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Research paper

Antiprotozoal properties of Indonesian medicinal plant extracts

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ABSTRACT

Tithonia diversifolia, Cyclea barbata, Tinospora crispa, Arcangelisia flava, Pycnarrhena cauliflora are plants used in Indonesia for the traditional treatment of malaria. In the search for new antiparasitic drugs, the parts traditionally used of these 5 plants were extracted with ethanol and then fractionated with various solvents and evaluated *in vitro* against *Plasmodium falciparum* and also against *Babesia divergens* and *Leishmania infantum.* Seven crude plant extracts out of 25 tested displayed high antimalarial activities with $IC_{50} < 5$ µg/ml and in the cases of some of them an interesting selectivity regarding their cytotoxicity against mammalian cells. *A. flava* appeared to be the most promising antiplasmodial plant with the highest antiplasmodial activity (IC_{50} values less than 3 µg/ml) and the weakest cytotoxicity. By contrast, only *P. cauliflora* radix, through its dichloromethane and methanol fractions also demonstrated a high activity against *L. infantum*, with IC_{50} values around 3 µg/ml; their high selectivity index, especially on VERO cells, hypothesises a specific parasiticidal action. Moreover, for all the extracts showing antiplasmodial activity, a positive correlation was demonstrated with antibabesial activity, suggesting that these antiplasmodial extracts could be a potential source of antibabesial compounds. These preliminary results confirm the antiplasmodial interest of some of these plants used in traditional medicine but also their effects on leishmaniasis and babesiosis. Ongoing phytochemical investigations should allow identification of the chemical series responsible for these activities.

1. Introduction

Malaria is the most prevalent parasitic disease in the world and continues to be one of the largest public health problems, especially in the developing countries. There is an estimated 3.2 billion people at risk of being infected and leading to an estimated 584,000 malaria deaths 78 % of which concern children under 5 years (WHO, 2014c). *Plasmodium falciparum*, the most deadly species of malaria parasite already shows resistance to all antimalarial drugs, including artemisinin and its derivatives, which are the latest and the most effective treatments of malaria (Straimer et al., 2015; WHO, 2014c; Witkowski et al., 2009; Wongsrichanalai et al., 2002). Unfortunately the use of artemisinin-based combination therapies (ACTs) advocated by the World Health Organization (WHO) did not allow for the emergence and the spread of multi-resistance in the Greater Mekong region (Cambodia, Vietnam, Thailand, Myanmar and Laos) threatening the strategies of malaria eradication in the other endemic areas (WHO, 2014b). Consequently there is an urgent need to discover new antimalarial agents, and crude extracts of natural products appear to be a promising route to follow as they are more affordable for people in developing countries (Benoit-Vical, 2005; Benoit-Vical et al., 2008, 2003).

Indonesia is rich in medicinal plants which the population use traditionally for curing diseases including malaria. This study, based on ethnobotanical data, was carried out on 5 medicinal plants selected as potential sources of antimalarial agents: *Tithonia diversifolia, Cyclea barbata, Tinospora crispa, Arcangelisia flava, Pycnarrhena cauliflora*.

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Except *T. diversifolia,* the 4 other plants are from the Menispermaceae family studied largely for their use in traditional medicine and principally for malaria treatment (Verpoorte et al., 1982b).

Some of these plants have already been investigated for their antiplasmodial activities and pharmacological properties but no standardised studies have yet been performed with them. The infusion of *T. diversifolia* leaves is traditionally used for lowering blood glucose (Miura et al., 2005), flatulence, malaria fever (Njoroge and Bussmann, 2006), and also the healing of wounds. An ethanol extract of *T. diversifolia* exhibited anti-malarial activity by its leaf extracts (Oyewole et al., 2008). *C. barbata* leaves have been used by the Javanese for gastric problems and as a prophylactic against malaria fever (Manilal and Sabu, 1985; Saxena et al., 2003). *T. crispa* is traditionally used for the treatment of fever, rheumatic arthritis, hepatitis, anti-hyperglycaemia, and also malaria by using stem infusions (Pathak et al., 1995; Pushpangadan and Atal, 1984). *T. crispa*, which is abundant in the Philippines, is used freely by the natives under the name of *makabuhay* (meaning “You may live”), as a panacea, especially valuable in malarial fevers (The Southwest School of Botanical Medicine, 1918). Supporting the traditional uses, *T. crispa* scientifically proved as anti-hyperglycaemia (Noor and Ashcroft, 1998) and also both *in vitro* and *in vivo* antimalarial activity (Najib Nik et al., 1999; Rungruang and Boonmars, 2009). *A. flava* is traditionally used as a stem decoction for typhoid fever (Mandia, 1999), hepatitis, gastric disturbance and malaria (Subeki et al., 2005). The anti-hepatotoxicity (Wongbutdee et al., 2003) and anti-malarial activity have been profiled by several studies (Lovin et al., 2012; Nguyen-Pouplin et al., 2007; Vennerstrom and Klayman, 1988; Verpoorte et al., 1982a). *P. cauliflora* is a Dayak tribe folk medicine used for flavouring, flatulence (by placing the soaked leaves on the stomach), fever and malaria (by drinking the leaf infusion). Only local accounts published in Indonesian can be cited for these uses. One study of *P. cauliflora* has just revealed its cytotoxic activities related to apoptosis and cell cycle arrest (Masriani and Adnyana, 2011), but its anti-parasitic properties have not yet been established. Indeed, despite widespread traditional use of *P. cauliflora* reported on malaria treatment in Indonesia, to the best of our knowledge, no studies have been conducted on this plant. Other *Pycnarrhena* species have been studied for their biologically active principles (Siwon et al., 1981b; Verpoorte et al., 1978).

