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Laboratory and field trials at Ifakara (Kilombero District, Tanzania) on the plant molluscicide *Swartzia madagascariensis*

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Summary

The molluscicidal activity of crushed seed pods of *Swartzia madagascariensis* was assessed by laboratory and field trials. Mature dry seed pods were ground and extracted in tap water for 24 h. Water extracts exerted a significant molluscicidal activity against *Bulinus globosus* up to dilutions of 100 mg of ground pods per litre. The chromatographically isolated saponin (*1*) responsible for the molluscicidal activity showed a toxicity of LC 100 at 3 mg/l after exposure of *B. globosus* and *Biomphalaria glabrata* for 24 h. Saponin (*1*) could be identified by FAB-MS and ^{13}C-NMR-spectroscopy as oleanolic acid-3-O-β-D-glucuronopyranosyl (1→3)-α-L-rhamnopyranoside.

Two field trials with *S. madagascariensis* pod extracts in ponds (60 and 160 m^3) harbouring dense populations of *B. globosus* compared well with the laboratory findings and showed the efficiency of the molluscicide in a natural habitat. A single application of the plant molluscicide significantly reduced the populations of *B. globusus.* The toxicity of *S. madagascariensis* pod extracts to non-target organisms remains an obstacle for its use in certain situations where schistosomiasis control is envisaged and where *S. madagascariensis* is found. However, *S. madagascariensis* is a valid candidate molluscicide which may be applied in selected epidemiological settings as part of integrated schistosomiasis control measures.

Key words: plant molluscicide; *Swartzia madagascariensis;* saponin; *Bulinus; Biomphalaria;* schistosomiasis control.

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Introduction

Plant molluscicides are gaining increased attention as they seem to be less expensive than synthetic molluscicides and, thus, appropriate for snail control as part of integrated control measures against schistosomiasis (reviewed by Kloos and McCullough, 1982; Marston and Hostettmann, 1985). More than 1000 plant species have been tested for molluscicidal activity (Kloos and McCullough, 1982). However, studies on long-term toxicity against non-target organisms (including man) including observations on the mutagenicity/carcinogenicity of these plant molluscicides are rare (McCullough and Mott, 1983).

Endod, *Phytolacca dodecandra,* is one of the best studied molluscicides of plant origin. The fruits of endod contain triterpenoid saponins of high molluscicidal properties (reviewed by Marston and Hostettmann, 1985). Promising field trials to control schistosomiasis were undertaken with endod in Ethiopia (Goll et al., 1983). Further field trials were performed with *Ambrosia maritima* in Egypt (El Sawy et al., 1984) and with *Anacardium occidentalis* in Mozambique (Webbe and Lambert, 1983).

The different plants which exhibit promising molluscicidal properties show distinct geographical distribution patterns. Some of them are not necessarily abundant in the different areas where schistosomiasis is endemic. This emphasizes the need to search for other plant molluscicides which will meet the recently defined prerequisites of WHO (1983) and Marston and Hostettmann (1985).

Urinary schistosomiasis is endemic in the Ifakara division, Kilombero river plain, southeastern Tanzania (Zumstein, 1983). It causes significant morbidity (Tanner et al., 1983; Degrémont et al., 1985). *Bulinus globosus* was identified as the only intermediate host of importance (Zumstein, 1983). Studies on the population dynamics of *Bulinus globosus* indicated that repeated local molluscicide might be appropriate (Marti et al., 1985). Such interventions should be cost-effective, integrated with the local health services and community-based (Tanner, 1984; Mott, 1984).

Among the different plants in Ifakara division with molluscicidal activities (Haerdi, 1964), *Swartzia madagascariensis* was selected for our investigations as it met best the selection criteria outlined by WHO (1983).

The aims of the present study were to investigate the molluscicidal activity of the seed pods of *S. madagascariensis* against *B. globosus* and *Bi. glabrata* and to test the efficiency of water extracts of the fruits within natural habitats harbouring *B. globosus* populations.

Materials and Methods

Study area

The present study was undertaken in Kikwawila village approximately 16 km north of Ifakara, the capital of the Kilombero District (Morogoro Region, southeast Tanzania). Ifakara is situated in the Kilombero river plain at an altitude of 250 m. This study area has already been described in detail

(Jätzold and Baum, 1964; Zumstein, 1983; Marti et al., 1985); it is endemic for urinary schistosomiasis, other helminth infections and holoendemic for malaria. Other health problems are manifold in the rural communities around Ifakara (Tanner et al., 1982).

Snail surveys

The habitats chosen for the field trials of *S. madagascariensis* were searched for *Bulinus globosus* by two experienced field workers for 20 min using standardized sieves (Marti et al., 1985). The relative densities of *B. globosus* were calculated as snails/man/10 min according to Olivier and Schneidermann (1956).

The species determination of *B. globosus* was performed on morphological grounds and by starch gel electrophoresis (Jelnes, 1980; Marti, 1984).

Snails

Bulinus globosus used for laboratory experiments were collected from rivers and ponds at Kikwawila village. Colonies were established at the Swiss Tropical Institute in Ifakara and Basel.

