International Organization for Chemical Sciences in Development

Working Group on Plant Chemistry

CHEMISTRY, BIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF AFRICAN MEDICINAL PLANTS

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African traditional healer and *Harpagophytum procumbens* (Pedaliaceae)

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22. Antimalarial active principles of *Spathodea campanulata*

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Introduction

Spathodea campanulata P. BEAUVAIS (Bignoniaceae) is one of the important plants used in traditional medicine, whose chemical analysis has been recommended (Oliver-Bever 1960). This plant is used in traditional medicine for the management of malaria and the blood schizontocidal action of the alcoholic extract of its leaves against *Plasmodium berghei berghei* in mice has been described (Makinde *et al.* 1987). Extracts of the stem bark of the tree also demonstrated antimalarial activity against *P. berghei berghei* in mice both in early and established infections (Makinde *et al.* 1988).

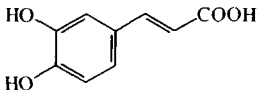
Column chromatography was effective for the isolation of three fractions of the stem bark which demonstrated antimalarial properties (Makinde *et al.* 1990). Two of which, fractions B and C were obtained from the chloroform extract while one fraction (Z) was obtained from the hexane extract of the stem bark (Makinde *et al.* 1990). Phytochemical investigation has led to the characterization of the antimalarial active principles in the leaves and in the three fractions of the stem bark of *Spathodea campanulata* using spectroscopic methods and chemical transformations. The isolation of these antimalarial compounds from the stem bark of *S. campanulata* is noteworthy in the current search for new antimalarial drugs since these compounds have never been reported to have antimalarial action.

Results

Fresh leaves and stem bark of *S. campanulata* were collected and sun-dried. Details of the method of extraction and biological screening for antimalarial property have been published elsewhere (Makinde *et al.* 1988).

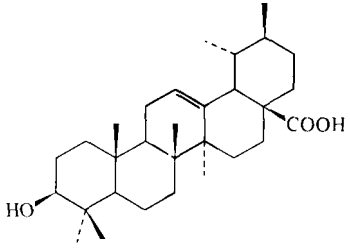
The aqueous methanol extract of the leaves (8 g) which was active in biological screening was fractionated on a silica gel column chromatography with ethyl acetate-methanol (3:1) as solvent to afford an orange crystalline solid,

recrystallized in aqueous alcohol and identified as caffeic acid.



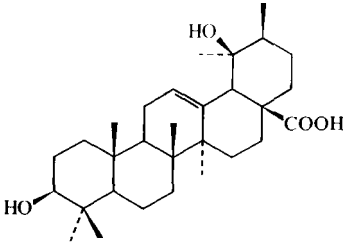
Caffeic acid

The chloroform extract of the stem bark of *S. campanulata* (15 g) has been separated in different fractions by column chromatography on silicagel. Elution with hexane-ethylacetate (2:3) yielded fraction B, which afforded after further purification on a short column of alumina and recrystallization twice in methanol a white amorphous powder identified as 3,20β-dihydroxyurs-12-ene-28-oic acid (20β-hydroxyursolic acid, **1**).



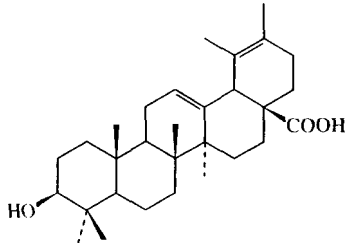
(**1**)

Fraction C was eluted by ethylacetate-methanol (9:1) from the same column than fraction B. Further purification on a short column of alumina, preparative TLC and crystallization in ethanol afforded white crystals characterized as 3β-hydroxyurs-12-19-dien-28-oic acid (tomentosolic acid, **2**).



(**2**)

The hexane extract (12 g) of *S. campanulata*, eluted on silicagel column chromatography with ethylacetate-methanol (9:1) afforded fraction Z. Purification on a short column of alumina and recrystallization in ethanol gave white crystals identified as ursolic acid (**3**).



(**3**)

The three triterpenes isolated from the stem bark of *Spathodea campanulata* exhibited antimalarial activity in different assays and Table 22.1. shows the results of the action of these three compounds against *Plasmodium berghei berghei* in Fink and Kretschmar's test (Makinde *et al.* 1990). Each triterpene demonstrated a marked dose-dependent suppressive effect and high mean survival times.

