Journal of Ethnopharmacology 185 (2016) 235–242

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

In vitro inhibition of *Plasmodium falciparum* early and late stage gametocyte viability by extracts from eight traditionally used South African plant species

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ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form
11 March 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords:
Gametocytes
Plant leaf extracts
Malaria
Plasmodium falciparum

ABSTRACT

Ethnopharmacological relevance:: Extracts of plant species, used traditionally to treat malaria, have been extensively investigated for their activity against *Plasmodium* intraerythrocytic asexual parasites in search of new antimalarial drugs. However, less effort has been directed towards examining their efficacy in blocking transmission. Here, we report the results of the *in vitro* screening of extracts from eight selected plant species used traditionally to treat malaria in South Africa for activity against *Plasmodium falciparum* NF54 early and late stage gametocytes. The species used were *Khaya anthotheca, Trichilia emetica, Turraea floribunda, Leonotis leonurus, Leonotis leonurus* ex Hort, *Olea europaea* subsp. Africana, *Catha edulis* and *Artemisia afra.*

Aim of the study: To investigate the activities of extracts from plant species traditionally used for malaria treatment against *P. falciparum* gametocytes.

Material and methods:: Air-dried and ground plant leaves were extracted using acetone. Primary two point *in vitro* phenotypic screens against both early and late stage gametocytes were done at 10 and 20 μg/ml followed by full IC_{50} determination of the most active extracts. Inhibition of gametocyte viability *in vitro* was assessed using the parasite lactate dehydrogenase (pLDH) assay.

Results:: Of the eight crude acetone extracts from plant species screened *in vitro*, four had good activity with over 50–70% inhibition of early and late stage gametocytes' viability at 10 and 20 μg/ml, respectively. *Artemisia afra* (Asteraceae), *Trichilia emetica* (Meliaceae) and *Turraea floribunda* (Meliaceae) were additionally highly active against both gametocyte stages with IC_{50} values of less than 10 μg/ml while *Leonotis leonurus* ex Hort (Lamiaceae) was moderately active ($IC_{50} < 20$ μg/ml). The activity of these three highly active plant species was significantly more pronounced on late stage gametocytes compared to early stages.

Conclusion:: This study shows the potential transmission blocking activity of extracts from selected South African medicinal plants and substantiates their traditional use in malaria control that broadly encompasses prevention, treatment and transmission blocking. Further studies are needed to isolate and identify the active principles from the crude extracts of *A. afra*, *T. emetica* and *T. floribunda*, as well as to examine their efficacy towards blocking parasite transmission to mosquitoes.

1. Introduction

For the past decades, the management of malaria has primarily relied on vector control and chemotherapeutic drugs such as chloroquine and the artemisinin-based combination therapies (ACT's). While the benefits of adopting these measures have been evident (Bhatt et al., 2015), unfortunately so too has been the

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http://dx.doi.org/10.1016/j.jep.2016.03.036
0378-8741/

emergence of insecticide resistant mosquitoes (Benelli, 2015; Dai et al., 2015; Edi et al., 2012; Ranson et al., 2011) and drug resistant strains of *Plasmodium falciparum* parasites (Ashley et al., 2014; Dondorp et al., 2009; Tun et al., 2015). There is thus a dire need to advance the efficacy of these approaches with novel interventions, drugs and insecticides in the fight against this disease. In the current era of renewed calls for malaria elimination and eradication, there is a need to devise new strategies to complement current interventions (Roberts and Enserink, 2007). One such identified strategy is to block transmission from the human host to the mosquito vector (Alonso et al., 2011).

Human-to-mosquito transmission blocking entails targeting the sexual stages of the parasite, the gametocytes. This is the only stage of *Plasmodium* that can infect a female *Anopheles* mosquito for the sexual development of the parasite into sporozoites that can in turn infect humans and thereby perpetuate the lifecycle (Baker, 2010). Inhibiting gametocyte development would significantly reduce the number of infective mosquitoes and thus the number of newly infected patients. A single low dose primaquine (0.25 mg/kg) remains the only gametocytocidal drug recommended by the World Health Organisation against *P. falciparum* gametocytes (White et al., 2014). However, its use is restricted due to adverse effects on patients with glucose-6-phosphate dehydrogenase deficiency (Baird and Hoffman, 2004), a genetic disorder that is prevalent among populations in malaria endemic areas (Nkhoma et al., 2009). While some antimalarial drugs are active against the early stages of *P. falciparum* gametocytes (Abay, 2013; Butcher, 1997; Lucantoni et al., 2013; Mackerras and Ercole, 1949; Price et al., 1996; White et al., 2014), they are not effective at clinically relevant concentrations on the late stage, transmittable gametocytes. There is therefore an urgent need to find new safe and efficacious transmission blocking drugs that can target late stage gametocytes.