Biomphalaria glabrata came from a laboratory colony maintained for four years at the School of Pharmacy (Lausanne); the snails originated from a colony established by F. Hoffmann-La Roche Ltd., Basel.

Molluscicides

Swartzia madagascariensis: S. madagascariensis is found in the East-African *Brachystegia* woodland (Miombo), (Watt and Breyer, 1962); it is abundant in the Kilombero District. The trees flower after the short rainy season (December to February). The seed pods grow up to 30 cm in length (February to April) and dry out between July and August when they fall down. The tree is well-known to the local population as a medical plant and is used for fishing as well (Haerdi, 1964). It is known as "munyenye" (Kimbunga), "mtagalala" (Kipogoro). Young pods (green) and dried ones (dark brown) were collected by knocking them down with a long bamboo pole. An average yield was approx. 30 kg of dry pods per tree. They were subsequently dried in the sun and ground by hand in a local pounder used by the population (Kiswahili: "kinu") to produce rice and maize flour. The ground pods were passed through a sieve (mesh size 8 mm) and the sieved material was stored at ambient temperature in a dry environment.

The *extraction* of the ground pods followed the procedure outlined in Fig. 1. The extracts were tested in the laboratory for molluscicidal and haemolytic activity (as described below).

Laboratory assays. Freshly prepared concentrated aqueous extract (40 g ground pods per l) was diluted with tap water to 800 mg–50 mg ground fruit/l. Samples of 50 ml of each dilution were placed in crystallization dishes (⌀ 6 cm). *B. globosus* of 6–10 mm size were placed in the dishes (usually 5 per dish) with the different dilutions of the *S. madagascariensis* pod extracts. After exposure (standard assay 24 h) the snails were carefully rinsed with tap water and transferred to 50 ml tap water without molluscicide for further 24 h. The snails which sank to the bottom of the dish and which were completely retracted after the 24 h exposure to tap water were considered dead. The molluscicidal activity against *Bi. glabrata* was similarly tested. The snails were considered dead when there was no observable heart beat on microscopic investigation.

Test for haemolytic activity. It was intended to establish a simple method to estimate the concentrations of *S. madagascariensis* pod extracts in water samples. Four ml of a water sample were collected and 200 µl of 18% NaCl solution was added. Water samples from field trials which showed turbidity were filtered before erythrocytes were added (1st step: Coffee filter 1×10, 67 g/m^2, Melitta Bentz & Co., Egerkingen, CH; 2nd step: Millipore 0,45 µm, type HAWP, Millipore Corp., Molsheim, F). Human erythrocytes were collected with 5 ml EDTA-Vacutainers (No. 606452 Becton Dickinson, Basel, CH), centrifuged and washed twice with 0,9% NaCl (10 min, 300×*g*). 50 µl of washed erythrocytes were added to the water samples to be tested. The samples were mixed and left for 6 h. They were subsequently centrifuged (300×*g*) and assessed for haemolysis by naked eye:

++ = complete haemolysis, no erythrocytes at the bottom,
+ = partial haemolysis, erythrocytes in the pellet,
0 = no haemolysis.

Characterization of active principle of S. madagascariensis fruit extracts

General remarks. Thin layer chromatography (TLC) was undertaken on silica gel-precoated Al-sheets (Merck, Darmstadt, FRG). Detection with Godin reagent (Godin, 1954) showed a violet colour for oleanolic acid derivatives. For normal-phase column chromatography, silica gel 60, 40–63 μm (Merck) was used and reversed-phase chromatography was performed on a Lobar Lichroprep RP-8 column (40–63 μm i.d. 2.5×27 cm, Merck) equipped with a Duramat-80 pump (Chemie und Filter, Regensdorf, CH).

The ^{13}C-NMR spectra were recorded on a Bruker AM-400 apparatus at 100.6 MHz in (D_5) pyridine as solvent.

Fast-atom-bombardment (FAB) MS was obtained on a ZAB-1S spectrometer. The target was bombarded with 5-KeV Xe-atoms. Samples were suspended in thioglycerol.

Isolation. Dried ground pods (40 g) of *S. madagascariensis* were extracted with 200 ml of water for 24 h to give 20 g of aqueous extract, of which 2×5 g were partitioned between water and n-butanol (500:900 ml).

