethnopharmacological approaches seem to be promising in finding new antimalarial candidates (Bero and Quetin-Leclercq, 2011; Nogueira and Lopes, 2011; Saxena et al., 2003).

Indonesia is the second country with the the largest biodiversity in the world with the forests where 28,000 species of plants are found. It was estimated that around 2,500 plants species have potential medicinal efficacy (Elfahmi et al., 2014). Cep-cepan plant (*Castanopsis costata* (Blume) A.DC.) is one of plants that were used empirically as an antimalarial drug by the people of North Sumatra, Indonesia. However, the administration of *C. costata* as an antimalaria medication has not been scientifically investigated. As a member of Fagaceae family, *C. costata* has been known to have several therapeutic properties, such as antifever, digestion disorder relieving, wound healing, and pain killing effects (Salim et al., 2017). Previous studies reported that *C. costata* has a variety of pharmacological activities such as antibacterial (Nurtjahja et al., 2013), antioxidant (Alkandahri et al., 2016), analgesic (Salim et al., 2017), and anti-inflammatory effects (Alkandahri et al., 2018). While the anti-malaria effect of *C. Costata* has not been studied yet, its close family called *Quercus laceyi* was reported to have antimalarial activity against *Plasmodium falciparum* (Cai et al., 2016; Pan et al., 2018).

Since *C. costata* has similarities with *Q. laceyi* that is known for its antimalarial activity, we investigated the antimalarial activity of *C. costata* in experimental animals infected with *Plasmodium berghei* ANKA (*PbA*).

**Materials and Methods**

All of the experiments in this study were performed in Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, Padjadjaran University, Bandung, Indonesia. The experimental animal works have been approved by the Research Ethics Committee of Padjadjaran University Bandung (No. 507/UN6.KEP/EC/2018) and were exactly conducted according to the “Principles of Laboratory Animal Care” (NIH Publication No. 86-23).

**Plant determination**

Fresh leaves (10 kg) of *C. costata* were collected from Namu Keling Village, North Sumatera and transported to Phytochemistry and Pharmacology Laboratory, Padjadjaran University, for cleaning, air drying, milling, and extraction process. The plants were then identified as *C. costata* by Herbarium unit, Department of Biology, Faculty of Mathematics and Science, Padjadjaran University (Code: 219/HB/04/2017).

**C. costata extraction and fractionation**

The *C. costata* leaves powder (1 kg) was exhaustively macerated in 70% ethanol for 72 hr. The liquid extract was obtained and concentrated using rotary evaporator at 50°C to produce around 9.87% concentrate (fixed weights of the extracts were divided by simplicia weights multiplied by 100%).

AJP, Vol. 9, No. 5, Sep-Oct 2019 475
The extract was stored at 4°C before being used for the experiment. The dried ethanolic extract was dissolved in distilled water for preparing various doses. After dissolved in an ethanol-water (1:3) mixture, the ethanol extract of *C. costata* leaves (50 g) was fractionated by liquid–liquid partition, using ethyl acetate (EA) (4 × 150 ml), and hexane (4 × 150 ml). This procedure produced three fractions including *n*-hexane (16.54 g, 33.08%), ethyl acetate (EA) (19.68 g, 39.36%) and water (10.17 g, 20.34%).

**Phytochemical screening test**

The extract and fractions of *C. costata* leaves were qualitatively screened to identify the presence of secondary metabolites including alkaloids, flavonoids, terpenoids, phenolic compounds, tannins, saponins, and anthraquinone glycosides (Yadav and Agarwala, 2011).

**Animals**

Thirty two male Balb/C (25-30 g and 8-12 weeks old) were obtained from animal house of School of Pharmacy, Bandung Institute of Technology (Bandung, Indonesia) and housed under standard conditions with 12 hr-12 hr light-dark cycles. Four mice were housed in each cage and they had free access to food and water *ad libitum*. In order to acclimatize to the environment, the animals were transferred to laboratory 7 days before initiation of the experiments. Animal procedures were performed according to guidelines for the care of experimental animals of Faculty of Medicine, Padjadjaran University.