Table 22.1. Blood schizontocidal action in mice of compounds isolated from the stem bark of *S. campanulata* on *P. berghei berghei* using Fink and Kretschmar's tests

	Dose ($mgKg^{-1}$ day^{-1})	% suppression ± SE	% suppression of parasitaemia	Mean survival time (days)
Control (2.5 % Tween 80)	-	25.7 ± 2.9		7.0 ± 1.4
20ß-hydroxyursolic acid (**1**)	20	23.0 ± 3.8	10.5	8.0 ± 1.2
	40	14.9 ±3.6	42.0	12.6 ± 2.8
	80	12.2 ± 0.8	52.5	12.2 ± 1.3
Tomentosolic acid (**2**)	5	25.8 ± 0.7	-0.4	7.8 ± 1.2
	10	16.8 ± 2.8	34.6	10.0 ± 1.3
	20	8.7 ± 2.9	66.1	12.4 ± 1.7
	40	4.7 ± 1.9	81.7	19.2 ± 1.8
	80	6.1 ± 1.4	76.3	1.8 ± 1.1
Ursolic acid (**3**)	15	16.9 ± 3.3	34.2	12.8 ± 3.3
	30	8.5 ± 2.0	66.9	17.8 ± 5.1
	60	0.8 ± 0.7	96.9	25.3 ± 3.5
Chloroquine	10	0.4 ± 0.3	98.4	25.8 ± 1.2

Similar results were obtained from the blood schizontocidal action of the isolated compounds in an established infection test (Rane test). There was a fall in parasitaemia in groups of mice treated with these products while the control (blank) group showed increase in parasitaemia. The mean survival time produced by the control and triterpenes **1**, **2** and **3** are as shown in Table 22.2.

Table 22.2. Mean survival time in mice produced by triterpenes of *S. campanulata* stem bark extracts on *P. berghei berghei* in Rane test

	Dose ($mgKg^{-1}day^{-1}$)	Mean survival time (days)
Control (Tween 80)	-	7.2 ± 1.0
20ß-hydroxyursolic acid (**1**)	20	5.6 ± 0.3
	40	14.4 ± 4.1
	80	16.4 ± 4.2
Tomentosolic acid (**2**)	5	8.8 ± 2.0
	10	13.2 ± 4.1
	20	17.0 ± 4.8
	40	18.4 ± 4.2
Ursolic acid (**3**)	15	8.6 ± 0.5
	30	19.8 ± 4.5
	60	24.0 ± 4.0
Chloroquine	5	26.0 ± 2.0

Discussion

The isolation of caffeic acid as the antimalarial principle in the leaves of *S. campanulata* is noteworthy because caffeic acid is already known to have some antimalarial properties. It demonstrated antipyretic property and suppressed malaria in chicks (Helbecque *et al.* 1963). The use of the alcoholic decoction of the leaves of *S. campanulata* in the treatment of malaria in traditional medical practice has therefore some scientific basis.

Purification of fraction B, C and Z of the stem bark of *S. campanulata* yielded 3,20β-dihydroxyurs-12-en-28-oic acid, 3-hydroxyurs-12,19-dien-28-oic acid and 3-hydroxyurs-12-en-28-oic acid respectively. These compounds are structural analogues and are biogenetically related (Drake and Duvall 1936, Barton *et al.* 1962). The isolation of ursolic acid and two of its derivatives as antimalarial agents is a major step in the search for new antimalarials because ursolic acid or any other triterpenoids has never been reported as antimalarial agent. The only terpenoids reported to be active against *Plasmodium* sp. are the sesquiterpenoid artemisinin or qinghaosu and its structural analogues which have been found effective for the treatment of some drug-resistant strains of the malarial parasite (Klayman 1985).

Ursolic acid is well tolerated in the body. It is non-toxic when fed to rats, guinea pigs, chickens, rabbits at levels of 1000 - 5000 mg/kg body weight and to humans at a dose of 20 mg/kg/day (Lubitz and Fellers 1941).

It is also important to note that the isolation of the three triterpenoids of the urs-12-ene series from *S. campanulata* has never been reported.

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