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Cyclamin, a new Molluscicide from the tubers of *Cyclamen purpurascens* Mill. tested against the snail *Biomphalaria glabrata* (Say)

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Keywords : *Biomphalaria glabrata, Cyclamen purpurascens,* cyclamin, molluscicide, saponin, schistosomiasis.

Cyclamin, a monodesmosidic triterpenoid saponin isolated from the tubers of *Cyclamen purpurascens* Mill. was found to possess molluscicidal activity against the schistosomiasis-transmitting snail *Biomphalaria glabrata* (Say). The lowest concentration showing 100 % mortality to snails was 21 mg/l. The haemolytic activity and the molluscicidal potency of cyclamin were compared with those of primulic acid with the result that high haemolytic activity will not automatically imply a strong molluscicidal potency.

Mots clés : *Biomphalaria glabrata, Cyclamen purpurascens*, cyclamine, mollusquicide, saponine, schistosomiase.

Cyclamine, un mollusquicide nouveau isolé des tubercules de *Cyclamen purpurascens* Mill., testé contre l'escargot *Biomphalaria glabrata* (Say)

La cyclamine, une saponine monodesmosidique et triterpénoïde isolé des tubercules de *Cyclamen purpurascens* Mill., a fait preuve d' une activité mollusquicide contre *Biomphalaria glabrata* (Say), un hôte intermédiaire de *Schistosoma* sp. La plus petite concentration causant une mortalité de 100 % était 21 mg/l. L'activité hémolytique et l'activité mollusquicide de la cyclamine étaient comparées aux résultats correspondants de l'acide primulique. Il s'est avéré qu'une grande valeur de l'activité hémolytique n'implique pas forcément une forte activité mollusquicide.

1. Introduction

Many attempts are presently being made to control schistosomiasis by killing the transmitting intermediate host snails of this dangerous parasite. Schistosomiasis is endemic throughout Asian, African and South-American countries affecting more than 200 million people.

One of the main methods for control of the disease has always been the use of molluscicides to reduce the number of snails and therefore the transmission of the parasite to man. The presently available compounds or formulations tend to be generally biocidal, affecting many of the plants or animals (or both) in the snail habitats (WHO 1965). Although chemotherapy is one of the most common methods for control of schistosomiasis, synthetic compounds are more expensive than natural products such as plant extracts in developing countries (Takeda et al. 1987).

Since the early thirties more than 1100 plant species have been tested for molluscicidal activity (Kloos & McCullough 1987). Of this huge number only about 70 natural products with recognized molluscicidal activity have been isolated. Some of these are saponins, terpenoids, tannins, chalcones, coumarins and alka-

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loids (Marston & Hostettmann 1985). Since the discovery of highly potent saponins in the berries of *Phytolacca dodecandra* L'Herit saponin molluscicides have received remarkable attention (Parkhurst et al. 1973). Of particular interest are triterpenoid and spirostanol saponins. Without doubt, the main attention is paid to plants which grow abundantly in the endemic area of the disease but a preliminary study of a defatted and freeze dried methanolic crude extract of tubers from *Cyclamen purpurascens* Mill. (Primulaceae), a medicinal plant in Europe, has shown a distinct molluscicidal activity (200 mg/l) against the snail *Biomphalaria glabrata* (Say). Literature revealed that this plant contains the monodesmosidic triterpenoid saponin cyclamin (Tschesche et al. 1969).

In this paper we report the isolation of the saponin from tubers as well as the laboratory evaluation of the molluscicidal potency of the sample against *Biomphalaria glabrata.*

2. Material and methods

2.1. Plant material

Tubers of *Cyclamen purpurascens* were collected in late spring 1992 in a forest near Andritz, a northern district of Graz, Austria.

2.2. Snails

Bioassays were carried out with snails of the species *Biomphalaria glabrata* reared in aquaria; water temperature 24 ° C. Snails of uniform sizes were used (average diameter of the shell 9 mm). The tests were carried out by placing five snails in a water solution of known concentration of cyclamin at a temperature of 27 ° C. According to Hostettmann et al. (1982) distilled water was used. Exposure time and recovery period in distilled water were 24 h each. After these time intervals, the snails were placed on a petri dish, light was shone from underneath and the heart-beat was checked by a microscope. The number of replicates for each test was two.

