

Investigation of Indian Euphorbia Latexes for Antiplasmodial Properties

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Abstract

This article reports the antiplasmodial activities of *n*-hexane, ethyl acetate and methanol extracts of latexes belonging to three Indian *Euphorbia* species, namely *Euphorbia antiquorum*, *E. nerifolia* and *E. tirucalli* against chloroquine-resistant (K1) strain of *Plasmodium falciparum*. Chloroquine diphosphate was used as positive control. Out of screened extracts, only the *n*-hexane extract of *E. tirucalli* was found to have significant activity with $IC_{50}=8 \pm 2.7 \mu\text{g/mL}$. These results support the traditional usage of *E. tirucalli* in the treatment of severe fevers (or) malaria and therefore, is worthy of further investigation.

Keywords: Malaria; *Plasmodium falciparum*; Latex; *Euphorbia antiquorum*; *Euphorbia nerifolia*; *Euphorbia tirucalli*

Introduction

Malaria is a life-threatening parasitic disease transmitted by female *Anopheles* mosquitoes. According to the World Malaria Report 2012 [1], a significant reduction in malaria incidence and mortality found in half of the malaria endemic countries, but, these countries account for only 3% of the total estimated malaria cases i.e., about 219 million cases (range 154-289 million) per every year world-wide. Of which, 40% of cases were found in the Democratic Republic of the Congo, India and Nigeria. About 660 000 deaths (range 490,000-836,000) estimated to occur every year globally, of which, 90% deaths are occur in the WHO African Region (sub-Saharan Africa), with children under five years of age and pregnant women most severely affected. According to the “*National Health Profile, 2015*” of India, the number of cases as well as deaths due to the disease dropped significantly every year between 2010 and 2013. But, they again elevated between 2013 and 2014. India recorded an estimated 535 malaria deaths in 2014, as compared to 440 in 2013. Malaria cases also rose from 881 730 to 1 070 513 between 2013 and 2014 [2]. The World Malaria Report also notes that resistance of *Plasmodium falciparum* to artemisinin and its derivatives, the key compounds in artemisinin-based combination therapies (ACTs), has been detected in four South East Asian countries, in addition to existing chloroquine resistance. Hence, due to the importance given to public health throughout the world, creating a new antimalarial drug is imperative and beneficial. The majority of natural products or derivatives of natural products in clinical use today originated from traditional medicinal plants. More than 150

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constituents from higher plants with significant antiplasmodial activities have been reported during the last ten years and a number of semi-synthetic derivatives of artemisinin are now in clinical use. At the same time, there has been a new interest in the development of herbal antimalarials as a source of affordable and effective treatments for malaria [3].

Euphorbia is one of the most important genera of the Euphorbiaceae. The species of Euphorbiaceae are extensively used as a folk remedy in many countries around the world to cure numerous diseases such as cancer, epithelioma, sarcoma, skin tumours, diabetes, diarrhoea, heart diseases, hemorrhages, hepatitis, jaundice, malaria, ophthalmic diseases, rheumatism, scabies, asthma, cough, earache and etc., [4-6]. Especially, the latexes of three species out of 68 species found in India, namely *E. antiquorum* L., *E. nerifolia* L. and *E. tirucalli* L., are extensively used in the Indian system of medicine, such as Ayurveda and Siddha to treat a variety of diseases including severe fevers [7-9]. In view of our special interest in developing antimalarials [3], these euphorbia latexes have been collected and subjected for anti-plasmodial screening.

Materials and Methods

Plant material

A survey has been carried out in and around Visakhapatnam region (17°69' N, 83°22' E) to identify the latex species of *Euphorbia* during July-August 2014. The species viz. *E. antiquorum* L., *E. nerifolia* L. and *E. tirucalli* L. were identified for antimalarial screening. The identity of these plants was confirmed by a taxonomist and voucher specimens (140801, 140802 and 140701, respectively) were kept available in the Central Research Laboratory, GIT, GITAM University, Visakhapatnam, India. The fresh latexes from the above plants were collected by tapping individually from their respective single plant sources during morning and evening hours.