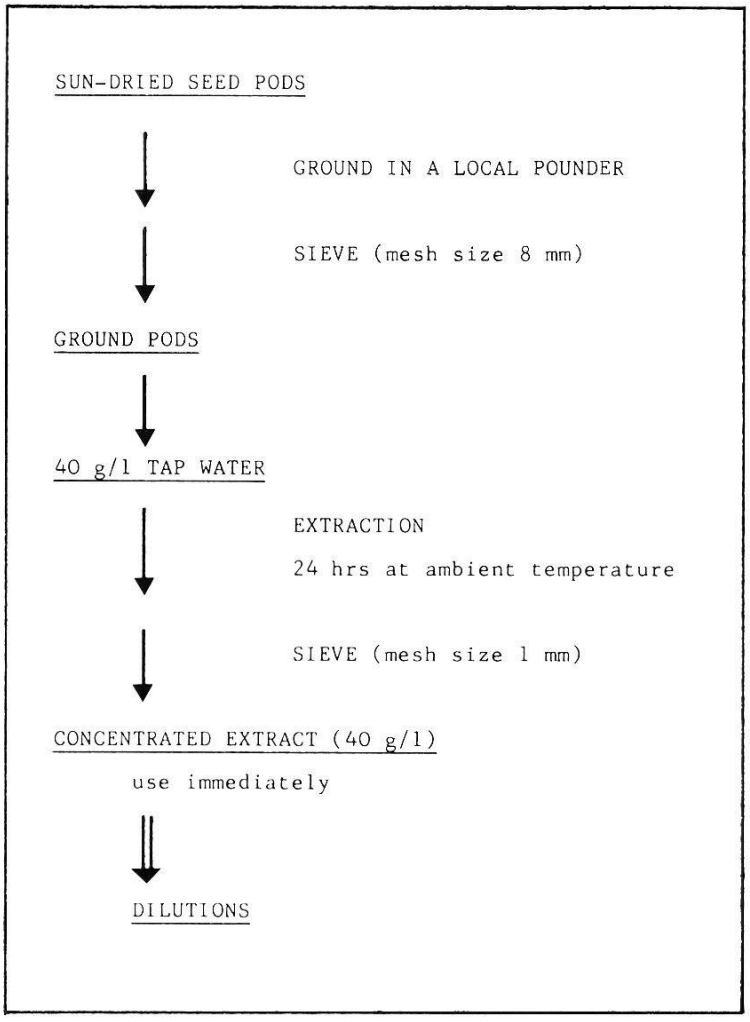

Fig. 1. Extraction of *S. madagascariensis* fruits.

The n-butanol extract (3 g) was separated on a silica gel column (⌀ = 3 cm; l = 115 cm) with $CHCl_3$/MeOH/H_2O (58:35:7) followed by an increase in polarity to $CHCl_3$/MeOH/H_2O (65:40:10). Separation was monitored by TLC and 6 fractions were obtained. The bioactive fraction II (230 mg) was chromatographed in two batches on a Lobar RP-8 column with MeOH/H_2O (75:25) and afforded 70 mg of pure saponin (*1*).

Acidic hydrolysis. Saponin (*1*) (2 mg) was dissolved in MeOH (3 ml) and refluxed in 10 ml of 4N HCl for 4 h. The aglycone was extracted with Et_2O and identified by TLC with diisopropyl ether/acetone (75:30) as oleanolic acid by comparison with an authentic sample. The aqueous layer was then adjusted to pH 6 with $NaHCO_3$. After evaporation to dryness, the sugars were extracted from the residue with pyridine and analysed by TLC with AcOEt/H_2O/MeOH/AcOH (65:15:15:20); detection with p-anisidine phthalate.

Water samples. Collected samples of water were filtered through a coffee filter 1×10 (Melitta). The filtrate (100 ml) was extracted in a separating funnel (500 ml) with 200 ml of n-butanol. After setting (6 h) and decantation, the organic layer was evaporated under reduced pressure (Rotavapor RE 120; Büchi Ltd., Flawil, CH). The residue was dissolved in MeOH and chromatographed on TLC with $CHCL_3$/MeOH/H_2O (65:35:5). In order to estimate semiquantitatively saponin concentrations in water samples, reference dilutions of crude extracts were treated as described above and were compared by TLC with water samples collected in the field. The following classification was applied: 0 = not detected, + ≤ 50 mg ground fruit/l, ++ ≥ 100 mg ground fruit/l.

Structure elucidation. Saponin *1*: (oleanolic acid-3-O-β-D-glucuronopyranosyl-(1→3)-α-L-rhamnopyranoside, $C_{42}H_{66}O_{13}$) white powder. Acidic hydrolysis afforded oleanolic acid D-glucuronic acid and L-rhamnose. FAB-MS: (negative ions) 777 ($[M-H]^-$); 631 ($[M-H)-146]^-$); 455 ($[(M-H)-322]^-$). ^{13}C-NMR (100 MHz(D_5) pyridine): Chemical shifts of the aglycone correspond to those previously described for oleanolic acid (Tori et al., 1974). Sugar signals: 173.0 (C(6)); 107.0 (C(1)); 102.9 (C(1′)); 82.2 (C(3)); 77.3 (C(5)); 76.0 (C(2)); 74.2 (C(4′)); 72.8* (C(4′)); 72.6* (C(3′)); 71.9 (C(2′)); 69.9 (C(5′)); 18.8 (C(6′)); C(X): β-D-glucuronopyranosyl; C(X′): α-L-rhamnopyranosyl (* assignments may be interchanged).

Field trial I. A pond known to harbour large populations of *B. globosus* was chosen. The snail densities in this pond were followed monthly since April 1981 (Table 5, Marti et al., 1985). The habitat is connected to the neighbouring river only during the rainy season; it is not frequented by the human population as revealed by studies on human water contact pattern (Lwihula, 1985).

