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The traditional medicine *Spilanthes acmella*, and the alkylamides spilanthol and undeca-2*E*-ene-8,10-diynoic acid isobutylamide, demonstrate *in vitro* and *in vivo* anti-malarial activity

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Abstract

Spilanthes spp. are used as traditional herbal medicines in Africa and India to treat malaria. Yet, to date, there is no data on active constituents or most effective extraction methods for this indication. The isolated alkylamides, spilanthol and undeca-2*E*-ene-8,10-diynoic acid isobutylamide, found in *S. acmella* Murr., were shown to have IC₅₀s of 16.5 µg/mL and 41.4 µg/mL on *Plasmodium falciparum* strain PFB and IC₅₀s of 5.8 µg/mL and 16.3 µg/mL for the chloroquine resistant *P. falciparum* K1 strain, respectively. Further investigations revealed that at relatively low concentrations, spilanthol and the water extract of *S. acmella* reduced the parasitemia 59% and 53% in mice infected with *P. yoelii yoelii* 17XNL at 5 mg/kg and 50 mg/kg, respectively. Unexpectedly, the 95% ethanol extract of *S. acmella* was less effective (36% reduction in parasitemia) at 50 mg/kg. These results provide the first evidence supporting *S. acmella* against malaria and demonstrating active constituents in *S. acmella* against *P. falciparum*.

Keywords

Plasmodium falciparum; alkylamides; deca-2*E*,6*Z*,8*E*-trienoic acid isobutylamide; malaria; traditional medicine; phytotherapy

INTRODUCTION

The majority of countries coping with malaria spend less than US \$10 per capita annually on healthcare, resulting in a situation where drug costs of 50 cents or less are economic deterrents to treatment (White, 2004). Perhaps due to such economics, and the lack of access to healthcare facilities, medicinal plant preparations remain a well utilized option for the

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CONFLICT OF INTEREST

None reported.

treatment of malaria by the rural poor (Spelman, 2009). *Spilanthes acmella* Murr. (Asteraceae; syn. *Blainvillea acmella* (L.) Philipson) is one such plant from the traditional pharmacopoeia that is reported to be useful in the treatment of malaria. A related species, *S. oleracea* L., is a component of a formula known as Malarial-5, produced and sanctioned by the National Institute of Public Health in Mali for the treatment of malaria, relying primarily on ethnobotanical indications as evidence for treatment (Keita et al., 1990).

Several bioactive compounds have thus far been isolated and characterized from *S. acmella* which includes alkylamides and flavonoids. The *N*-alkylamides are fatty acid derivatives and have been identified in several species of *Spilanthes* (Greger, 1988). Early work found spilanthol, also known as affinin or deca-2*E*,6*Z*,8*E*-trienoic acid isobutyl amide, a local anesthetic, as the main lipidic component (Gerber, 1903). More recent work has found acetylenic alkylamides such as undeca-2*E*-en-8,10-diyenoic acid isobutylamide (UDA) in lower quantities (Bae et al., 2010). However, these compounds and the extracts of *S. acmella*, have never been assessed for antiplasmodial activity.

MATERIALS AND METHODS

Plant Material and Extractions

Cultivation of *S. acmella* took place in Williams, OR at Horizon Herbs. Fresh, whole plants of *S. acmella* were harvested. Species was verified by Richard Cech (Horizon Herbs, Williams, OR) and voucher specimens were submitted to the University of North Carolina Herbarium in Chapel Hill, NC (accession numbers 583423 and 583424). The plants were one year old at time of harvest. A typical protocol for the manufacture of dry root ethanolic extracts was followed and has been described previously (Spelman et al., 2009b). After extraction, extracts were concentrated to 10 mg/mL by speedvac for *in vitro* and *in vivo* experiments.

Isolation of spilanthol

A CombiFlash system (Teledyne, Lincoln, Nebraska, USA) with a 130 g C18 column was used for the separation. A gradient of isocratic separation was conducted with mobile phase composition of 50% A and 50% B, where A = 1% acetic acid (Fisher Chemical, Pittsburgh, PA, USA) in nanopure water and B = HPLC grade acetonitrile (Pharmaco-AAPER, Shelbyville, KY, USA). A flow rate of 0.2 mL/min was used, the injection volume was 10 μ L, detection wavelength 229 nm and the total analysis time was 10 min. An ion trap mass spectrometer with electrospray ionization source (LCQ Advantage, ThermoFisher, San Jose, CA) was employed for quantification of spilanthol content and for identification of other alkylamides and has been published previously. The purity of the spilanthol was estimated to be 84% spilanthol and 13% spilanthol isomers, and an alkylamide of (MH⁺) of 236 m/z at 2.2% previously identified as deca-2*E*,6*Z*,8*E*-trienenoic acid 2-methylbutylamide (Bae et al., 2010). UDA was purchased from Chromadex (Santa Ana, CA, USA).

Biological assays-*in vitro* and *in vivo*.

