



Phytochemical and antimalarial screening of *Brucea javanica* plant extracts

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ABSTRACT

The leaf, root, and stem extracts of *Brucea javanica* were screened for phytochemical properties and antimalarial activity, after extractions with various organic solvents. Qualitative analysis of the phytochemical constituents showed the presence of the following secondary metabolites: terpenoids, saponins, flavonoids, steroids, phenols, glycosides, alkaloids, and anthraquinones. The extracts showed very strong in vitro activity against the malaria parasite *Plasmodium falciparum*. The phytochemical constituents in the extracts were also assessed. The acetone extract of the roots showed excellent antimalarial activity with an IC_{50} value of 0.28 $\mu\text{g/ml}$, whereas stem and leaf extracts had high antimalarial activities with IC_{50} about 2.5 $\mu\text{g/ml}$.

Keywords: *Brucea javanica*, phytochemical screening, antimalarial activity

INTRODUCTION

Malaria remains one of the most serious world health problems and the major cause of morbidity and mortality in the endemic regions. According to the World Health Organization (WHO) [1], there are 97 countries and territories with ongoing malaria transmission, and 7 countries in the prevention of reintroduction phase, making a total of 104 countries and territories in which malaria is presently considered endemic. Globally, an estimated 3.4 billion people are at risk of malaria. The WHO estimates that 207 million cases of malaria occurred globally in 2012 (uncertainty range 135-287 million) causing 627,000 deaths (uncertainty range 473,000-789,000). Most of the cases (80%) and most of the deaths (90%) occurred in Africa, while the deaths were dominantly (77%) of children under 5 years of age. In Africa, the use of indigenous plants plays an important role in the traditional treatments of malaria, and these plants are good sources for the discovery of antimalarial natural compounds [2-5]. The therapeutic properties ascribed to most medicinal plants are linked to their phytochemical compounds. Phytochemicals such as alkaloids, glycosides, phenols, saponins, triterpenoids, and flavonoids might have antimalarial properties [6-8].

Brucea javanica belongs to the family Simaroubaceae, and occurs naturally in several Southeast Asian countries. This plant has been used for the treatments of malaria, dysentery, and cancer [9-10]. As a part of our search for bioactive metabolites from Thai medicinal plants [11-15], we investigated the phytochemical screening of hexane and acetone soluble extracts from the stems, the roots, and the leaves of *B. javanica*. These extracts exhibited significant antimalarial activity when evaluated against *Plasmodium falciparum*.

EXPERIMENTAL SECTION

Plant Materials

Samples of the leaf, the root, and the stem from *Brucea javanica* were collected in Prince of Songkla University, Suratthani campus, Thailand, in May 2013. A voucher specimen (number WU-0239) was deposited in the herbarium of Walailak University, Thasala, Nakhon Si Thammarat, Thailand.

Preparation of Extracts

Dried and ground leaves, roots, and stems of *B. javanica* (each 500 g) were extracted with hexane and acetone, with maceration at room temperature over five days in each solvent. The extracts were concentrated under reduced pressure to dry residues that were stored in sterile vials, pending phytochemical and antimalarial screening.

Phytochemical Screening

The hexane and acetone extracts were subjected to phytochemical screening for the presence of terpenoids, saponins, flavonoids, steroids, coumarins, glycosides, alkaloids, and anthraquinones according to standard procedures [16-20].

Terpenoids: An 0.5 g plant extract sample was mixed with 2 ml of chloroform in a test tube. Then 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to the mixture to form a layer. An interface with a reddish brown coloration was formed when terpenoids were present.

Saponins: An 0.5 g plant extract sample was dissolved in boiling water, allowed to cool, and shaken vigorously. The froth was mixed with 3 drops of olive oil, and the formation of an emulsion indicated the presence of saponins.

Flavonoids: A few drops of 1% ammonia solution were added to the extract sample in a test tube. A yellow coloration indicated the presence of flavonoids.

Glycosides: A 10 ml quantity of 50% conc. H_2SO_4 was added to 0.5 g of the extract in a test tube. The mixture was heated in a boiling water bath for 5 min. Then 10 ml of Fehling's solutions (5 ml of solutions A and B each) was added, and the mixture was boiled. A brick red precipitate indicated the presence of glycosides.

Phenolic compounds: In a test tube containing about 1 ml alcoholic solution of the extract, a few drops of 10% aqueous ferric chloride solution were added. The emergence of blue or green color indicated the presence of phenolic compounds.