**Parasite inoculation**

Animals were infected with *PbA* by injecting each mouse with 0.2 ml of *PbA*-infected blood (1×10⁶ *PbA* parasitized red blood cells) intraperitoneally, and parasitemia levels was then observed daily. Day-0 was defined when parasitemia levels reach at least >2%.

**Drug administration**

Depending on the group, the antimalarial standard drug (artesunate), *C. costata* extract and *C. costata* fractions were administered directly into the mice stomach via oral gavage technique. All treatments were started when parasitemia >2% (day 0) and performed once a day for 5 consecutive days (day 0 to day 4).

**Evaluation of in vivo antimalarial activity of extract and fractions of *C. costata* leaves (4-day test)**

Antimalarial activity of the extract and fractions of *C. costata* leaves was evaluated using the method described by Knight and Peters (Knight and Peters, 1980). The animals were divided into eight groups. Briefly, after having >2% parasitemia (day 0), the mice were treated with 100, 200 and 400 mg/kg/day doses of the *C. costata* leaves extract, 108 mg/kg/day dose of the *C. costata* leaves fractions (n-hexane, ethyl acetate, water), 36.4 mg/kg/day of artesunate (Guilin Pharmaceutical Co., Ltd) and 1% PGA (Pulvis Gummi Arabicum) suspension (control) for five consecutive days (day 0 to 4). Thin blood smears were made from the tail blood of each mouse every day for 5 days (day 0 until day 4) and stained using 10% giemsa to observe the infected-red blood cells under microscope. The average of parasitemia and % parasitemia inhibition were calculated and expressed using the following formula (Fidock et al., 2004).

\[
\text{% Parasitemia} = \frac{\text{Total number of parasitized red blood cells}}{1000 \text{ red blood cells}} \times 100\% \\
\text{% Parasitemia inhibition} = \frac{\text{Parasitemia in control group} - \text{Parasitemia in study group}}{\text{Parasitemia in control group}} \times 100\%
\]
Inhibitory effect of Castanopsis costata extract on Plasmodium berghei

Microscopic observation
Parasitemia levels of thin blood smear of all mice were analyzed under the microscope (Nikon Eclipse E100, Japan) with 1000x magnification. In addition, the morphology of each growth phase of P. berghei ANKA in host red blood cells were also observed.

Statistical analysis
Data were analyzed using SPSS software (version 22). For statistical analysis, one way analysis of variance (ANOVA) followed by Tukey’s was applied. The results are presented as mean±S.E.M and p-values less than 0.05 were considered significant.

Results
Phytochemical screening
The phytochemical screening of C. costata extract revealed the presence of chemical constituents such as phenolics, alkaloids, flavonoids, saponins, tannins, triterpenes, and anthraquinone glycosides, whereas the C. costata leaves water fraction contained phenolics, flavonoids, saponins, and tannins. Meanwhile, ethyl acetate fraction of C. costata leaves showed the presence of phenolics, flavonoids, saponins, tannins, and triterpenes. Furthermore, n-hexane fraction of C. costata leaves contained alkaloids and triterpenes. A summary of the phytochemical screening of extract and fractions of C. costata leaves is presented in Table 1.

Antimalarial activity
Parasitemia observation
Our observation on day 0 to 4 revealed that both mice groups treated with ethanolic extract (Figure 1) and fractions of C. costata (Figure 2) showed lower increases in parasitemia compared to the control group, indicating that administration of ethanolic extract and fractions of C. costata inhibited P. berghei development in mice. Although progressive inhibition of parasitemia was observed, this effect was still below parasitemia inhibition produced by artesunate (Figures 1 and 2).

