

## Antiplasmodial activities of some products from *Turreanthus africanus* (Meliaceae)

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### SUMMARY

We investigated the antiplasmodial activity of some pure compounds of *Turreanthus africanus* (Meliaceae), a plant that is used in traditional medicine to treat malaria in Southwest Cameroon. A phytochemical analysis of the methylene chloride:methanol (1:1) extract of the seeds of the plant yielded seven compounds. Four of them, which were oils, were subjected to *in vitro* bioassays on *Plasmodium falciparum* F 32, chloroquine sensitive strain. Compound 1 (16-oxolabda-8 (17), 12(E)-dien-15-oic acid), showed the highest antiplasmodial activity, two others (methyl-14,15-epoxylabda-8 (17), 12(E)-diene-16-oate, and turreanin A), had moderate activity and one was inactive. These findings are consistent with the use of *T. africanus* in the traditional treatment of *P. falciparum* malaria.

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### Introduction

The high burden of malaria with an estimated 300 to 500 million cases and a mortality rate of about a million deaths annually mostly in children is increasing [1, 2, 3]. *Plasmodium falciparum* causes the most severe form of malaria and is responsible for the majority of all clinical cases [4]. Its control in recent years has been rendered more difficult by the emergence and spread of drug resistant strains [5, 6]. Medicinal plants are widely used in the treatment of microbial infections in African traditional medicine [7] and some of these plants have been shown to have considerable antimalarial activity [8, 9, 10]. In continuation of our search for new antiplasmodial agents from plant sources, we investigated the compounds isolated from *Turreanthus africanus*

(Meliaceae), a timber tree of the rainy forests of West and Central Africa. In Cameroonian traditional medicine, the trunk bark and seeds of this plant are boiled together with *Carica papaya* (Caricaceae) leaves, the seeds of *Aframomum melegueta* (Zingiberaceae) and lime and used for treatment of malaria and other fevers, by drinking and or as a steam bath. The bark of this tree is also used as a fish poison in small freshwater streams. It is also said to have insecticidal activity. Some of the compounds isolated from this plant have been described from *Renealmia alpinia* (Zingiberaceae) and are known to have cytotoxic activity [11]. Herein, we report on the antiplasmodial activities of the compounds isolated from the seeds of *T. africanus*

## Materials and Methods

### Plant Material

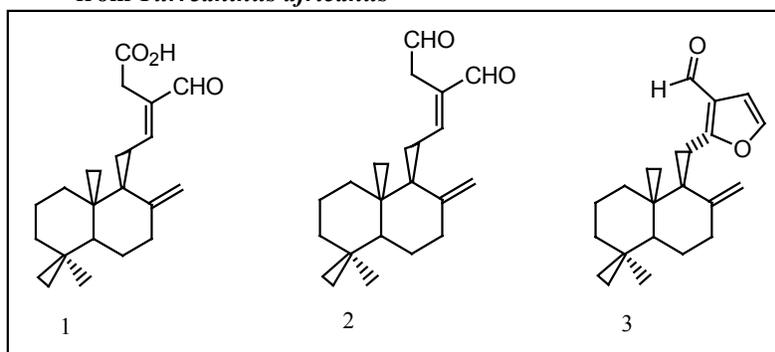
The seeds of *T. africanus* were collected in Buea on the flanks of Mount Cameroon in August 2003, and authenticated by Mr. Ndivé Elias, a Botanist of the Limbe Botanic Garden, Cameroon, where a voucher specimen was deposited.

### Extraction and Isolation

The air-dried and coarsely ground seeds (3.5 kg) of *T. africanus* were defatted in hexane for 48 hours, followed by maceration in methylene chloride: methanol mixture (1:1) for 5 days. Filtration and concentration *in vacuo* on a rotary evaporator yielded 150 g of extract, which was subjected to vacuum liquid chromatography on

silica gel, eluting with a gradient of ethylacetate in hexane. This gave seven major fractions, which were variously purified by gel permeation through Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub> and/or CH<sub>2</sub>Cl<sub>2</sub>/MeOH), and collecting 50 ml for each elution. Fraction 1 obtained by 10–15 % ethylacetate in hexane afforded 16-oxolabda-8 (17), 12(E)-dien-15-oic acid, **1** (22 mg), as an orange oil [11], while fraction 2 (20–25% ethylacetate in hexane) gave methyl-14, 15-epoxylabda-8 (17), 12(E)-diene-16-oate, **2** (150 mg), as a yellowish oil and turreanin A, **3** (100 mg), as an orange oil, shown in Figure 1, [12,13].