The water volume was estimated at 60 m^3, according to the methods described by Olivier and Uemura (1973) before molluscicide which took place in October 1984 (end of dry season). In order to achieve an initial concentration of 300 mg/l of the *S. madagascariensis* pod extract, 18 kg of ground fruits were extracted with water from the pond to be treated in a 200 l drum according to Fig. 1. The filtered extract was subsequently dispersed in the pond by thrashing the water with sticks.

Field trial II. A second field trial was performed in another pond within the area of Kikwawila village in October 1984. The *B. globosus* densities were followed in this pond since April 1981 (Marti et al., 1985). Again, there was no significant human water contact observed at this site (Lwihula, 1985). The pond was connected to the adjacent river only during rainy season (Marti et al., 1985). The water volume was calculated to 160 m^3 two days before application. In order to achieve a concentration of 500 mg/l, 80 kg of ground *S. madagascariensis* pods were extracted for 24 h with water from the pond and the filtered extract was applied as described for field trial I.

Follow-up of field trials. Before (as controls) and immediately after dispersing the *S. madagascariensis* extract in the ponds, five cages (14×8×5 cm, mesh size 1 mm), each containing 10 *B. globosus* (size 6–10 mm, collected outside the study ponds), were placed at different sites in the pond (4 cages at the surface with 1 cm emerging above the surface, 1 cage 50 cm below the surface) and left for 24 h. The viability of the snails was assessed as described for the laboratory tests. The procedure with 5 new cages each was repeated 6, 12, 24, 48 and 72 h after the application of the *S. madagascariensis* extract.

In addition, water samples were collected at the same sites where encaged snails were exposed immediately after the application of the *S. madagascariensis* extracts and again after 6, 12, 24 and 48 h. The samples were brought to the laboratory and tested, as described above, for the presence of saponins by TLC and haemolysis.

B. globosus densities were followed weekly for the first ten weeks following molluscicidal and thereafter at monthly intervals.

Results

The results of laboratory experiments revealed that an extraction time of 24 h in tap water at ambient temperature is required to achieve 100% snail mortality using dilutions of 100 mg/l (Fig. 1, Table 1). An extraction time between 6 and 24 h gave the best results. The extraction was not improved by heating to 50° C. Boiling for 30 min or 1 h decreased the molluscicidal activity of *S. madagascariensis* fruit extracts (data not shown). There was a significant difference between young (green) pods and mature (dark-brown) seed pods when extracted according to Fig. 1 (Table 3). Consequently, mature, sun-dried seed pods were used for all the following experiments and field trials. Ground seed pods could be stored for 1 year without loss of molluscicidal activity (data not shown).

Table 2 shows that a 24 h water extract of 100 mg/l exerts a high molluscicidal activity (snail mortality >90%) to *Bulinus globosus* exposed for 6, 12 or 24 h. Short exposure of *B. globosus* leads to high mortality only at high concentrations of *S. madagascariensis* extracts (≥400 mg/l, Table 2).

Table 1. Mortality among *B. globosus* exposed to *S. madagascariensis* pod extracts at different concentrations and extraction times. Results of 5 independent experiments with 5 snails per experimental group. Arithmetic mean % (±SD)

Extraction time (h)	Concentrations (mg ground pods/l)				
	400	200	100	50	0
0.25	100.0 (0)	32.0 (46.0)	12.0 (17.9)	4.0 (8.9)	0 (0)
0.5	100.0 (0)	64.0 (32.7)	20.0 (34.6)	4.0 (8.9)	0 (0)
1.0	100.0 (0)	84.0 (16.7)	24.0 (43.4)	4.0 (8.9)	0 (0)
3.0	100.0 (0)	100.0 (0)	24.0 (43.4)	4.0 (8.9)	0 (0)
6.0	100.0 (0)	100.0 (0)	96.0 (8.9)	4.0 (8.9)	0 (0)
12.0	100.0 (0)	100.0 (0)	96.0 (8.9)	4.0 (8.9)	0 (0)
24.0	100.0 (0)	100.0 (0)	100.0 (0)	28.0 (4.5)	0 (0)
48.0	100.0 (0)	80.0 (28.3)	36.0 (38.5)	20.0 (34.6)	0 (0)
72.0	76.0 (26.1)	24.0 (26.1)	8.0 (17.9)	0.0 (0)	0 (0)
96.0	56.0 (45.6)	24.0 (26.1)	8.0 (10.9)	8.0 (10.9)	0 (0)
192.0	36.0 (43.4)	24.0 (16.7)	4.0 (8.9)	8.0 (10.9)	0 (0)

Further experiments indicated that an initial concentration maximum of 200 g/l of ground pods could be used in order to obtain 100% snail mortality at 100 mg/l after 24 h extraction. Higher initial concentrations of ground pods for extraction did not improve the molluscicidal activity of the 24 h extracts.