Furthermore, because *Plasmodium* and *Babesia* belong to the Apicomplexa phylum they share many common biochemical pathways. We can therefore hypothesise the possibility of finding common active compounds for both pathogens. *Babesia divergens* babesiosis is a widespread illness transmitted by livestock ticks. This pathogen is the main agent of bovine babesiosis in Europe but can also affect splenectomized humans (Ceci et al., 2014; L'Hostis et al., 1995; Zintl et al., 2014). Infections can occur without producing symptoms, but babesiosis may also be severe and sometimes fatal due to the intraerythrocytic development of the parasite (Melhorn and Schein, 1984). There are effective babesiacides, but imidocarb dipropionate is practically the only drug available on the market and therefore the most widely used. More specific new fast-acting treatments for babesiosis should now be developed (Vial and Gorenflot, 2006).

Leishmaniasis, identified as a “Neglected Tropical Disease” by WHO, is another parasitic disease. The pathogen is endemic in 98 countries and territories, with the number of new cases estimated at 1.3 million per year (WHO, 2014a). Nowadays, malaria and leishmaniasis are responsible for over a million deaths a year and threaten more than 350 million people worldwide, mostly, but not only in tropical and subtropical countries.

Here we offer a standardised study that focuses on the search for new antiparasitic drugs targeting *Plasmodium, Leishmania* and *Babesia*. Indeed, even though the five selected plants are traditionally used for the treatment of malaria in Indonesia, the antiplasmodial activities of their different extracts were not totally investigated. The extracts showing the best antiplasmodial activity were also tested on *Babesia divergens* cultures to investigate another Apicomplexa parasite. Moreover, in some tropical zones, malaria and leishmaniasis infections are largely co-endemic, the extracts were thus tested on both *P. falciparum* and *L. infantum* to evaluate their anti-parasite activities as potential sources of new active compounds. The cytotoxicity of these extracts was assessed in parallel in order to evaluate the specificity of their anti-parasitic activities.

2. Materials and methods

2.1. Plant material

The plants were identified in the biology laboratory of the Department of Pharmacy, Islamic University of Indonesia, Yogyakarta-Indonesia (Herbarium of Laboratory of Biology, Department of Pharmacy, Faculty of Science and Mathematics, UII, Yogyakarta, Indonesia). Samples were air-dried and powdered.

Tithonia diversifolia (Hemsl.) A. Gray. (Asteraceae; voucher specimen 007-02/L-PB/UII/2013) was collected in the Sleman district of Yogyakarta. *T. diversifolia* is a shrub, locally called “kembang bulan”.

Cyclea barbata Miers (Menispermaceae; voucher specimen 008-03/L.S-PB/UII/2013) was collected in the Bantul district of Yogyakarta and locally known as “Cincau rambat” (Javanese). *C. barbata* can be easily distinguished by its deltoidovate, hispid leaves with acuminate apex, finely mucronate acumen and long male and female inflorescences with dense capitate flowers.

Tinospora crispa (L.) (Menispermaceae; voucher specimen 009-03/S-PB/UII/2013) known by the vernacular name of “brotowali”, was collected from Yogyakarta. *T. crispa* is a woody tropical liana with shiny green leaves.

Arcangelisia flava (L.) Merr. (Menispermaceae; voucher specimen 010-04/S-PB/UII/2013), collected in South Borneo, is a large, woody, glabrous and dioecious liana, up to 20 m long; with a stem of up to 5 cm in diameter and with yellow wood exuding a yellow sap when cut.

Pycnarrhena cauliflora (Miers.) Diels (Menispermaceae; voucher specimen 011-01/S.R-PB/UII/2013) was collected in Sintang district, West Borneo. This plant can be found in primary or secondary forests and tend to grow in clusters. From 8 species in the genus *Pycnarrhena,* only 4 (*P. australiana, P. ozantha, P. manilensis, P. longifolia*) have been investigated in any detail.

2.2. Preparation of plant extract

For *T. diversifolia* (leaves), *C. barbata* (stem and leaves), *T. crispa* (stem), and *A. flava* (stem), powdered samples were extracted by maceration in 70% ethanol for 24 h, 300 g of plant powder with 1.5 L of solvent (Fig. 1). This process was repeated for two days consecutively using fresh 70% ethanol. Each ethanol extract was filtered and filtrates were evaporated to dryness using a rotary vacuum evaporator. Dried ethanol extracts were solubilized in ethanol to be fractionated by *n*-hexane/by liquid–liquid separation method which created two separate layers (*n*-hexane and first ethanol layer). The *n*-hexane layer gave the *n*-hexane fraction after evaporation. The fractionation process was continued by fractionation of the first ethanol layer using ethyl acetate (liquid–liquid separation) which produced ethyl acetate and second ethanol layers giving respectively after evaporation, the ethyl acetate fraction and ethanol fraction residue.

For *P. cauliflora* powdered radix part (Fig. 2) was directly treated by *n*-hexane to extract lipophilic compounds. After filtration the *n*-hexane extract was evaporated to dryness. The precipitate resulting of *n*-hexane extraction was further put back in suspension/solubilized using dichloromethane to obtain dichloromethane extract after filtration. The new precipitate was submitted to a methanol extraction followed by a filtration and an evaporation of the filtrate.

This extraction method was employed to investigate the possible active compounds responsible for plant activities. Particularly for *P.*