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Isolation, Characterization and *In Vivo* Antimalarial Evaluation of Anthrones from the Leaf Latex of *Aloe percrassa* Todaro

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Abstract

Malaria remains a critical problem in global public health and continues to remain among the top three infectious diseases (along with tuberculosis and HIV) due to resistance of the pathogen to antimalarial drugs. *Aloe percrassa* Tod is an indigenous species of Ethiopia traditionally used for the treatment of malaria, wounds and gastric problems. The present study focused on isolation and characterization of antiplasmodial constituents from the latex of *A. percrassa* using Peter's 4-day suppressive test. After a four day treatment of *Plasmodium berghei* infected mice with the latex at doses of 100, 200 and 400 mg/kg/day, chemosuppression of 45.9%, 56.8% and 73.6% was observed, respectively. Further phytochemical examination of the latex by preparative TLC over silica gel resulted in the isolation of two anthrones, viz. aloin A/B and microdontin A/B, whose structures were determined on the basis of UV, MS, ^{1}H, and ^{13}C NMR spectral data. Antimalarial activities of both aloin A/B and microdontin A/B were lower than that of the latex suggesting that the two compounds may have acted synergistically. No adverse sign of toxicity and mortality was observed in mice within the first 24 h as well as for the following 14 days after the test substances were orally administrated up to a dose of 2 g/kg for the pure compounds and 5 g/kg for the latex. The strong antimalarial activities coupled with a relative safety of the leaf latex and isolated compounds of *A. percrassa* may confirm the claim by traditional practitioners for the use of the plant against malaria infection.

Keywords: *Aloe percrassa*, aloin A/B, anthrones, *In vivo* study, microdontin A/B, Peter's 4-day suppressive test

1. Introduction

Malaria is an ancient and one of the major fatal parasitic killer diseases of the world, having been recorded as early as 1500 B.C [1]. Malaria is caused by five species of parasites of the genus *Plasmodium* (*P. falciparum, P. knowlesi, P. malariae, P. ovale* and *P. vivax*) that affect humans [2]. *P. falciparum* is the most virulent parasite, and is responsible for the majority of malaria related mortality. It is found in all malaria endemic regions of the world and is the most common human malaria parasite in Africa [3] and estimated to be the direct cause of 500 million cases and over 1 million deaths per year, mostly in women and children under the age of 5 years. In children, progression of the disease from mild to severe is particularly rapid [4].

Malaria is the number one public health problem in Ethiopia and a major cause of illness and death. Due to the high population pressure and depletion

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of agricultural land in highland areas, there has been a massive population movement to the lowlands, particularly in the last two decades. Recent information on the epidemiology of malaria indicates that the disease has encroached to areas that were free of malaria [5]. Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies of plant origin for their healthcare. [6].

The genus *Aloe* comprises about 600 species, of which 46 are indigenous to Ethiopia and Eriterea [7]. *Aloe percrassa*, locally known as Ere-Senay, is traditionally used for treatment of malaria, wounds, and gastro intestinal problems [8]. *A. percrassa* is an indigenous species belonging to a group of aloes that include *A. debrana*, *A. rivae* and *A. trigonantha*, which are stemless and often have secondarily branching inflorescences, with up to more than 50 racemes. *A. percrassa* is distinguished from the rest of the group by the large bracts, which are 10–16(–20) mm long [7].

In the present study, the antimalarial activity of latex of *Aloe percrassa* as well as the compounds isolated thereof have been investigated for their in vivo antimalarial activities using Peter's 4-day suppressive test.

2. Materials and Methods

2.1 General

Ultraviolet (UV) spectra were determined using a Shimadzu UV 1800 spectrometer (200–400 nm) at room temperature. ESI-MS were recorded on Ultimate 3000 LC-MS. The measurement was carried out by an electro spray ionization method with negative mode. The source voltage and temperature were fixed at 3kV and 250°C. NMR spectra were recorded on Bruker Avance DMX400 FT-NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C at room temperature using deuterated methanol.

2.2 Plant Material

The leaf latex of *A. percrassa* was collected from and around the town of Edagahamus on the road to Gunda Gundo, eastern zone of Tigray, northern Ethiopia. The authenticity of the plant material was confirmed by Professor Sebsibe Demissew, the National Herbarium, Department of Biology, Addis Ababa University, where voucher specimen (collection number GG 001) was deposited.

2.3 Experimental Animals and Parasite

Healthy adult Swiss albino mice of either sex (22–28 g and 6–8 weeks of age) bred and maintained at the Ethiopian Health Nutrition and Research Institute (EHNRI) were used. They were maintained under standard condition (temperature of 22 ± 3°C, relative humidity of 40–50% and 12 h light/12 h dark cycle), with food and water *ad libitum* in the animal house of the Department of Biology, College of Natural Sciences, Addis Ababa University. Animals were acclimatized for one week to the experimental environment, and each animal was housed individually in a cage under standard environmental conditions.

