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Evaluation of the biological activity of the molluscicidal fraction of *Solanum sisymbriifolium* against non target organisms

Jean-Jacques M. Bagalwa [a], Laurence Voutquenne-Nazabadioko [b,*], Charlotte Sayagh [b], Augustin S. Bashwira [a]

[a] *Laboratory of Malacology, Department of Biology, Centre de Recherche en Sciences Naturelles (CRSN) at Lwiro, Democratic Republic of Congo. C/o. Petit Séminaire de Mugéri, P.O.Box 02, Cyangugu, Rwanda*

[b] *Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 6229, Bat.18, BP 1039, 51687 Reims Cedex 2, France*

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ABSTRACT

The evaluation of the biocidal activity of the fruit of *Solanum sisymbriifolium* involving non target organisms such as aquatic insects, fish and snails lead to the isolation of the steroidal alkaloids, solamargine and β-solamarine, from the active fractions. The fractions A3 and C, with biological activity against fish, snail and aquatic insect and larvae, are able to affect the good functioning of ecosystem found on alimentary chain. The fraction B seems to be less toxic to fish and aquatic insect and larvae. The fraction B could thus be used as molluscicide in the future.

1. Introduction

Shistosomiasis is a parasitic disease that affects over 250 million people in tropical and subtropical areas of the world. The most efficient method of preventing the spread of the disease is destruction of the host snails by use of synthetic molluscicides or plant molluscicides. During our screening of Congolese plants for their possible use as molluscicidal agents, we noted that the ethanolic and aqueous extract of the fruit of *Solanum sisymbriifolium* (Solanaceae) were lethal *in vitro* against snail intermediates of *Schistosoma mansoni* found in the Eastern Democratic Republic of Congo. The saponin fraction showed a powerful molluscicidal activity at 1 mg/l and was planned for use in snail control in Democratic Republic of Congo where schistosomiasis is widespread [1–4]. This new drug from *Solanum sisymbrilifolium* must be evaluated for biocidal activity before use to avoid noxious effects on human beings, animals or plants and also to prevent contamination of the environment [5,6]. No toxicity was reported for people who drank the infusion prepared from the ground dried fruit to treat ascites, mycosis, ringworms, whooping cough, cough, mumps, malaria, madness, edemas, myalgia and also to stop lactation [7].

Solanum sisymbriifolium is a large schrub native of South America, with yellow prickles and, when ripe, globulose shiny red berries enveloped by a dense prickly accrescent calyx [8]. It is distributed throughout the greater part of the Bushi area, South Kivu province, Democratic Republic of Congo. It is known as a medicinal plant that is used by the native healers both in veterinary and human medicine [7,9]. *Solanum* species are well known to synthesize steroidal alkaloids and spirostane derivatives and some of which have shown molluscicidal activity [10]. Studies of some Brazilian *Solanum* species have shown that aerial parts of *S. sisymbriifolium* have significant molluscicidal activity [11]. Previous chemical studies on *S. sisymbriifolium* have reported the presence of

* Corresponding author. Tel.: +33 326918209; fax: +33 326913166.
E-mail address: laurence.voutquenne@univ-reims.fr
(L. Voutquenne-Nazabadioko).