This first series of experiments led to the extraction procedure as outlined in Fig. 1.

The molluscicidal activity of *S. madagascariensis* fruit extract was further analyzed as follows.

Mature pods of *S. madagascariensis* (40 g) were extracted with water (200 ml). The aqueous extract was separated into 6 fractions by gas chromatography (Still et al., 1978) on silica gel. Fraction II was shown by bioassay to possess the most important molluscicidal activity. From this fraction the pure saponin (*1*) (70 mg) was obtained by low-pressure reversed-phase chromatography.

Table 2. Mortality among *B. globosus* after various exposure times to different concentrations of *S. madagascariensis* pod extracts (24 h extraction). Results of 5 independent experiments with 5 snails per experimental group. Arithmetic mean % (±SD)

Exposure (h)	Concentrations (mg/l)				
	400	200	100	50	0
0.1	0.0 (0)	0.0 (0)	4.0 (8.9)	4.0 (8.9)	0 (0)
0.25	0.0 (0)	4.0 (8.9)	0.0 (0)	4.0 (8.9)	0 (0)
0.5	36.0 (43.4)	4.0 (8.9)	0.0 (0)	4.0 (8.9)	0 (0)
1.0	92.0 (17.9)	68.0 (41.5)	4.0 (8.9)	4.0 (8.9)	0 (0)
3.0	100.0 (0)	96.0 (8.9)	56.0 (38.5)	0.0 (0)	0 (0)
6.0	100.0 (0)	100.0 (0)	92.0 (10.9)	4.0 (8.9)	0 (0)
12.0	100.0 (0)	100.0 (0)	96.0 (8.9)	4.0 (8.9)	0 (0)
24.0	100.0 (0)	100.0 (0)	100.0 (0)	4.0 (8.9)	0 (0)

Table 3. Mean mortality of *B. globosus* exposed to different concentrations of extracts from *S. madagascariensis* young or mature seed pods

	Concentrations (mg/l)					
	800	400	200	100	50	0 (control)
Young (green) seed pods	100±0 (15/15)*	100±0 (15/15)	100±0 (15/15)	40±20 (6/15)	0 (0/15)	0 (0/15)
Mature (dark-brown) seed pods	100±0 (15/15)	100±0 (15/15)	100±0 (15/15)	100±0 (15/15)	0 (0/15)	0 (0/15)

* mean mortality % ±SD (dead snails/tested snails), three independent experiments with 5 snails each

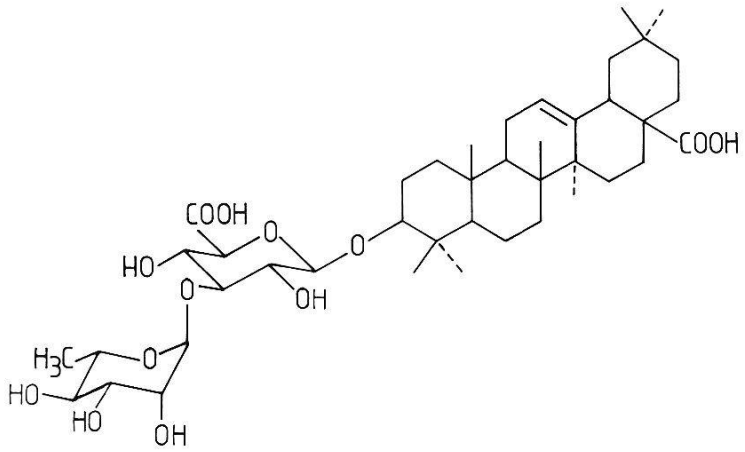

Fig. 2. Structural formula of oleanolic acid-3-O-β-glucuronopyranosyl-(1→3)-α-L-rhamnopyranoside = saponin (*1*) isolated from seed pods of *S. madagascariensis* and exerting molluscicidal activity against *B. globosus* and *Bi. glabrata.*

Acid hydrolysis of saponin (*1*) afforded oleanolic acid (Tori et al., 1974) as aglycone and glucuronic acid and rhamnose as sugars. Hydrolysis products were identified by comparison with authentic samples (TLC) (cf. Materials and Methods).

The molecular weight (778) of the saponin was established by FAB-MS (matrix: thioglycerol, negative ion mode). A quasi-molecular ion was observed at m/z 777 ($[M-H]^-$). Signals at m/z 631, 455 correspond to the loss of one rhamnose and one glucuronic acid unit, respectively.

The interglycosidic linkages as well as the position of substitution of the aglycone by the sugar chain were established by ^{13}C-NMR (Fig. 2). Thus, substitution of oleanolic acid at C(3) was indicated by the appearance of the signal at 89.2 ppm (Rui-Lin Nie et al., 1984). The free carboxylic group at C(28) was in the spectrum at 180.3 ppm (Rui-Lin Nie et al., 1984). Two anomeric carbon atoms appeared at 107.0 and 102.8 ppm for the glucuronic acid and the rhamnose, respectively. Permethylation, followed by GC-MS, established that the interglycosidic linkage was rhamnopyranosyl (1→3) glucuronopyranosyl (Borel et al., in preparation). Saponin (*1*) presented a high molluscicidal activity killing *B. globosus* and *Bi. glabatra* at a concentration of 3 mg/l within 24 h.