**Fig 1. Chemical structures of three of the four pure compounds isolated from *Turreanthus africanus***



### Preparation of Solutions

The compounds were weighed (10 mg of each) and dissolved in 100 to 200  $\mu$ l dimethylsulphoxide (DMSO) at 37° C overnight, then sterile gentamycin-free medium (RPMI 1640), was added to give stock solutions of 1 mg/ml final concentration. The stock solutions were sterile-filtered through 0.45  $\mu$ m filter and stored in the dark at 4° C. Chloroquine diphosphate (1 mg/ml) was prepared similarly.

### Culture of the Malaria Parasite

*Plasmodium falciparum* F32 strain (chloroquine-sensitive) originally obtained from

Tanzania was used in this study. This strain has been cultured and maintained in the Department of Immunology, Stockholm University, Sweden. The parasite was cultured by the candle jar technique of Trager and Jensen [14], using malaria culture medium (MCM: 10.43 g RPMI 1640, 2 g NaHCO<sub>3</sub>, and 0.5 ml of 50 mg/ml Gentamycin, per litre of double distilled water, with 10% human serum), group O<sup>+</sup> erythrocytes and incubated at 37° C. Synchronization and subculturing of the parasite were done as previously described [15] and synchronised cultures were used in the growth inhibition assay.

### *In Vitro Growth Inhibition Assay*

This was done as described with some modification [16]. 100 µl of sterile RPMI (gentamycin-free) was added in duplicate to all required wells in a 96 well flat-bottom plate (Costar, Corning, NY), 100 µl of extract solution was doubled diluted in the wells to give concentrations from 250 to 2 µg/ml. Chloroquine diphosphate was used as positive control (in the range 0.016 – 0.025 µg/ml). DMSO was tested at 0.125% final concentration. Negative control (without extract) was included. 100 µl of 1% F32 parasitised blood was added to all the wells. The plate was incubated in a candle jar for 48 hours and the cells fixed on 8-well multi-test slides and infected erythrocytes (Ei) counted by fluorescence microscopy using acridine orange stain. The number of Ei in 25 microscopic fields (approximately 5000 erythrocytes) per well was counted (i.e. a total of 40,000 erythrocytes per test concentration). The percentage parasitaemia after incubation was calculated as  $(Ei \div 40,000) \times 100$ . The percentage inhibition per concentration was calculated using the formula [17]:

$$\left[ \frac{(\% \text{ parasitaemia in control wells} - \% \text{ parasitaemia of test wells})}{\% \text{ parasitaemia of the control}} \right] \times 100$$

### *Acute Toxicity Test*

The most active product, compound **1**, was tested for acute toxicity *in vivo* in 6N C57 BL mice (approximately 20 g body weight). The compound (60 µg/g body weight of mouse > 2 x IC<sub>50</sub>) was injected intraperitoneally in each of six mice. DMSO (0.25% per gram body weight) was also tested in another group of mice. This test was conducted in accordance with the European Community guidelines [18].

### *Statistical Analysis*

The experiment was done twice for the most active product (compound **1**, Figure 1), each concentration was tested in duplicate. The average percentage inhibition of invasion of the two experiments for each concentration is shown on Table 1. The Mann-Whitney test was used to test for significance in the inhibition of erythrocyte invasion by *P. falciparum* F32 at

each concentration of compound **1**, by comparing the eight average Ei values corresponding to the eight wells of the multi-test slide for each concentration with those of the control.