Table 1. Phytochemical screening of extract and fractions of C. costata leaves.

| Phytochemical screening | Extract Water fraction | Ethyl acetate fraction | n-Hexane fraction |
|-------------------------|------------------------|------------------------|-------------------|
| Phenolics               | +                      | +                      | +                 |
| Alkaloids               | +                      | -                      | -                 |
| Flavonoids              | +                      | +                      | +                 |
| Saponins                | +                      | +                      | -                 |
| Tannins                 | +                      | +                      | +                 |
| Triterpenes             | +                      | -                      | +                 |
| Anthraquinone glycosides| +                      | -                      | -                 |

(++=Contained, (-)=Not contained)
The effect of extract and fractions of *C. costata* leaves on *PbA*-infected mice during 4-day Peter’s suppression test

Figure 3 shows that the parasitic inhibition effect of the extract of *C. costata* leaves was dependent on the administered dose. The administration of *C. costata* extracts (100, 200 and 400 mg/kg) inhibited parasitemia (p<0.05), but their impacts were lower than that of artesunate. The higher doses showed stronger parasitemia inhibition.

From the results of the probit analysis, it was found that an effective dose (ED50) to inhibit parasite growth was reached upon oral administration of 108 mg/kg/day *C. costata* extract (Figure 4).

The administration of 108 mg/kg/day *C. costata* fractions inhibited the parasitic growth. Its inhibitory effect was dependent on the type of the administered fraction. There was higher parasite growth inhibition upon the administration of fractions/artesunate compared to the control group (p<0.05) (Figure 5), but the effects of the three *C. costata* leaf fractions (108 mg/kg) were less than the effect produced by artesunate.

Parasites that infect red blood cells produce several changes including enlargement of red blood cells, alteration of the color of red blood cells and the appearance of spots on certain staining. (Cooke et al., 2001). There are differences in parasite morphology among the groups during 4 days of testing. Morphological differences among the test groups can be seen from the parasitic shape and the color of infected red blood cells (Figure 6).
Inhibitory effect of Castanopsis costata extract on Plasmodium berghei

Figure 6. Morphology of each growth phase of PbA for 4 days under a microscope with a magnification of 1000X. A) Normal red blood cells; B) Ring phase; C) Trophozoite phase; D) Schizont phase.

Discussion

Our results revealed antimalarial activity of C. costata leaves extract and fractions in PbA-infected mice during 5 days administration. These results also indicated that extracts and fractions of C. costata leaves are able to inhibit parasite growth in mice. The ethanolic extract of C. costata leaves was significantly active against PbA in a dose-dependent manner with the inhibitory activity observed in the C. costata-treated mice at doses 100 mg/kg (% inhibition: 42.66%), 200 mg/kg (% inhibition: 66.21%) and 400 mg/kg (% inhibition: 80.99%) of the extract (Figure 3).

Since the administration of C. costata leaves extract resulted in >50% parasitemia suppression at doses of 500, 250, and 100 mg/kg body weight, C. costata can be classified as an agent with very good antimalarial activity (Bantie et al., 2014). Meanwhile, the ethyl acetate fraction of C. costata leaves also showed higher parasitic growth inhibition (% inhibition: 79.85%) if compared to the water fraction (% inhibition: 51.19%) and n-hexane fraction (% inhibition: 62.77%) (Figure 5).