2.3. Extraction and isolation

Tubers of the plant (150 g) were ground (mean size of particles: 2 µm) and defatted with light petroleum at 60 ° C and filtered. The residue was stirred overnight in aqueous methanol (methanol : water = 80 : 20), filtered and the filtrate evaporated in vacuo at low temperature (ca. 30 ° C). The crude cyclamin product (50 g) was dissolved in warm water (300 ml) and extracted five times with n-butanol (300 ml) and concentrated under reduced pressure. Pure cyclamin (8 g : 16 % of the weight of the crude product) was obtained as white crystals after repeated recrystallisation from ethanol/water (4 : 1).

2.4. General

Melting point (m.p.) was determined using a BÜCHI 530 apparatus. Optical rotation ($[\alpha]^{20}$) was measured with a JASCO DIP - 370 digital polarimeter. Thin layer chromatography was performed on aluminium plates coated with Silicagel 60 F_{254} (Merck, prod. no. 5554) using methanol/ethylacetate (2 : 1) as solvent. For detection vanillin-sulfuric acid or cerium (IV) sulfate/ammonium molybdate was used.

2.5. Haemolytic activity

The determination of the haemolytic activity of cyclamin was carried out according to procedure of the Europäisches Arzneibuch (Anonymous 1975). For this purpose, blood of a freshly slaughtered cow and a standard saponin [Schweizer Standardsaponin with a known haemolytic activity of 25000 (Fluka)] were used.

3. Results and discussion

Cyclamin represents a neutral monodesmosidic triterpenoid saponin with cyclamiretin A as aglycone and glucose (3 Mol), xylose (1 Mol) and arabinose (1 Mol) as the sugar moiety (Tschesche et al. 1969). The physical characteristics of the compound we isolated from the tubers of *Cyclamen purpurascens* are :

m.p. 280 ° C ; $[\alpha]^{20}$ - 10.5 ° (c 1.0 H_2O).

Comparison with data known from literature (Tschesche et al. 1969) :

m.p. 280-281 ° C ; $[\alpha]^{20}$ - 10.1 ° (c 1.46 H_2O).

In general only monodesmosidic triterpenoid saponins exhibit strong molluscicidal activity whereas bidesmosidic saponins (sugar chains in position C-3 and C-28) are completly inactive. This was shown by a series of 24 different saponins isolated from various medicinal plants which have been tested against *Biomphalaria glabrata* (Hostettmann et al. 1982). As cyclamin is monodesmosidic (only one sugar molecule is attached to the aglycone at position C-3) it seemed very likely that this compound possess considerable molluscicidal activity.

No snails were killed in the two control groups. The lowest concentration of cyclamin showing 100 % mortality (LC 100) to snails was 21 mg/l.

Very low 100 % lethal concentrations for *Biomphalaria glabrata* could be stated for some other compounds too. A sesquiterpene lactone from *Podachae-*

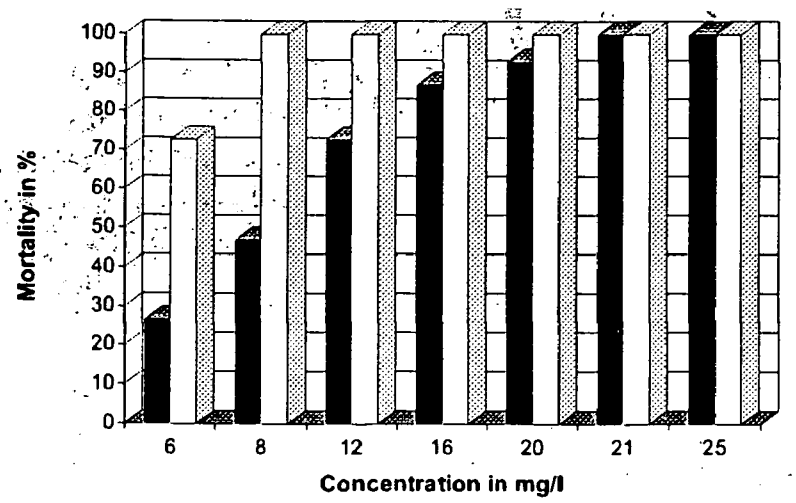

Fig. 1. Dosage mortality for *Biomphalaria glabrata* snails exposed to different concentrations of cyclamin in distilled water. Mean of the test and two replicates, 5 snails in each.
Black bars : percentage of snails killed within 24 h exposure to aqueous solution of cyclamin. White bars : percentage of snails killed within further 24 h in distilled water.