## Results

### *Antiplasmodial Activity*

All the isolated compounds were tested on trophozoites. The average percentage inhibition of erythrocyte invasion by *P. falciparum* F 32 in the culture for the four compounds ranged from 16 to 77 % at the highest concentration of 62.5 µg/ml tested (Table 1). Compound **1** was the most active with an IC<sub>50</sub> of 26 µg/ml (Figure 2). The invasion of erythrocytes by the parasite was significantly reduced at each of the four concentrations of compound **1** tested relative to the control ( $p = 0.001$  to  $0.002$ ). The effect of compound **1** on erythrocyte invasion is illustrated in Figure 2. The negative control had a growth rate (parasitaemia) of close to three fold after 48 hours incubation (1.5 – 2.9%). We recorded an IC<sub>50</sub> for chloroquine of approximately 0.02 µg/ml. DMSO did not inhibit parasite growth at the highest concentration (0.125% w/v) used in this assay.

### *Acute Toxicity Test: -*

No death was recorded in the three groups of six mice treated with compound **1**, DMSO and the untreated control respectively, during the seven days of the acute toxicity test. The physical activity of the treated mice was indistinguishable from the control.

## Discussion

The extracts prepared from the seeds of *T. africanus* (Meliaceae), yielded seven pure compounds. Four of them, which were oils, were subjected to an *in vitro* bioassay on *Plasmodium falciparum* F 32, chloroquine-sensitive strain. The highest activity was observed with Compound **1**, with IC<sub>50</sub> of 26 µg/ml (Table 1). Compounds **2** and **3** showed lower antiplasmodial activities (IC<sub>50</sub>: 51 and 60 µg/ml respectively), while the fourth compound, TA3, was inactive. *T. africanus* is used to treat malaria

by people of the Bakweri ethnic group based in the Fako Division in the South West Province of

**Table 1. Inhibition of *Plasmodium falciparum* F 32 (trophozoite stage) invasion of erythrocytes by the four compounds isolated from *T. africanus***

N <sup>o</sup>	Compound	Concentration of compound ( $\mu\text{g}/\text{mL}$ )				Approximate  IC <sub>50</sub>  ( $\mu\text{g}/\text{mL}$ )
		62.5	31.25	15.125	7.8	
		% Inhibition of Invasion				
1	<b>1<sup>b</sup></b>	76.8 <sup>c</sup>	52.7 <sup>c</sup>	42.8 <sup>d</sup>	34.6 <sup>d</sup>	26
2	<b>2</b>	54	13.3	a	a	51
3	<b>TA 3</b>	16.6	a	a	a	>>60
4	<b>3</b>	51.6	33.3	32.1	26.9	60

<sup>a</sup>Slides not counted due to very low inhibition observed at higher concentration.

<sup>b</sup>The four concentrations of compound **1** tested produced significant inhibition of erythrocyte invasion;

<sup>c,d</sup>Mann-Whitney statistical significance(p): <sup>c</sup>p = 0.001, <sup>d</sup>p = 0.002

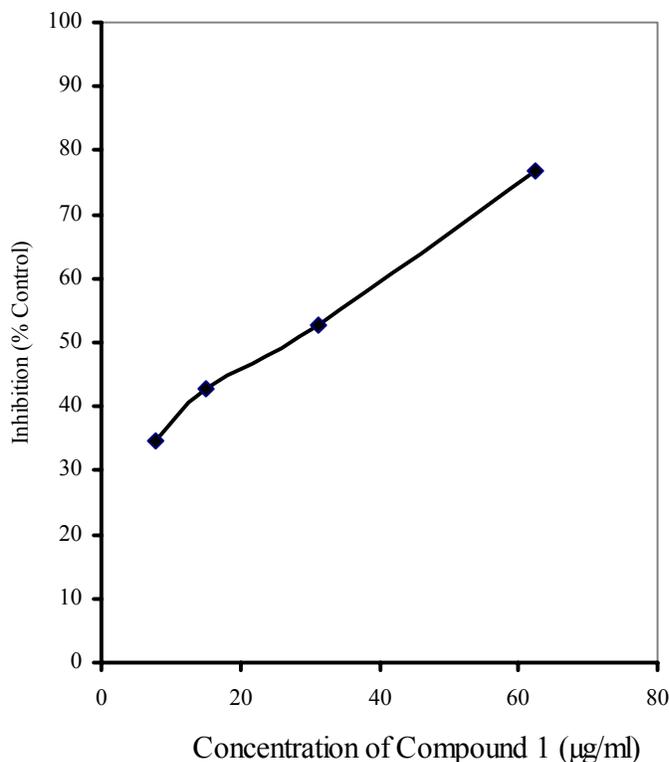
Cameroon. The Bakweri people call it “mokomo” in their native tongue (Mokwe). We recorded an average IC<sub>50</sub> of 0.02  $\mu\text{g}/\text{ml}$  for chloroquine (n =10), almost identical to the value (0.021  $\mu\text{g}/\text{ml}$ ) recorded by Muregi et al against chloroquine-sensitive *P. falciparum* K39, isolated from a Kenyan patient [9], using the <sup>3</sup>H-hypoxanthine incorporation method. This demonstrates the reliability of the fluorescence microscopy technique that was used in this study. On this basis, the IC<sub>50</sub> of compound **1** is high when compared with chloroquine (IC<sub>50</sub> = 0.02  $\mu\text{g}/\text{ml}$ ) The results show that the individual products present in *T. africanus* have weak antiplasmodial activity. In the traditional medicine practice of the Bakweris, the trunk bark and seeds of this plant are boiled together with *Carica papaya* leaves, the seeds of *Aframomum melegueta* and lime and used for treatment of malaria. The results serve as evidence that at least there is some antimalarial activity which supports the claim of traditional medicine practitioners and users that the plant is active against malaria. The acclaimed