The anti-malarial activity of the extract and fractions of C. costata leaves could be due to the presence of several secondary metabolite compounds in extract and fractions of the leaves of C. costata, especially alkaloid compounds, flavonoids, triterpenoids, phenolics, tannins, saponins, and anthraquinone glycosides. Some secondary metabolites were proven to have anti-malarial activities. Some alkaloids have been known to inhibit parasite growth by inhibiting parasite growth through intracellular choline transport in the blood schizont phase (Hilou et al., 2006). In addition, sesquiterpenoid and triterpenoid can inhibit parasitic growth by inhibiting heme polymerization into hemoglobin through free radical formation of sesquiterpenic lactone which will alkylate heme to form hemartemin complex. Moreover, sesquiterpenoid and triterpenoid have been known to inhibit the respiration process in mitochondria, protein synthesis in parasitic cells and Ca^{2+} ion transporters called PfATP6, that is a sarcoplasmin reticulum of calcium-dependent ATPases (SERCA) which is only found in P. falciparum (Meschnick et al., 1996; Pouplin et al., 2007). Furthermore, flavonoid compounds were also reported to inhibit the growth of malaria parasites by inhibiting the nutrient transport required by the parasite in the intraeritrocytic stage (i.e. blood schizont) (Kirk, 2001; Kirk, 2004) and inhibit hemoglobin degradation and heme detoxification in food vacuoles of malaria parasites (Biagini et al., 2003; Bilia, et al., 2006; Frolich et al., 2005).

Phenolic compounds and tannins in the extracts and fractions of C. costata leaves are also expected to play a role in inhibiting parasite growth via their antioxidant actions (Kim et al., 2003). The antioxidant activities of phenolic and tannin compounds are mainly caused by the redox nature of these compounds, which can play an important role in absorbing and neutralizing free radicals, and decomposing peroxide compounds (Laphookhieo et al., 2009). Furthermore, antioxidant activity of phenolic compounds and tannins can inhibit parasite protein synthesis and also
neutralize oxidative damage induced by malaria parasites (Builders et al., 2010).

Our study proved that the extract and fractions of the leaves of *C. costata* have the ability to inhibit the growth of malaria parasites in Balb/C mice infected with *PbA*. This suggests that *C. costata* can be potentially used to develop new agents to combat malaria. However, further studies are required to elucidate the mechanisms behind such effects.

**Acknowledgment**

This work was funded by fundamental research grant of Padjadjaran University. We would like to thank all the persons who helped us in this work.

**Conflicts of interest**

Authors declare no conflict of interest.

**References**

Alkandahri MY, Nisriadi L, Salim E. 2016. Secondary metabolites and antioxidant activity of methanol extract of *Castanopsis costata* leaves. Pharmacol Clin Pharm Res, 1: 98-102.

Alkandahri MY, Siahaan PN, Salim E, Fatimah C. 2018. Anti inflammatory activity of cep-cepan leaves (*Castanopsis costata* (Blume) A.DC). Int J Current Med Sci, 8: 424-429.

Bantie L, Assefa S, Teklehaimanot T, Engidawork E. 2014. In vivo antimalarial activity of the crude leaf extract and solvent fractions of croton macrostachyus hoehst. (Euphorbiaceae) against *Plasmodium berghei* in mice. BMC Complement Altern Med, 14: 2-10.

Bero J, Quetin-Leclercq J. 2011. Natural products published in 2009 from plants traditionally used to treat malaria. Plant Med, 77: 631-40.

Biagini GA, O'Neill, Nzila PM, Ward SA. 2003. Antimalarial chemotherapy: young guns or back to the future. Trends Parasitol, 19: 479-487.

Bilia AR, Melilo de Malgalhaes P, Berganzi MC, Vincieri FF. 2006. Simultaneous analysis of artemisinin and flavonoids or several extract of *Artemisia annua* L. obtained from a Commercial Sample and Selected Cultivar. J Phytomed, 13: 487-493.

Builders MI, Wannang NN, Ajoku GA, Builders PF, Orisadipe A, Aguiyi JC. 2010. Evaluation of antimalarial potential of *Vernonia ambigua* kotschy and peyr (Asteraceae). Int J Pharmacol, 18: 1-9.

Burke E, Deasy J, Hasson R, McCormack R, Randhawa V, Walsh P. 2003. Antimalarial drug from nature. J Trinity Student Med: 1-8.