The IC₅₀s of spilanthol and UDA were determined against the chloroquine-resistant K1 strain and the mildly chloroquine-resistant strain PFB of *Plasmodium falciparum* using a previously established method (Desjardins et al., 1979). The growth inhibition was assessed in triplicate by comparison of the radioactivity incorporated into the treated culture with that in control culture from the same plate. The *in vivo* experiments were carried out with 5 week old male and female Swiss mice weighing 23.2 g (\pm 0.67) (Elevage Janvier, La Genest Saint Isle, France) were housed in standard environmental conditions (24 \pm 1.8 C, 55% humidity). The experiments were conducted in accordance with the EEC Directive of 1986 (86/609/EEC) on laboratory animals. Briefly, extracts of *Spilanthes acmella* and spilanthol (25 mg/

kg and 2.5 mg/kg twice daily, respectively) in a PBS methylcellulose solution (1%) were injected intraperitoneally into each animal 2 hours after inoculation with 10^7 *Plasmodium yoelii yoelii* parasites. Injections were continued twice a day for 4 days and experimental groups consisted of 5 mice (1 male and 4 females) per group. Parasitaemia was monitored daily by microscopic examination of Giemsa stained-thin blood smears.

Statistical analysis

Data is expressed as the mean \pm SEM and comparison of means was conducted using a two tailed *t*-test for paired data when differences were observed. The mean values were considered significantly different if $p < 0.01$.

RESULTS AND DISCUSSION

Figure 1 illustrates the IC50s for the tested alkylamides, spilanthol and UDA on *P. falciparum* *in vitro*. For the Brazilian mildy chloroquine resistant strain PFB (Figure 1A), the IC50s for spilanthol and UDA are 16.5 and 41.4 $\mu\text{g/mL}$, respectively. While for the Thaiianese chloroquine resistant strain K1 (Figure 1B), the effect of the alkylamides is significantly greater, with IC50s of 5.8 and 16.3 $\mu\text{g/mL}$, respectively.

Because *Spilanthes* remedies are commonly prepared as tea, further studies into *S. acmella* were performed *in vivo* on *P. yoelii yoelii*-infected mice using a whole plant galenic extract (an extract of herb/vegetable origin) of 100% water (10 mg/mL). In addition, another form of traditional remedy, a fresh plant ethanolic extract (10 mg/mL, 70% ethanol final volume) was also utilized. Ethanolic extracts have been shown to contain ten times the concentrations of spilanthol as compared to aqueous extracts (Bae et al., 2010). As seen in Figure 2, the control group had an average parasitemia of 17.7% (± 3.3) five days after infection. A significant reduction of parasitemia by treatment with spilanthol (5 mg/kg) and *S. acmella* water extract (50 mg/kg) was observed with parasitemia decreased to 7.3% ± 1.4 and 8.4% ± 1.7 , respectively ($p < 0.001$). The average parasitemia of the *Spilanthes acmella* ethanol extract (50 mg/kg) group after 5 days was 11.3% ± 2.0 ($p = 0.01$). Thus, isolated spilanthol and water extract exhibited the highest activity, 59% and 53% reductions in parasitemia, while the ethanol extract showed a 36% reduction in parasitemia under these experimental conditions.

This suggests that in addition to spilanthol, there may be water soluble constituents that are also active against *Plasmodium*. Common hydrophilic phytochemicals have previously been shown to potentiate known antimalarials (Soh et al., 2009). Moreover, there is likely multiple modes of activity for the observed effect of *Spilanthes* extract. For example, the treatments could have also induced immunological activity contributing to a reduction in parasitemia. Recent studies on alkylamides structurally similar to spilanthol, and UDA specifically, demonstrate immunological activity, particularly cytokine modulation, at concentrations below 1.5 μM (Matthias et al., 2008; Spelman et al., 2009a). Further investigations are necessary to determine the viability of this traditional medicine, and its lead compounds, for the treatment of malaria.

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Abbreviations

K1	a strain of <i>Plasmodium falciparum</i> originating from Thailand
PFB	a strain of <i>Plasmodium falciparum</i> originating from Brazil
UDA	undeca-2 <i>E</i> -en-8,10-diyonic acid isobutylamide