As saponins are responsible for the molluscicidal activity of *S. madagascariensis* pod extracts, an attempt was made to establish a simple test which could be used to measure semiquantitatively *S. madagascariensis* saponins when applied in the field. Haemolysis was chosen as indicator (Hostettmann et al., in prep.) for the field trials.

Two field trials were performed in order to test the molluscicidal activity of *S. madagascariensis* pod extracts in a natural habitat known to harbour *B. globosus.* The densities of *B. globosus* were recorded in these ponds since April 1981 prior to the trials which were undertaken in October 1984 (Tables 5, 7). These data show a distinct seasonal pattern of *B. globosus* densities with a peak

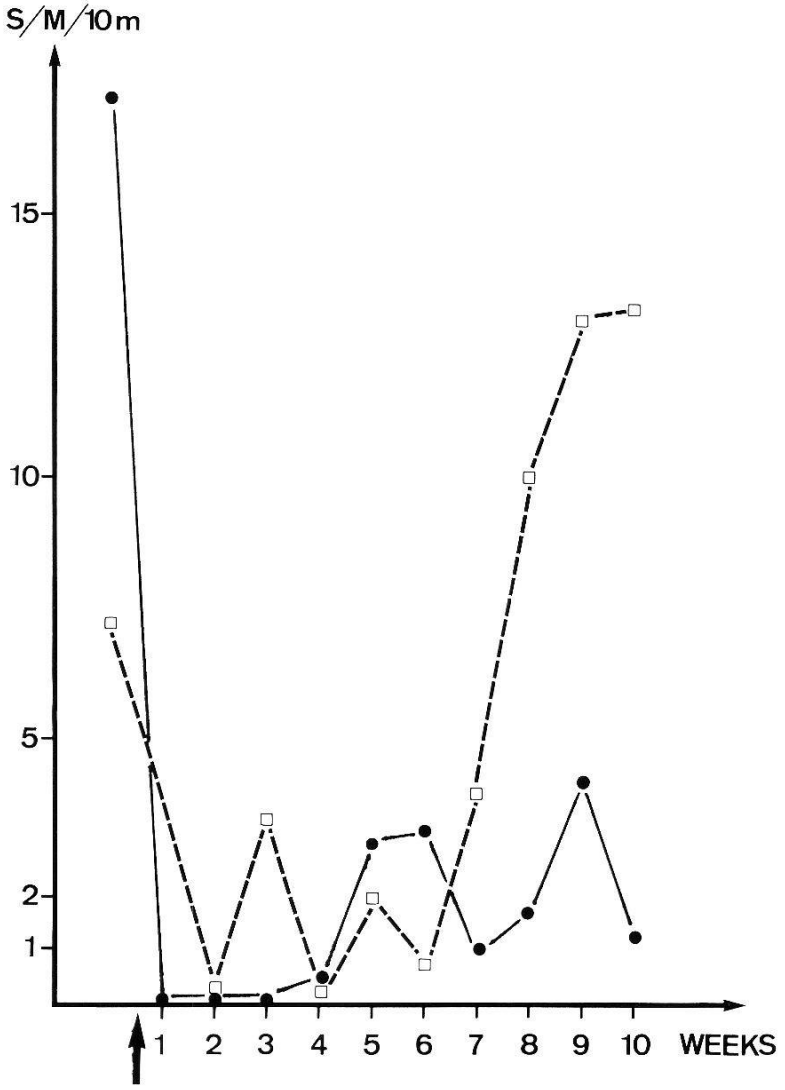

Fig. 3. Short-term follow-up of field trials: *Bulinus* spp. densities (snails/man and 10 min; s/m/10 m) followed before and for ten weeks after the application of *S. madagascariensis* fruit extracts (↑). ●——● = Field trial I; □- - - -□ = Field trial II.

during the short rainy season, October–February. Early October was chosen for the application of the molluscicide in these field trials as *B. globosus* densities started to increase during this month; the mollusciciding effect could thus be demonstrated by the absence of the distinct *B. globosus* density peak during the short rainy season. Furthermore, the water level in the ponds was very low at the end of the dry season. The habitats were not then connected with the adjacent river, thus preventing the dispersion of the molluscidide into the river. Although laboratory experiments indicated high molluscicidal activity of *S. madagascariensis* at 100 mg/l, higher initial concentrations were applied in the field trials in order to compensate for possible inaccuracy in water volume measurements and for the deposits of silt in the ponds.

The results of the first field trials showed that an initial molluscicide concentration of not less than 100 mg/l was reached (Table 4). Complete haemolysis

was observed during the first 12 h after the application and all exposed, encaged snails died within the same period. The analysis of water samples by TLC paralleled these findings. However, haemolysis was no longer observed and the mortality rates of subsequently exposed snails decreased from 24 h after the application onwards.