Extraction

The Fresh latexes were subjected for extraction individually by stirring successively with cold n-hexane, ethyl acetate and methanol. These extracts were filtered and then concentrated individually under vacuum using a rotary evaporator at 40°C. These extracts were weighed and the yields were expressed as percentages (w/w).

Sample preparation

Crude extracts and control drugs stock solutions (1 mg/mL) were prepared by weighing 1 mg accurately, dissolved in 50 µL of dimethyl sulphoxide (DMSO) or ethanol and made up to 1 mL with double distilled water. Later these solutions were diluted in complete medium.

Chemicals and reagents

Solvents are analytical grade of Merck and all other the materials for the antiplasmodial assays were purchased from Sigma-Aldrich.

Antiplasmodial assay

Cultures containing predominantly early ring stages of *P. falciparum* were used for testing. After adding sample solutions (initial well concentration=500 µg/ml) to 96-well microtiter plates in duplicate, two-fold serial dilutions were made with RPMI 1640 medium and infected erythrocytes were added to give a final volume of 100 µL with 2.5% hematocrit and 1%

parasitemia. Chloroquine diphosphate was used as positive control (initial well concentration=0.5 µg/ml). In each test, uninfected and infected erythrocytes without sample solutions and positive control solution were incubated. Plates were placed into a modular incubator gassed with 93% nitrogen, 3% oxygen and 4% carbon dioxide and incubated at 37°C for 48 hr. Parasite growth was assessed by measuring parasite lactate dehydrogenase activity (pLDH) [3]. The reagent used contained the following in each mL, acetylpyridine adenine dinucleotide (APAD), 0.74 mg; lithium lactate, 19.2 mg; diaphorase, 0.1 mg; Triton X-100, 2 µL; nitroblue tetrazolium, 1 mg; and phenazine ethosulfate, 0.5 mg. Fifty microliters of this reagent was added to each well and mixed. Following incubation at 37°C for 10-15 minutes, optical densities were read at 550 nm using a Dynatech Laboratories MRX microplate reader. IC₅₀ values were determined using linear regression analysis (Microsoft Excel). Data are expressed as the mean ± standard deviation (S.D.) of the IC₅₀ of three independent experiments on different days.

Results and Discussion

The latexes of *Euphorbia nerifolia* L., *E. antiquorum* L. and *E. tirucalli* L. are popularly known by the common name Snuhi and they are milky in nature and difficult to distinguish, morphologically. The collected fresh latexes (10 g each) from the above plants were subjected for extraction and the concentrated extracts were recorded in percentages in TABLE 1. The *n*-hexane extract was yielded in maximum quantity (16%-45%) by all latexes, whereas ethyl acetate and methanol extracts were obtained as negligible quantities. In the present study, these extracts were subjected for antiplasmodial screening for the first time. The activities of the extracts and control drugs are shown in TABLE 1. The data is expressed as the mean ± standard deviation of the IC₅₀ of three independent experiments on different days. Only the *n*-hexane extract of *E. tirucalli* was possessed activity against *P. falciparum* (strain K1) with significant IC₅₀=8 ± 2.7 µg/mL value and other extracts are found to be inactive (IC₅₀>500 µg/mL). The antiplasmodial activity of *E. tirucalli* supports its usage in the traditional treatment for severe fevers/malaria [4].

TABLE 1. Extractive values and antiplasmodial activities of *Euphorbia* latexes.