Fig. 1. Mortalité de *Biomphalaria glabrata* exposé à différentes concentrations de cyclamine diluée avec de l'eau distillée. Moyenne de l'expérience et de deux réplicats, chacun avec cinq escargots. Barres noires : pourcentage des escargots tués après un séjour de 24 h dans des solutions aqueuses de cyclamine. Barres blanches : pourcentage des escargots tués après les 24 h suivantes dans l'eau distillée.

nium eminens (Lagasca) Schultz-Bip. killed the snails at the 1.0 mg/l level within 24 h (Fronczek et al. 1984). Warburganal and Muzigadial, two sesquiterpenes isolated from the East African plant *Warburgia ugandensis* Sprague killed *Biomphalaria glabrata* within 2 h at a concentration of 5 mg/l (Nakanishi & Kubo 1977). Coumarins from *Ethulia conyzoides* L. possessed a LC 90 between 19 and 23.5 mg/l depending on the age of the snails against *Biomphalaria glabrata* (Kady et al. 1992).

Recently published results of the molluscicidal activity of the diterpene milliamine L (LC 100 2.5 µg/l/d), isolated from the latex of *Euphorbia milii var. hislopii* against the snail *Biomphalaria glabrata*, indicates that there has been a major advancement in the search of a highly potent plant molluscicide (Zani et al. 1993).

In comparison with other triterpenoid saponins cyclamin does not rank as the most potent molluscicidal saponin but it is a molluscicide of average potency.

Although saponins are often highly haemolytic their oral toxicity to homeotherm animals (e.g. mammals) is usually very low as a result of little resorption (Marston & Hostettmann 1991). Gessner & Orzechovski (1974) mention that pigs feed tubers of *Cyclamen purascens* without damaging their state of health whereas cyclamin is very toxic to fish and man.

The investigation of the haemolytic activity yielded 300000 and was compared with the value for primulic

Table 1. Efficacy of extracts of plants containing recognized molluscicidal triterpenoid saponins against *Biomphalaria glabrata*.
* In some plants more than one saponin was found to possess molluscicidal activity.

Tableau 1. Efficacité des extraits de plantes contenant des saponines mollusquicides et triterpénoïdes sur *Biomphalaria glabrata*.
* Quelques plantes contiennent plus d'une saponine avec activité mollusquicide.

Plant	Family	LC 100 (mg/l)*	Reference
Aesculus indica L.	Hippocastanaceae	10	Sati & Rana 1987
Clerodendrum wildii Moldenke	Verbenaceae	25	Toyota et al. 1990
Cussonia spicata Thunb.	Araliaceae	12.5, 100	Gunzinger et al. 1986
Hedera helix L.	Araliaceae	3, 8, 12, 15	Hostettmann 1980
Lonicera nigra L.	Caprifoliaceae	2	Domon & Hostettmann 1983
Phytolacca dodecandra L'Herit	Phytolaccaceae	3, 6, 25	Dorsaz & Hostettmann 1986
Sesbania sesban Merrill	Fabaceae	3, 25	Dorsaz et al. 1988
Swartzia simplex Spreng	Fabaceae	3	Borel et al. 1987
Talinum tenuissimum Dinter	Portulacaceae	1.5	Gafner et al. 1985
Tetrapleura tetraptera Taub.	Mimosaceae	2.5, 5, 20	Maillard et al. 1989
Xeromphis spinosa (Thunb.)	Rubiaceae	15, 20	Sati et al. 1987

acid (25000 - 29000), a saponin from *Primula elatior* (Primulaceae), published by Steiner & Holtzem (1955) when using a standard saponin with an activity of 12500 - 17000. Primulic acid kills *Biomphalaria glabrata* within 24 h at a concentration of 8 mg/l (Hostettmann et al. 1982).

It is obvious from this comparison that high haemolytic activity of a particular saponin does not automatically imply it will exhibit a strong molluscicidal potency.

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