The densities of *B. globosus* dropped from 17.2 snails/man/10 min just before molluscicide to 0 one week after the single application of *S. madagascariensis* extracts (Fig. 3). *B. globosus* were observed only at low densities and never reached the initial density during the short-term (Fig. 3) and long-term (Table 5) follow-up of five months. When compared to the data obtained from the same pond for three consecutive years before treatment, the density of *B. globosus* did not show the distinct peak between October–February and remained at very low levels (Table 5).

The results of the second field trial in a larger pond (160 m^3) compared initially well to the first field trial. Haemolysis was observed up to 6 h after the single application of the *S. madagascariensis* extract which was paralleled by the mortality of the exposed, caged *B. globosus* and by TLC (Table 6).

The density of *B. globosus* dropped from 7.2 (snails/man/10 min) to 0.2 and it reached the initial density only 8 weeks after the application (Fig. 3). When compared to the data obtained from 1981–1983, the *B. globosus* density remained markedly lower after molluscicide and the distinct peak October–January was no longer observed. However, the *Bulinus* population increased from January onwards reaching densities exceeding those from previous years (Table 7), clearly indicating the need for continuing applications of molluscicide.

Discussion

The present study confirms the molluscicidal properties of *S. madagascariensis* pods as previously indicated by Mozley (1939, 1952). *S. madagascariensis* pods are a candidate for a plant molluscicide. The plant meets most of the criteria proposed for plant molluscicides (Kloos and McCullough, 1982; WHO, 1983; Marston and Hostettmann, 1985). Simple water extracts of sun dried mature seed pods show significant molluscicidal activity against *B. globosus* (Tables 1, 2). The isolated saponin (*1*) responsible for the molluscicidal activity shows a toxicity of LC_{100} = 3 mg/l after exposure of *B. globosus* and *Bi. glabrata* for 24 h. This is within the range of the molluscicidal activity of endod (*Phytolacca dodecandra,* reviewed by Marston and Hostettmann, 1985) which proved to be successful in a pilot control project in Ethiopia (Goll et al., 1983).

By a combination of different chromatography methods, the main molluscicidal compound, saponin (*1*), was isolated from the pods of *S. madagascariensis* and identified by FAB-MS and ^{13}C-NMR spectroscopy as oleanolic acid-3-O-β-D-glucuronopyranosyl-(1→3)-α-L-rhamnopyranoside.

The activity of this monodesmosidic compound is in concordance with the general structure-activity relationship of other molluscicidal saponins (Hostettmann et al., 1982). More details on the structure elucidation of saponin (*1*) as well as the isolation of further saponins from *S. madagascariensis* will be described elsewhere (Borel et al., in preparation).

The present study shows that the pods of the perennial *S. madagascariensis* are easily obtained at high yields and can be stored after drying in the sun. They can also be efficiently extracted with water by a procedure that does not demand any sophisticated apparatus or highly trained personnel (Fig. 1). Furthermore, the plant is abundant in the East-African *Brachystegia* woodland (Watt and Breyer, 1962; Haerdi, 1964) and it is well known to the local population as a medicinal plant and fish poison (Haerdi, 1964). The application of extracts is easy and the initial concentration of the molluscicidal activity can be monitored by a simple, semiquantitative haemolysis test which parallels the concentration of saponins (Tables 4, 6). The two field trials together show the efficiency of the pod extracts of *S. madagascariensis* against *B. globosus* (Tables 4–7). However, the results (especially field trial II, Table 7, Fig. 3) indicate that the extracts are most probably not active against *B. globosus* egg masses, as also observed for endod (Kloos and McCullough, 1982). This was confirmed during recent laboratory experiments (Suter, in prep.) and implies the need of at least one sub-

Table 4. Field trial I: application of *S.madagascariensis* fruit extracts in a 60 m^3 pond, showing haemolysis, mortality of *Bulinus globosus* and semiquantitative determinations of saponins by thin-layer chromatography (TLC)

	Before	Hours after application of *S.m.* extracts					
		0	6	12	24	48	72
Haemolysis[a]							
A	0	++	++	++	0	0	ND
B	0	++	+	+	0	0	ND
Mortality of snails[b]							
dead/total	0/10	50/50	50/50	50/50	43/50	14/50	6/50
TLC	0	++	++	++	+	0	0

[a] Haemolytic capacity of water samples collected from 5 different sites of the pond and at different times after the application of *S. madagascariensis* extracts; ++ = total haemolysis, + = partial haemolysis, 0 = no haemolysis, A = undiluted water samples, B = 1:2 diluted, each result represents the mean of the 5 sites.

[b] Mortality of encaged *Bulinus globosus*, dead/total No. of exposed

TLC = Thin-Layer chromatography for saponins; 0 = not detected, + = ≤50 mg ground fruits/l, ++ = ≥100 mg ground fruits/l

ND = Not done

Based on measurements of the water volume, *S. madagascariensis* fruit extracts were applied to reach an initial concentration of 300 mg ground fruits per 1 pond water.