Chloroquine (CQ) sensitive strain of *Plasmodium berghei* ANKA strain obtained from the Department of Biology, College of Natural Sciences, Addis Ababa University was used. The parasite was subsequently maintained in the laboratory by serial blood passage from mouse to mouse on weekly bases. At the end of each experiment the animals were euthanized with diethyl ether. All procedures complied with the Guide for the Care and Use of Laboratory Animals [9] and approved by the Institutional Review Board of the School of Pharmacy, Addis Ababa University.

2.4 Collection of Leaf Latex

The leaf latex of *A. percrassa* was collected by cutting the leaves transversally near the base and arranging them concentrically around a plate. The latex was then left in open air for 1–3 days to allow evaporation of water, which yielded a dark brown powder.

2.5 Isolation of Compounds

Isolation of compounds was performed by Preparative Thin Layer Chromatography (PTLC) over silica gel using chloroform and methanol (4:1) as a solvent system. The chromatographic zones were visualized first in daylight and then under ultraviolet light of wavelengths 254 and 366 nm. Each band was carefully scrapped off separately from the plate and dissolved in methanol and chloroform (1:1). Finally the mixture was filtered and the solvent evaporated using rotavapor.

2.6 Oral acute Toxicity Testing

Acute oral toxicity study was conducted as per the internationally accepted protocol drawn under OECD guidelines 425 [10]. Fifteen healthy Swiss female mice weighing 23–25 g were randomly divided into 3 groups of 5 mice per group. After being fastened for 3 h, mice in the first group were given 5 g/kg of the leaf latex, the second group 2 g/kg of microdontin A/B and the third group 2 g/kg of aloin A/B orally and observed for any signs of toxicity for 14 days to assess safety of the test substances. The mice were observed for gross behavioral changes such as loss of appetite, hair erection, lacrimation, mortality and other signs of toxicity manifestation.

2.7 *In vivo* Antimalarial Activity

2.7.1 Parasite Inoculation

P. Plasmodium berghei infected albino mice with parasitaemia level of 20–30% were used as donor. Parasitized erythrocytes were obtained from a donor infected mouse by cardiac puncture with a sterile needle and syringe. The blood was then diluted with physiological saline (0.9%) based on parasitemia level of the donor mice to prepare in such a way that 1 ml blood contains 5×10^7 infected erythrocytes. Twenty five male Swiss albino mice were selected and inoculated intraperitonealy with infected blood suspension (0.2 ml) containing 1×10^7 infected erythrocytes.

2.7.2 4–Day Suppressive Test

A modified Peter's 4–day suppressive test against chloroquine sensitive *P. berghei* infection in mice was employed. The mice were divided into five groups with groups I (negative control), II, III and IV receiving the latex at doses of 100, 200, and 400 mg/kg/ of body weight per day, respectively, while group V (positive control) were treated with chloroquine at a dose of 25 mg/kg/day in a volume of 0.5 ml. All the test substances were administered through oral route by using oral gavages. Treatment was started 3 h after infection on day 0 and then continued daily for four days (i.e. from day 0 to day 3). On the fifth day (D_4) thin blood smears were prepared fixed in methanol and stained with 10% Geimsa solution to be examined under the microscope with an oil immersion objective of 100x magnification power to evaluate the percent suppression [11–13].

Percentage growth inhibition of the parasites was calculated by the following formula:

$$\text{Growth inhibition}(\%) = \frac{\text{Parasitemia in negative control} - \text{Parasitemia in study group}}{\text{Parasitemia in negative control}} \times 100$$

2.7.3 Monitoring of Body Weight and Mean Survival Time

For a 4–day suppressive test, additional parameters, such as body weight and mean survival time, were also used to evaluate the antimalarial activity of the test substances in animal model. Body weight of each mouse was measured before infection (day 0) and on day 4 on healthy mice at the tested doses using a sensitive digital weighing balance. Mean survival time was also recorded from day 1–28 after infection.

2.8 Data Analysis

Results of the study were expressed as mean ± standard error of mean (M ± SEM). Data were analyzed using Windows SPSS Version 20. Comparison of parasitaemia among groups and statistical significance was determined by one-way ANOVA and student t-test at a 95% confidence interval ($\alpha = 0.05$). The results were considered significant when $P < 0.05$.