Tannins: About 0.5 g of the extract was boiled in 10 ml of water in a test tube. A few drops of 0.1% ferric chloride were added, and the emergence of brownish green or bluish black coloration was the indication of presence.

Alkaloids: About 1 ml of 1% hydrochloric acid was added to 3 ml of the extract in the test tube, and the mixture was treated with few drops of Dragendorff's reagent. The formation of a reddish brown precipitate indicated the presence of alkaloids.

Steroids: About 0.5 g of the plant material was extracted in 10 ml chloroform. First 2 ml of acetic anhydride was added, and then 2 ml of conc. H_2SO_4 . A color change from violet to blue or green indicated the presence of steroids.

Anthraquinones: About 0.5 g of the extract was boiled with 10 ml of conc. H_2SO_4 and filtered. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was removed, and then 1 ml of 10% aqueous ammonia was added. The emergence of pink, violet, or red tint in the solution indicated the presence of anthraquinones.

Antimalarial activity *in vitro*

The malarial parasite, *Plasmodium falciparum* (K1, multidrug resistant strain), was cultured according to the method of Trager and Jensen [21]. The quantitative assessment of malarial activity *in vitro* was based on the microculture radioisotope technique, following Desjardins [22]. The inhibitory concentration IC_{50} is that concentration of an antimalarial compound that reduces parasite growth by 50%, and is a conventional measure of activity with lower values being better. The amount of *P. falciparum* parasites was determined by the *in vitro* uptake of [3H]-hypoxanthine, and an active reference compound included in the tests was dihydroartemisinin ($IC_{50} = 2.51$ nM), while inhibition was relative to untreated control.

RESULTS AND DISCUSSION

The phytochemical analyses of *B. javanica* leaf, root, and stem extracts revealed the presence of terpenoids, saponins, flavonoids, steroids, phenols, glycosides, alkaloids, and anthraquinones (Table 1), in the hexane and acetone extracts that were tested separately. All the plant parts investigated were rich in saponins, flavonoids, and phenolic compounds. The differences between the two extraction solvents in the results of phytochemical screening might be attributed to solvent effects on solubility, as reported previously for phytochemicals [23-24]. Saponins, flavonoids, and phenols were detected in all the extracts screened. The contents of active constituents in a plant can vary widely within the plant species, for example by geographical location, maturity or growth stage of the plant, season, climate, soil, and propagation method [25]. This is corroborated by the observation, that the collection

season of plant parts affects their medicinal effectiveness [26]. The *in vitro* antimalarial screening, in the current study, indicates that the leaf, the root, and the stem extracts had very good activities against *P. falciparum*. The acetone extracts of *B. javanica* stems and leaves had IC₅₀ values of about 2.5 µg/ml, while the root extract showed excellent activity with IC₅₀ = 0.28 µg/ml (Table 2). These findings support the suggestions that *B. javanica* contains active constituents that target specific metabolic activities of the parasitic schizont stage [27]. Our results confirm that this plant that is used in the traditional medicine against malaria possesses significant *in vitro* antimalaria potential, and justifies its use in traditional medicine.

Table 1. Qualitative phytochemical analysis of *B. javanica*

Phytochemical constituents	Leaf extracts		Root extracts		Stem extracts	
	Hexane	Acetone	Hexane	Acetone	Hexane	Acetone
Terpenoids	-	+	-	+	+	-
Saponins	+	+	+	+	+	+
Steroids	+	-	-	-	-	-
Flavonoids	+	+	+	+	+	+
Alkaloids	-	-	-	+	-	-
Phenols	+	+	+	+	+	+
Glycosides	-	+	-	+	-	+
Anthraquinones	-	+	-	+	-	+
Tannins	+	-	-	-	-	+

+ = Present; - = absent

Table 2. Antimalarial activity of *B. javanica* crude extract

Plant extract	IC ₅₀ (µg/ml)	
	Hexane	Acetone
Leaf extracts	3.24	2.55
Root extracts	0.56	0.28
Stem extracts	5.66	2.52

CONCLUSION

The present work demonstrated by screening the presence of phytochemicals in *B. javanica* and the *in vitro* antimalarial activity of these constituents. The current study could be extended by isolating active compounds against malaria and elucidating their structures, assuming such singly active compounds exist, after which the expensive proposition of testing for toxicity could be considered.