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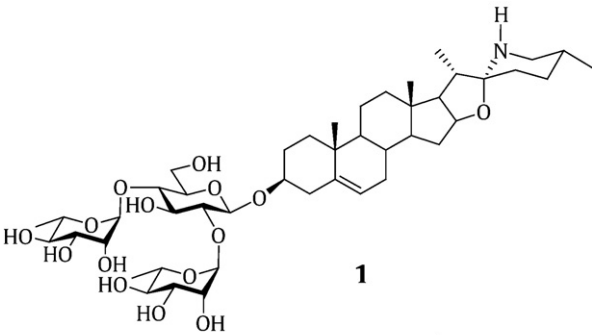
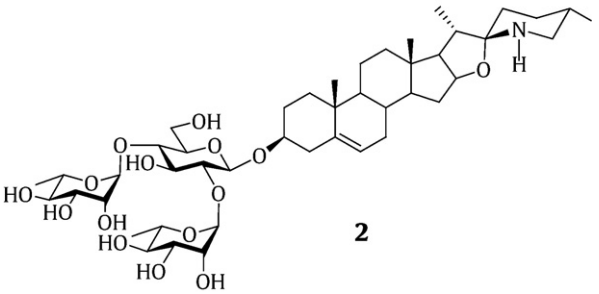
alkaloids [12], and a spirostane saponin, named nuatigenosido in roots [13–15], a steroidal alkaloid, solasodine, in leaves and berries [16,17], as well as neolignan and sterols in berries [17]. In our study, the methanolic extract of the fruit of *S. sisymbriilofium* was fractionated and the biological activity of each fraction was tested against non target organisms such as aquatic insects, fish and snails. Two known compounds, solamargine (1), a chacotriose solasodine, and β-solamarine (2), a chacotriose tomatidenol, isolated from the active fraction B are reported for the first time in *S. sisymbriifolium*. This type of investigation would constitute a means to revaluate the African traditional ethnomedical systems on the one hand and Congolese resource on the other hand.





2. Material and methods

2.1. General

NMR spectra were recorded on a Bruker Avance DRX-500 spectrometer at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. ESIMS and HRESIMS were recorded on a ESI-Q-TOF Micromass spectrometer. Column chromatography was performed with Kieselgel 60 (63–200 µm, Merck) silica gel or LiChroprep RP-18 (40–63 µm, Merck) silica gel. TLC analysis was run on 60 F_{254} precoated silica gel plates (Merck) and spots were visualized by heating after spraying with 50% H_2SO_4. Snails, aquatic insects (larvae and adults) and fish were identified by reference to the collection of the Laboratory of Malacology and Hydrobiology (Department of Biology, CRSN at Lwiro) where voucher specimens are preserved.

2.2. Plant material

The fruit of *Solanum sisymbriifolium* Lam. (Solanaceae) were collected at Lwiro (2° 14,228′ S and 28° 48,441′ E) in March 2004. Plant identification was done by comparison with authentic samples from the Herbarium of the Laboratory of Botany of the CRSN at Lwiro (Kivu Province, DR Congo) that contains 12000 plant specimens of the studied area.

2.3. Extraction and isolation of the steroidal alkaloids

The dried and powdered fruit (300 g) was extracted with 96% methanol. The MeOH extract was concentrated and precipitated with acetone. Then, the crude saponin precipitate was dialyzed to give a saponin rich extract (2.69 g). This extract (2.5 g) was fractionated by VLC on reversed phase C_{18} (MeOH–H_2O, 60:40, 70:30, 80:20 and 100:0, each 300 ml) to give fraction A (1.52 g), B (680 mg), C (277 mg) and D (58 mg), respectively. Fraction A was repurified by RP-18 VLC (MeOH–H_2O, 40:60, 60:40, 80:20 and 100:0, each 200 ml) to give fraction A1 (1.08 g), A2 (64 mg), A3 (116 mg) and A4 (212 mg). A part of fraction B (100 mg) was chromatographed on silicagel CC (4 g) eluted with a gradient of $CHCl_3$–MeOH–H_2O (70:30:1 to 70:30:2) to give 1 (20 mg). A part of fraction C (70 mg) was purified by silicagel CC using a gradient of $CHCl_3$–MeOH (9:1 to 7:3) followed by preparative TLC on silicagel with $CHCl_3$–MeOH–H_2O (70:30:5) as eluant, to give solamargine (1) (13 mg) and β-solamarine (2) (5.8 mg).