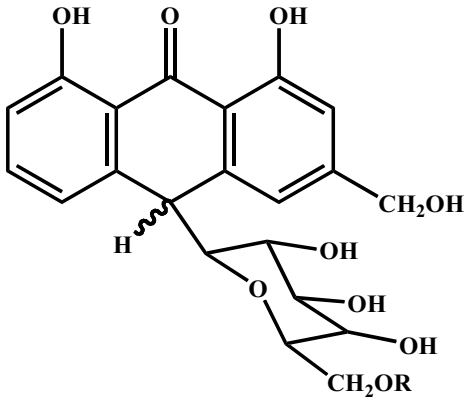
3. Results and Discussion

3.1 Acute Toxicity

No major signs of toxicity such as changes in general behaviour, variations in body weight and mortality were observed when the leaf latex and the isolated compounds were administrated orally at a dose of 5 and 2 g/kg, respectively. However, minor signs of toxicity such as temporary hair erection and diarrhoea were observed in a limited number of experimental animals.

3.2 Structural Elucidation of Isolated Compounds

Analysis of the leaf latex of *A. percrassa* by PTLC over silica gel led to the isolation of microdontin A/B and aloin A/B, which appeared bright yellow when viewed in daylight, dark under $UV_{254\ nm}$, and dark orange under $UV_{366\ nm}$. Microdontin A/B (Fig. 1) was isolated as a yellow amorphous solid with R_f value of 0.72 (Chloroform: Methanol (4:1));



Microdontin A/B: R = *p*-Coumaroyl
Aloin A/B: R = H

Fig. 1. Chemical structures of microdontin A/B and aloin A/B.

UV λ max (MeOH): 208, 303, 312 nm; -ve ESI-MS m/z: 563 $[M-H]^-$ ($C_{30}H_{28}O_{11}$). ^1H and ^{13}C NMR spectral data of microdontin A/B are identical with those data reported for the same compound by Farah *et al.* [14].

Aloin A/B (Fig. 1) was also obtained as a yellow amorphous solid with R_f value of 0.59 (Chloroform: Methanol (4:1)); UV λ max (MeOH): 208, 271, 299, 357; -ve ESI-MS m/z: 417 $[M-H]^-$ ($C_{21}H_{22}O_9$). ^1H and ^{13}C NMR spectral data of aloin A/B are identical with those data reported in the literature for the same compound [15, 16].

3.3 Antimalarial Activity

The results of the present study showed that the latex of *A. percrassa* possesses *in vivo* antimalarial activity against *P. berghei*, with the maximum parasitaemia suppression observed at a dose of 400 mg/kg. As shown in Table 1, percentage suppression of *P. berghei* in mice increases with increasing dose. Mice treated with chloroquine were completely free from the parasites on day four.

Mean survival time is another parameter that is commonly used to evaluate the efficacy of antimalarial plant extracts [17]. In the present study, the mean survival time of mice treated with the latex was longer when compared with vehicle treated animals. As shown in Table 2, the latex prolonged mean survival time of the experimental animals in a dose dependent manner. Similarly, body weight loss is one feature of rodent malaria infections [18]. The results of the present work showed that the latex has the capacity to prevent weight loss at all dose levels compared to the control. The mean weight of each group with the exception of the vehicle treated group improved on the fifth day of infection. In general, animals treated with higher doses of the latex gained more weight than those treated with lower doses (Table 3).

As shown in Table 1, the isolated compounds have also produced a dose dependent chemosuppresion. After

Table 1: Percentage suppression of *Plasmodium berghei* in mice after treatment of the leaf latex, microdontin A/B and aloin A/B

Test substances	Dose (mg/kg /day)	% Parasitaemia ± SEM	% Suppression
Distilled water	0.5 ml	32.9 ± 4.12	-
Latex	100	17.8 ± 1.23*a	45.9
	200	14.2 ± 1.84*a	56.8
	400	8.7 ± 1.28*a	73.6
Microdontin A/B	100	31.3 ± 2.11	25.8
	200	21.8 ± 2.49**a	48.3
	400	16.3 ± 2.45*a	61.4
Aloin A/B	100	29.2 ± 5.7*a	36.8
	200	22.6 ± 1.61*a	51.1
	400	15.3 ± 0.06*a	66.8
Chloroquine	25	0.00*a	100

Values are presented as M ± SEM; n =5; ** ($p < 0.01$ *($p < 0.001$); a = compared to negative control.