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REFERENCES

- [1] World Health Organization, World malaria report, WHO publications, Geneva, **2013**, 1-68.
- [2] JC Chukwujekwu; CA Lategan; PJ Smith; FR Van-Heerden; J Van-Staden, *S. Afr. J. Bot.*, **2009**, 75, 176-179.
- [3] A Hilou; OG Nacoulma; TR Guiguemde, *J. Ethnopharmacol.*, **2006**, 103, 236-240.
- [4] Y Ouattara; S Sanon; Y Traore; V Mahiou; N Azas; L Sawadogo, *Afr. J. Tradit. Complement. Altern. Med.*, **2006**, 3(1), 75-81.
- [5] ES Omoregie; BS Sisodia, *Bayero J. pure Appl. Sci.*, **2012**, 5(1), 90-97.
- [6] ES Omoregie; A Pal; B Sisodia, *Niger. J. Basic and Appl. Sci.*, **2011**, 19(1), 121-126.
- [7] SF Mbatchi; B Mbatchi; JT Banzouzi; T Bansimba; NGF Ntandou; JM Ouamba; A Berry; F Benoit-Vical, *J. Ethnopharmacol.*, **2006**, 104, 168-174.
- [8] L Tona; RK Cimanga; K Mesia; CT Musumba; T De-Bruyne; S Apers; N Hernans; S Van-Miert; L Pieters; J Totte; AJ Vlietinck, *J. Ethnopharmacol.*, **2004**, 93, 27-32.
- [9] Subeki; H Matsuura; K Takahashi; K Nabeta; M Yamasaki; Y Maede, *J. Nat. Prod.*, **2007**, 70, 1654-1657.
- [10] QJ Chen; MA Ouyang; QW Tan; ZK Zhang; ZJ Wu; QY Lin, *J. Asian Nat. Prod. Res.*, **2009**, 11(6), 539-547.
- [11] P Chumkaew; S Kato; K Chantrapromma, *J. Asian Nat. Prod. Res.*, **2010**, 12(5), 424-428.
- [12] P Chumkaew; S Kato; K Chantrapromma, *Chem. Pharm. Bull.*, **2006**, 54(9), 1344-1346.
- [13] P Chumkaew; S Kato; K Chantrapromma, *Chem. Pharm. Bull.*, **2005**, 53(1), 95-96.

- [14] T Srisawat; P Chumkaew; W Heed-Chim; Y Sukpondma; K Kanokwiroon, *Trop. J. Pharm. Res.*, **2013**, 12(1), 71-76.
- [15] T Srisawat; P Chumkaew; W Maichum; Y Sukpondma; P Graidist; K Kanokwiroon, *J. Med. Sci.*, **2013**, 13(2), 130-135.
- [16] S Anbuselvi; S Muthumani, *J. Chem. Pharm. Res.*, **2014**, 6(2), 380-383.
- [17] A Renjith Alex; K Ilango; BA Viswanath; R Shunmuga Sundaram; S Ganeshan, *J. Chem. Pharm. Res.*, **2014**, 6(3), 1115-1120.
- [18] AS Kamba; LG Hassan, *Afr. J. Pharm. Pharmacol.*, **2010**, 4(9), 645-652.
- [19] HB Yesufu; IZ Khan; FI Abdulrahman; YZ Abatcha, *J. Chem. Pharm. Res.*, **2014**, 6(5), 791-795.
- [20] S Nisa; Y Bibi; A Waheed; M Zia; S Sarwar; S Ahmed; M Fayyaz Chaudhary, *Afr. J. Biotechnol.*, **2011**, 10(6), 990-995.
- [21] W Trager; JB Jensen, *Science*, **1976**, 193, 673-675.
- [22] RE Desjardins; CJ Canfield; JD Haynes; JD Chulay, *Antimicrob. Agents Chemother.*, **1979**, 16, 710-718.
- [23] AH Kawo; A Mustapha; BA Abdullahi; LD Rogo; ZA Gaiya; AS Kumurya, *J. Pure. Appl. Sci.*, **2009**, 2(1), 34-40.
- [24] M Yusha'u; A Bukar; AL Balarabe, *Biol. Environ. Sci. J. Trop.*, **2008**, 5(3), 72-76.
- [25] OA Adoum; JA Akinniyi; T Omar, *Ann. Borno*, **1997**, 13(14), 199-207.
- [26] T Odugbemi, A textbook of medicinal plants from Nigeria, University of Lagos Press, Nigeria, **2008**, 20-323.
- [27] R Dhar; K Zhang; GP Talwar; S Grag; N Kumar, *J. Ethnopharmacol.*, **1998**, 61, 31-39.