Table 5. Six-month follow-up of field trial I: *Bulinus* spp. densities (snails/man/10 min) before (April 1981–October 1984) and after molluscicide (November 1984–March 1985, shaded area) with *S. madagascariensis* fruit extracts

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1981				2.8	34.5	7.5	7.0	9.8	7.7	59.3	73.0	117.3
1982	189.5	80.8	32.3	20.3	7.5	2.5	7.0	7.8	12.7	7.3	54.3	128.0
1983	144.3	106.5	45.3	ND	10.7	8.0	18.5	21.2	18.0	38.2	177.3	103.0
1984	40.2	14.7	3.7	1.3	5.5	1.7	11.0	7.3	4.2	17.2	3.3	8.2
1985	7.7	3.7	3.3	1.2								

Data April 1981–March 1983 from Marti et al. (1985)

Table 6. Field trial II: application of *S. madagascariensis* fruit extracts in a 160 m^3 pond, showing haemolysis, mortality of *Bulinus globosus* and semiquantitative determination of saponins by thin-layer chromatography (TLC)

	Before	Hours after application of *S.m.* extracts				
		0	6	12	24	48
Haemolysis[a]						
A	0	++	ND	+	0	ND
B	0	++	ND	+/0	0	ND
Mortality of snails[b]	0/10	50/50	ND	41/50	20/50	8/50
TLC	0	++	ND	++	+	0

[a] Haemolytic capacity of water samples collected from 5 different sites of the pond and at different times after the application of *S. madagascariensis* extracts; ++ = total haemolysis, + = partial haemolysis, 0 = no haemolysis, A = undiluted water samples, B = 1:2 diluted, each result represents the mean of the 5 sites.

[b] Mortality of encaged *Bulinus globosus*, dead/total No. of exposed

TLC = Thin-layer chromatography for saponins; 0 = not detected, + = ≤50 mg ground fruits/l, ++ = ≥100 mg ground fruits/l

ND = Not done

Based on measurements of the water volume *S. madagascariensis* fruit extracts were applied to reach an initial concentration of 300 mg ground fruits per 1 pond water.

sequent application of *S. madagascariensis* pod extract in order to achieve improved persistence of low snail densities.

The comparison of the results of our laboratory experiments with those of the two field trials indicates that the molluscicidal potency of *S. madagascariensis* pod extracts remains unchanged when applied in a natural habitat, i.e. when exposed to physiochemical influences such as pH, sunlight, temperature, organic matter. Under both conditions a half-life of 12–24 h is indicated (Tables 4, 6). Obviously a short half-life reduces the risk of toxicity to humans. The rapid

Table 7. Six month follow-up of field trial II: *Bulinus* spp. densities (snails/man/10 min) before (April 1981–October 1984) and after molluscicide (shaded area, November 1984–March 1985) with *S. madagascariensis* fruit extracts

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1981				0.5	2.0	0.8	1.5	2.7	0.7	3.0	4.0	1.0
1982	2.2	4.3	1.8	0.5	1.0	8.3	7.5	8.3	18.4	24.7	47.7	46.7
1983	4.3	2.7	2.3	ND	5.7	2.8	6.0	4.7	15.5	44.2	57.7	20.0
1984	9.7	7.7	1.7	2.7	4.7	4.0	2.0	5.0	7.8	7.2	2.0	13.0
1985	18.0	25.0	12.2	12.7								

Data April 1981–March 1983 from Marti et al. (1985)

biodegradability of *S. madagascariensis* water extract is of importance as preparation and application of this plant molluscicide is appropriate for community-based actions.

However, consideration must also be given to the toxicity of *S. madagascariensis* pod extracts to non-target organisms, although focal and seasonal molluscicide schedules are likely to be the rule and thus will minimize such a risk. Nevertheless, investigations on the toxicity and mutagenicity of this plant molluscicide are currently being undertaken. For example, *S. madagascariensis* contains highly piscicidal properties, a fact which is well known and exploited to the local people (Haerdi, 1964). However, as the *B. globosus* breeding sites and the *S. haematobium* transmission sites are not important for fishing in the Ifakara division (Marti and Tanner, 1982; Lwihula, 1985), *S. madagascariensis* pod extracts represent a cost-effective molluscicide for possible use in Ifakara, where *S. haematobium* is highly endemic (Zumstein, 1983). Comprehensive epidemiological studies have previously indicated that *B. globosus* is the only important intermediate host in the Ifakara division (Zumstein, 1983) where it is found in large numbers and in clearly defined pockets at the end of the dry season (Marti, 1984; Marti et al., 1985). Although most of these pockets are not connected to the adjacent river during the dry season (Marti et al., 1985), they act as important reservoirs for snail populations found in the adjacent rivers as the water level rises during the rainy season. Some of these pockets are also important transmission sites (Marti et al., 1985; Marti and Tanner, 1982).

S. madagascariensis pod extracts are now being used for repeated focal molluscicide of these breeding pockets/transmission sites during the dry season (Suter et al., in prep.). This is part of integrated transmission control measures within the aegis of a primary health care programme (Tanner, 1984).

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