Name of species	Part	% Extract	IC ₅₀ µg/mL (Mean ± SD)
			Against <i>P. falciparum</i> (strain K1)
<i>Euphorbia antiquorum</i> L.	<i>n</i> -hexane	38	>500
	ethyl acetate	<0.1	>500
	methanol	3	>500
<i>Euphorbia nerifolia</i> L.	<i>n</i> -hexane	45	>500
	ethyl acetate	<0.1	>500
	methanol	5	>500
<i>Euphorbia tirucalli</i> L.	<i>n</i> -hexane	16	8 ± 2.7
	ethyl acetate	<0.1	>500
	methanol	8	>500
Chloroquine diphosphate	control		0.36 ± 0.04

In addition to present activity, anti-arthritis, antimicrobial, antiviral, anti-herpetic, anti-oxidant, hepatoprotective, proteolytic, anticancer, molluscidal and larvicidal activities are also seen in earlier studies due to a great variety of chemical substances found in *E. tirucalli* besides its distinct medical folklore claims of different parts of the world [4,10]. The characteristic constituents of *E. tirucalli* are triterpenes, however, diterpenes, tannins and other class of compounds were also reported. The latex of this plant was reported to contain euphol, taraxasterol, tirucallol [11], 31-nortriterpene, cycloeuphordenol, cyclotirucanenol [11-13], lupeol, esters of (euphol and tirucallol), acetates of (euphol, tirucallol and lupeol), lupenone, friedelin, glutinol [14], euphorbinol and cycloeuphornol [15,16].

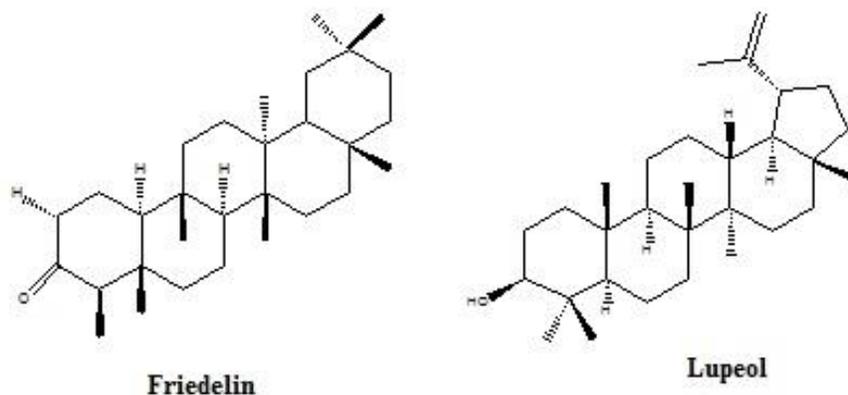


FIG. 1. Chemical structures of friedelin and lupeol.

In various studies, lupeol was reported to have weak activity against *P. falciparum* with IC_{50} values $27.7 \pm 0.5 \mu M$ (strain 3D7) [17], $30 \mu g/mL$ (strain FcM29-Cameroon) [18] and $25 mg/mL$ [19]. Though lupeol is a well-known cytotoxic substance, the inhibition of parasite growth is not through its toxic nature [17].

A study by Ziegler [17] confirmed that lupeol has an indirect action as it causes a transformation of the human erythrocyte shape toward that of stomatocytes and made the cells unsuitable for parasite growth. Friedelin was also reported to have potent antiplasmodial activity *in-vitro* with IC_{50} value $7.2 \pm 0.5 \mu M$ against chloroquine resistant strain (W2) of *P. falciparum*. [20] The presence of these two compounds (FIG. 1) may explain the antiplasmodial activity of *E. tirucalli* found in the present study. However, a detailed phytochemical investigation is required to find the constituents responsible for the activity.

Conclusion

The latexes of *E. antiqorum*, *E. nerifolia* and *E. tirucalli* were screened for antiplasmodial activity for the first time and *E. tirucalli* was found to be active with $IC_{50}=8 \pm 2.7 \mu g/mL$ against chloroquine-resistant (K1) strain of *Plasmodium falciparum*. This activity supports the traditional usage of *E. tirucalli* in the treatment of severe fevers/malaria. Therefore, further studies need to be done to isolate and characterize active constituents of extract.

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