Plant-derived natural products have played a fundamental role in the control of malaria. They have been a vital source of some of the mainstay drugs in malaria treatment such as the alkaloid, quinine and sesquiterpene lactone, artemisinin (Wells, 2011). These drugs were identified following intensive screens of hundreds of plant extracts for activity against the intraerythrocytic, asexual parasites of *Plasmodium* (Tu, 2011; Zhang, 2011). By contrast, less effort has been channeled towards examining extracts of plants for their activity against the sexual stages of the parasite. Currently, only three plant species have been directly screened for their malaria transmission blocking capacity: *Azadirachta indica* (Meliaceae) (Dhar et al., 1998; Jones et al., 1994; Lucantoni et al., 2010; Udeinya et al., 2006, 2008; Yerbanga et al., 2014), *Vernonia amygdalina* (Asteraceae) (Abay et al., 2015, 2013) and *Guiera senegalensis* (Combretaceae) (Yerbanga et al., 2014). *A. indica* has been comprehensively studied and shown to have good gametocytocidal activity *in vitro* (Dhar et al., 1998; Jones et al., 1994; Udeinya et al., 2008, 2006), *in vivo* (Lucantoni et al., 2010) and *ex vivo* (Yerbanga et al., 2014). The active components are a diverse range of limonoid compounds (Jones et al., 1994; Lucantoni et al., 2010; Yerbanga et al., 2014). *V. amygdalina* gametocytocidal activity was demonstrated *in vivo* with the active principles identified as the sesquiterpene lactones vernadalol and vernolide (Abay et al., 2015, 2013). *G. senegalensis* did not have any transmission blocking properties (Yerbanga et al., 2014).

Even given the complexities involved in plant-based screening for antimalarial activity, the paucity of information of plant species with potency against early and late stage gametocytes encourages studies to identify malaria transmission blocking capabilities of plant extracts. In the current study, we explored the anti-gametocyte properties of eight plant species traditionally used to treat malaria in South Africa. This was investigated by using a stage-specific *in vitro* phenotypic screen of crude extracts of plants for activity against early and late stage *P. falciparum* NF54 gametocytes.

2. Materials and methods

2.1. Plant selection, collection and crude acetone extract preparation

The subset of eight plant species investigated in this study was selected as follows: Plants were first and foremost chosen for being used traditionally for either malaria or fever treatment as documented in ethnobotanical studies (Alam et al., 2012; Clarkson et al., 2004; Van Wyk, 2008; Table 1). From the ethnobotanical set, plants were selected based on two parallel criteria: (1) previous reports of either good or moderate activity against the intraerythrocytic asexual parasites of *P. falciparum* ($IC_{50} \leq 10$ μg/ml – good; $20 \geq IC_{50} > 10$ – moderate) and (2) evidence of the presence of compounds (at plant family level) that are structurally similar to those known to be gametocytocidal either *in vitro*, *in vivo* or *ex vivo* (Abay et al., 2015; Adjalley et al., 2011; D'Alessandro et al., 2013; Duffy and Avery, 2013; Jones et al., 1994; Lucantoni et al., 2013, 2010; Sáenz et al., 2013; Sun et al., 2014; Yerbanga et al., 2014). Using these two parallel selection strategies, the ethnobotanical set was narrowed down to a hundred plant species from which eight were collected and screened in the current study.

Leaves of the eight plant species were collected from the Manie van der Schijff Botanical Garden at the University of Pretoria, Hatfield campus in July 2014. Plants were identified by a curator and voucher specimens were made and deposited at the H.G.W.J. Schweickerdt Herbarium of the University of Pretoria (Table 1) and

Table 1
Plant species selected and evaluated for potential inhibition of asexual or sexual stage *P. falciparum* parasites.

Plant species (Family)	Common names	Selection criteria	Extraction yield (%)	Voucher no.
Khaya anthotheca (Welw) C.DC. (Meliaceae)	Rooimahonie (Afrikaans), red mahogany (English)	c	11.05	PRU 121,391
Trichilia emetica Vahl subsp. Emetica (Meliaceae)	Umkhulu (Zulu), bosveldrooiessenhout (Afrikaans)	a, c	3.25	PRU 121,390
Turraea floribunda Hochst. (Meliaceae)	Umadlozane (Zulu), umhlatholana (Xhosa), kanferfoelieboom (Afrikaans)	a, c	7.12	PRU 121,387
Leonotis leonurus (L.) R. Br. (Lamiaceae)	Wilde dagga (Afrikaans), lion's ear (English)	a	5.85	PRU 121,393
Leonotis leonurus ex Hort (yellow) (Lamiaceae)	Wilde dagga (Afrikaans)	a	3.85	PRU 121,394
Olea europaea subsp. Africana (Oleaceae)	Wild olive (English), Olienhout (Afrikaans)	b	7.08	PRU 121,388
Catha edulis (Vahl) Endl (Celastraceae)	Umhlwazi (Zulu), igqwaka (Xhosa), khat (Afrikaans)	a	3.33	PRU 121,392
Artemisia afra Jacq. ex Wild. (Asteraceae)	African wormwood (English), Wilde-als (Afrikaans), Umhlonyane (Zulu)	a, c	6.38	PRU 121,389

a – good activity against asexual parasites ($IC_{50} \leq 10$ μg/ml); b – moderately active against asexual parasites ($20 \geq IC_{50} > 10$); c – chemical class type production. All plant species names have been checked and confirmed as acceptable on www.theplantlist.org.