effectiveness of the stem bark against malaria in traditional use is likely as a result of the combined effect of the different compounds present in it together with the antimalarial effects of the other plants used in conjunction with it. Bhat and Surolia [19] showed that the petroleum ether extracts of the rind and pulp of *Carica papaya* had IC<sub>50</sub>s of 15.19 and 18.09  $\mu\text{g}/\text{ml}$  respectively against *P. falciparum* FCF 2, a local Indian strain; the methanol and water extracts gave IC<sub>50</sub>s above 100  $\mu\text{g}/\text{ml}$ . We have recorded an IC<sub>50</sub> of 52.5  $\mu\text{g}/\text{ml}$  for *Carica papaya* leaves against *P. falciparum* F 32. Labdanes from *Aframomum latifolium* and *Aframomum sceptrum* of the Zingiberaceae family showed a modest *in vitro* activity against a chloroquine-sensitive *Plasmodium falciparum* strain [20]. *Aframomum melegueta* of the same family has been shown to have antipyretic, analgesic, anti-tussive and anti-hepatotoxic actions [21]; inhibits prostaglandin synthesis [22]; and has antibacterial and antifungal activity [23, 24] but apparently has not been extensively investigated for antimalarial activity. It is likely

that these plants used traditionally together with *T. africanus* contribute to the observed antimalarial activity, however this combined effect and its clinical efficacy remains to be demonstrated experimentally. Figure 2 shows a dose-related inhibition of erythrocyte invasion suggesting that compound **1** may be acting on a

specific target in the *Plasmodium* parasite. At each of the four concentrations of compound **1** there was a significant reduction of erythrocyte invasion relative to the control ( $p = 0.001$  to  $0.002$  from  $62.5$  to  $7.8 \mu\text{g/ml}$  respectively).

**Fig 2. Inhibition of *P. falciparum* F 32 (trophozoite stage) invasion of erythrocytes by Compound 1 ( $\text{IC}_{50} = 26 \mu\text{g/ml}$ )**



Compound **1** is known to be cytotoxic *in vitro* [11], but the compound did not show any acute toxicity in mice at greater than twice its  $\text{IC}_{50}$  per gram body weight ( $60 \mu\text{g/g}$  body weight). However a more extensive toxicity study is required to better assess the safety of the compound. From the above findings, compound **1** is the most active component from *T. africanus* probably acting in an additive mode or in synergy with the other compounds present in it and in the other plants with which it is combined in traditional use. *In vivo* biotransformation of compound **1** and the other compounds may produce intermediates that may be more active against the malaria parasite

compared to the *in vitro* activity but this also remains to be demonstrated.

## Conclusion

The results show that *T. africanus* has weak antiplasmodial activity, which probably when combined with other antiplasmodial plants (*C. papaya* and *A. melegueta*) results in an enhanced antimalarial effect which is exploited in the traditional medicine of the Bakweris as an alternative to standard treatment for clinical malaria.

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## RESEARCH ARTICLE

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