Table 2: Mean survival time of *Plasmodium berghei* infected mice after treatment with leaf latex, microdontin A/B and aloin A/B

Test substances	Dose mg/kg /day)	Mean survival time (days)
Distilled water	0.5 ml	7.0 ± 0.32
	100	8.6 ± 0.24*[a]
Latex	200	9.0 ± 1.58[ba]
	400	10.4 ± 0.51[ca]
	100	7.2 ± 0.37*[a]
Microdontin A/B	200	7.6 ± 0.4*[a]
	400	8.6 ± 0.4*[a]
	100	7.40 ± 0.4*[a]
Aloin A/B	200	8.0 ± 0.32*[a]
	400	9.0 ± 0.32[ba]
Chloroquine	25	ND

Values are presented as M ± SEM; n = 5; *p > 0.05; b = p < 0.05; c = p < 0.001; a = compared to negative control; ND = No death within the follow-up 28-days.

a four day treatment with aloin A/B and microdontin A/B at a dose of 400 mg/kg, the mean parasitaemia level of the test groups dropped to 15.3 ± 0.06% and 16.3 ± 2.45, respectively, from the corresponding value of the negative control group (32.9 ± 4.12). The mean survival time and body weight of mice receiving the isolated compounds have also improved in a dose dependent manner (Tables 2 and 3).

It is interesting to note that at all doses tested the latex showed better chemosupression effect than the individual compounds. This could be explained that the two compounds exert their actions synergistically or there may be other minor components in the latex which have stronger schizonticidal activity.

Other than direct parasiticidal effects, plants may possess other pharmacological benefits to the hosts, such as acting as analgesics, antipyretics or as immune stimulators [19]. Thus, protection from weight loss in the study animals could be due to suppression of the parasite, enhanced appetite and/or the above pharmacological effects. For example, aloe-emodin has been reported to possess various pharmacological and biological activities including antiparasitic, immunostimulation, anti-inflammatory and analgelsic, among others [20]. Therefore, the antiplasmodial activity of aloin A/B observed in this study could probably be supported by such pharmacological action as its structure is similar to aloe-emodin.

Several classes of natural products including naphthoquinones, anthraquinones, chalcones, flavanones, coumarins, and phenolic glycosides have been reported to possess antimalarial activity [21], which gives credence

Table 3: Body weight of *Plasmodim berghei* infected mice after the administration of leaf latex, microdontin A/B and aloin A/B

Test substances	Dose (mg/kg/day)	Wt D_0 ± SEM	Wt D_4 ±SEM	Mean difference
Distilled water	0.5 ml	24.20 ± 0.12	21.22 ± 0.37	–2.98 (–12.3)
	100	26.88 ± 0.42	27.50 ± 0.54	0.62 (2.3)
Latex	200	26.70 ± 0.38	27.76 ± 0.86	1.06 (4.0)
	400	26.28 ± 0.34	27.46 ± 0.40	1.18 (4.5)
	100	24.78 ± 0.28	25.00 ± 0.61	0.22 (0.9)
Microdontin A/B	200	25.64 ± 0.26	26.34 ± 0.40	0.70 (2.7)
	400	23.20 ± 0.25	23.90 ± 0.312	0.72 (3.1)
	100	24.42 ± 0.25	24.64 ± 0.36	0.22 (0.9)
Aloin A/B	200	24.10 ± 0.56	24.72 ± 0.66	0.62 (2.6)
	400	25.24 ± 0.30	26.20 ± 0.15	0.96 (3.7)
Chloroquine	25	26.98 ± 0.22	28.14 ± 0.27	1.16 (4.3)

Values are presented as M ± SEM; n = 5; Wt D_0: weight pre-treatment on day zero; Wt D_4: weight post-treatment on day five; values in parenthesis indicate % of change.

to the activity seen by the anthrone derivatives, aloin A/B and microdontin A/B. Some phenolic compounds like flavonoids have been shown to inhibit influx of L-glutamine and myoinositol into infected erythrocytes [22]. As aloin A/B and microdontin A/B have phenolic OH groups at C-1 and C-8, they may exert their action by the same mechanism. Due to their structural similarity with atovaquone, the mechanism of action for the isolated compounds could also involve inhibition of mitochondrial electron transport in cytochrome bc complex, which is linked to pyrimidine biosynthesis [23]. Moreover intercalation with parasite DNA due to cyclic planar structure is another possible mechanism for the antimalarial activity of anthraquinones [24], which could also be a mechanism for the antimalarial activity of the isolated compounds. However, as the mode of action within similar classes of compounds may vary extensively, it may be difficult to propose the exact mechanism(s) by which the isolated compounds exert their antimalarial effect.

4. Conclusion

The study confirmed that the latex of *A. percrassa* possesses genuine antimalarial activity which could be attributed in part or in full to the presence of the anthrones aloin A/B and microdontin A/B, providing scientific evidence for the traditional claim. The results also indicated that the test substances have wide safety margin making them potential leads for the development of safer, more potent and cost effective alternative drugs for the treatment of malaria.

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