References

- Bae SS, Ehrmann BM, Etefagh KA, Cech NB. A validated liquid chromatography-electrospray ionization-mass spectrometry method for quantification of spilanthol in *Spilanthes acmella* (L.) Murr. *Phytochem Anal.* 2010; 5:438–43. [PubMed: 20310072]
- Desjardins RE, Canfield CJ, Haynes JD, Chulay JD. Quantitative assessment of antimalarial activity in vitro by a semiautomated microdilution technique. *Antimicrob Agents Chemother.* 1979; 16:710 – 718. [PubMed: 394674]
- Gerber E. Ueber die chemischen Bestandteile der Parakresse (*Spilanthes olearacea*, Jacquin). *Arch Pharm.* 1903; 241:270–89.
- Greger, H. Comparative phytochemistry of the alkylamides. In: Lam, J.; Breteler, H.; Arnason, T.; Hansen, L., editors. *Chemistry and biology of naturally-occurring acetylenes and related compounds (NOARC)*. Vol. v7. Elsevier; Amsterdam; New York: 1988. p. 159-78.
- Keita A, Doumbo O, Koita N, Diallo D, Guindo M, Traore AK. Etude preliminaire sur la faisabilite d'un protocole d'essai clinique. *Bull Med Trad Pharm.* 1990; 4:139–46.
- Matthias A, Banbury L, Bone KM, Leach DN, Lehmann RP. Echinacea alkylamides modulate induced immune responses in T-cells. *Fitoterapia.* 2008; 79:53–8. [PubMed: 17855021]
- Soh PN, Witkowski B, Olagnier D, Nicolau ML, Garcia-Alvarez MC, Berry A, Benoit-Vical F. In vitro and in vivo properties of ellagic acid in malaria treatment. *Antimicrob Agents Chemother.* 2009; 53:1100–6. [PubMed: 19015354]
- Spelman K. “Silver Bullet” Drugs Vs. Traditional Herbal Remedies: Perspectives on Malaria. *HG J Am Bot Counc.* 2009; 84:44–55.
- Spelman K, Iiams-Hauser K, Cech NB, Taylor EW, Smirnov N, Wenner CA. Role for PPAR γ in IL-2 inhibition in T cells by Echinacea-derived undeca-2*E*-ene-8,10-diyonic acid isobutylamide. *Int Immunopharmacol.* 2009; 9:1260–1264. [PubMed: 19712756]
- Spelman K, Wetschler MH, Cech NB. Comparison of alkylamide yield in ethanolic extracts prepared from fresh versus dry *Echinacea purpurea* utilizing HPLC-ESI-MS. *J Pharm Biomed Anal.* 2009; 49:1141–9. [PubMed: 19321283]
- White NJ. Antimalarial drug resistance. *J Clin Invest.* 2004; 113:1084–92. [PubMed: 15085184]

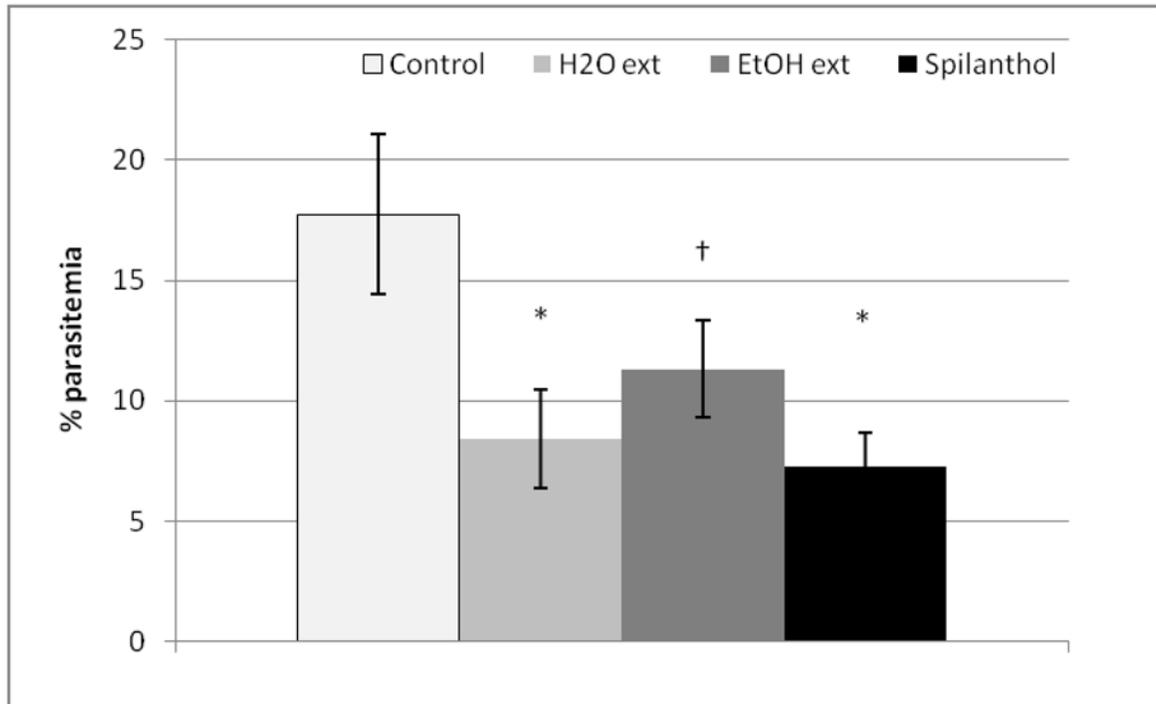


Figure 2. Spilanthol and extracts of whole plant *S. acmella* reduce parasitemia in *Plasmodium y. yoelli*-infected mice

Spilanthol, water extract, or (70%) ethanol extract of *Spilanthes acmella* inhibit parasitemia as compared to the control group. Mice were inoculated with *P. yoelli yoelli* 17XNL and treatment started 2 hours later. Treatments (twice daily spilanthol 2.5mg/kg; water extract 25 mg/kg; ethanol extract 25mg/kg) were given two times a day for four days. Parasitaemia was determined 5 days after infection by microscopic examination of Giemsa stained-thin blood smears. Values are mean ± S.E.M. (n = 5 for each group). †p = 0.01; *p < 0.001