2.3.1. Solamargine, (25R)-3β-{O-α-L-rhamnopyranosyl-(1−>2)-[O-α-L-rhamnopyranosyl-(1−>4)]-β-D-glucopyranosyloxy}-22αN-spirosol-5-ene (1)

White powder, $C_{45}H_{73}NO_{15}$ ESIMS$^+$ *m/z* (rel. int.): 869 $[M+2H]^+$ (100), 868 $[M+H]^+$ (50), 413 $[C_{27}H_{43}NO_2]^+$ (2). ^{1}H NMR spectral data (500 MHz, CD_3OD): δ 0.88 (3H, s, H-18), 1.06 (1H, d, J = 7.2, H-21), 1.09 (3H, s, H-19), 0.93 (3H, d, J = 6.3, H-27), 1.20 (1H, ddd, J = 13.9, 8.3, 5.6, H-14), 1.25 (1H, m, H-12a), 1.27 (3H, d, J = 6.2, H-6″), 1.29 (3H, d, J = 6.2, H-6‴), 1.37 (1H, td, J = 12.2, 6.0, H-15a), 1.48 (1H, td, J = 13.0, 3.8, H-24a), 1.85 (1H, dm, J = 13.2, H-12b), 1.86 (1H, dd, J = 8.1, 7.4, H-17), 2.05 (1H, m, H-15b), 2.33 (1H, td, J = 12.1, 1.6, H-4a), 2.33 (1H, ddm, J = 13.2, 2.5, H-4b), 2.67 (1H, t, J = 11.6, H-26a), 2.79 (1H, brd, J = 10.2, H-26b), 3.36 (1H, m, H-5′), 3.42 (1H, t, J = 9.4, H-4″), 3.43 (1H, t, J = 8.4, H-2′), 3.44 (1H, t, J = 9.4, H-4‴), 3.55 (1H, t, J = 9.2, H-4′), 3.62 (1H, t, J = 9, H-3′), 3.65 (1H, dd, J = 9.3, 3.6, H-3‴), 3.68 (1H, dd, J = 12.1, 3.5, H-6a′), 3.69 (1H, dd, J = 9.2, 3.6, H-3″), 3.83 (1H, dd, J = 12.1, 1.7, H-6b′), 3.87 (1H, dd, J = 3.6, 1.3, H-2‴), 3.96 (1H, dd, J = 3.6, 1.3, H-2″), 3.97 (1H, m, H-5‴), 4.15 (1H, dq, J = 9.6, 6.2, H-5″), 4.44 (1H, q, J = 7.3, H-16), 4.53 (1H, d, J = 7.8, H-1′), 4.86 (1H, brs, H-1‴), 5.23 (1H, brs, H-1″), 5.41 (1H, brd, J = 4.9, H-6). ^{13}CNMR spectral data (125 MHz, CD_3OD): δ 15.2 (C-21), 16.8 (C-18), 17.8 (C-6″), 18.0 (C-6‴), 19.4 (C-27), 19.8 (C-19), 21.9 (C-11), 30.4 (C-24), 30.7 (C-2), 31.0 (C-25), 32.8 (C-8), 33.0 (C-15), 33.2 (C-7), 34.4 (C-23), 38.0 (C-10), 38.6 (C-1), 39.5 (C-4), 40.8 (C-12), 41.8 (C-13), 42.8 (C-20), 47.8 (C-26), 51.7 (C-9), 57.7 (C-14), 61.9 (C-6′), 63.7 (C-17), 69.8 (C-5″), 70.7 (C-5‴), 72.2 (2C, C-2″,C-3‴), 72.4 (C-3″), 72.7 (C-2‴), 73.7 (C-4‴), 73.9 (C-4″), 76.5 (C-5′), 78.0 (C-3′), 79.2 (C-2′), 79.3 (C-3), 80.0 (C-4′), 81.7 (C-16), 99.7 (C-22), 100.4 (C-1′), 103.0 (C-1‴), 102.3 (C-1″), 122.5 (C-6), 141.9 (C-5). HRESIMS (positive ion mode) *m/z* 868.5054 $[M+H]^+$ (calcd. for $C_{45}H_{74}NO_{15}$, 868.5058).