Cai S, Risinger AL, Nair S, Peng J, Anderson TJ, Du L, Powell DR, Mooberry SL, Cicchewicz RH. 2016. Identification of compounds with efficacy against malaria parasites from common north american plants. J Nat Prod, 79: 490-498.

Cooke B, Mohandas N, Coppell R. 2001. The malaria-infected red blood cell: structural and functional changes. Advan Parasitol, 50: 1-86.

Eljahmi Woerdenbag HJ, Kayser O. 2014. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J Herb Med 4: 51-73.

Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. 2004. Antimalarial drug discovery: Efficacy models for compound screening. Nat Rev Drug Disc, 3: 509-520.

Frolich S, Schuberth C, Bienzle U, Siems KJ. 2005. *In vitro* Antiplasmodial activity of prenylated chalcon derivates and their interaction with haemin. J Antimicro Chemother, 55: 883-887.

Hayati EK, Jannah A, Ningsih R. 2012. Compounds identification and in vivo antimalarial activity of ethyl acetate extract from anting-anting plant (*Acalypha Indica* L.). Molekul, 7: 20-32.

Hilou A, Nacoulma OG, Guiguemde TR. 2006. *In vivo* antimalarial activities of extracts *Amaranthus spinosus* L. and *Boerhaavia erecta* L. in mice. J Ethnopharmacol, 103: 236-240.

Kim K, Lee S, Jung S, Park H, Shinkilt Y, Kim BK. 2003. Antioxidant activities of the extracts from the herbs of *Artemisia apiacea*. J Ethnopharmacol, 85: 69-72.

Kirk, K. 2001. Membran transport in the malaria infected erythrocyte. Physol. Review, 81: 495-537.

Kirk, K. 2004. Channels and transporters as drug targets in the plasmodium-infected erythrocyte. J Acta Trop, 89: 285-298.
Inhibitory effect of *Castanopsis costata* extract on *Plasmodium berghei*

Knight DJ and Peters W. 1980. The antimalarial action of N-benzyloxy dihydrotriazines: The action of cycloguanil (BRL50216) against rodent malaria and studies on its mode of action. Ann Trop Med Parasitol, 74:393-404.

Laphookhieo S, Maneerat W, Koysomboon S. 2009. Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Cratoxylum cochinichinense*. Molecule, 14: 1389-1395.

Meschnick SR, Taylor TE, Kamchonwongpaisan S. 1996. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. Microbiol Rev, 60: 301-315.

Mustofa. 2003. Molekul antimalaria alami: potensi dan tantangan pengembangannya sebagai obat baru untuk malaria. Trad Med J, 8: 8-18.

Nogueira CR, Lopes ML. 2011. Antiplasmodial natural products. Molecules, 16: 2146-2190.

Nurtjahja K, Kelana TB, Suryanto D, Priyani N, Rio G, Putra DP, Arbain D. 2013. Antimicrobial activity of endemic herbs from tangkahan conservation forest north sumatera to bacteria and yeast. Hayati J Biosci, 20: 177-181.

Pan WH, Xu XY, Shi N, Tsang SW, Zhang HJ. 2018. Antimalarial activity of plant metabolites. Int J Mol Sci, 19: 1-40.

Pouplin JN, Tran TH, Dolecek C, Phan TA, Farrar J, Carron P, Bodo B, Grelier P. 2007. Antimalarial and cytotoxic activities of ethnopharmacologically selected medicinal plants from south vietnam. J Ethnopharmacol, 109: 417-427.

Yadav RNS, Agarwala M. 2011. Phytochemical analysis of some medicinal plants. J Phytol, 3: 10-14.

Salim E, Fatimah C, Fanny DY. 2017. Analgetic activity of cep-cepan (*Saurauia Cauliflora* Dc.) leaves extract. J Nat, 17: 31-38.

Saxena S, Pant N, Jain DC, Bhakuni RS. 2003. Antimalarial agent from Plant Source. Curr Sci, 85: 1314-29.