Molluscicidal Activity of *Physalis angulata* L. Extracts and Fractions on *Biomphalaria tenagophila* (d'Orbigny, 1835) under Laboratory Conditions

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The main objective of this research is to evaluate the molluscicide activity of Physalis angulata *L.* Biomphalaria tenagophila *specimens under laboratory conditions. Extracts and fractions were supplied by the Laboratório de Química de Produtos Naturais, Farmanguinhos-Fiocruz. Experiments were performed according to the methodology described by the World Health Organization for molluscicide tests using the concentrations from 0.1 to 500 mg/l of the extracts, fractions and of a pool of physalins modified steroids present in this species. The results show that ethyl acetate and acetone extracts from the whole plant, the ethanolic extracts of the roots and the physalins pool from stems and leaves were active. Only the whole plant extracts were available in sufficient quantity for the determination of* LD_{50} *and* LD_{90} *values.*

Key words: molluscicidal activity - *Physalis angulata* L. - Solanaceae - *Biomphalaria tenagophila*

Schistosomiasis is a chronic and debilitating disease that affects people who have had contact with water harboring infected snails: rural workers, children, washerwomen from areas without a treated water supply and sewage system. The disease affects millions of people in several countries and is one of the world's main public health problems (OMS 1994).

The use of natural products in therapy through out the world is increasing, as well as the interest for research in this area.

Brazil, because of its wealth in biodiversity, has stimulated and focused research into new naturally derived drugs and this interest has extended to focus the field of vector control.

The search for more effective molluscicides, at lower costs and with less impact on the environment stimulated the search for potential active substances from native plants (Mott 1987). According to Baptista et al. (1994), 1,426 species of plants had been evaluated for molluscicide activity up to the time when he wrote his article. Notably active molluscicides are derived from specific parts of: *Phytolacca dodecandra* (Endod), *Croton macrostachys, Jatropha curcas, Ambrosia maritima, Anarcardium occidentale* (Kloos & McCullough 1987, Kuo 1987, Jurberg et al. 1989, Souza et al. 1992). *Euphorbia splendens* var. *hislopii* shows high bio-active potential (Vasconcellos & Schall 1986). Field work has been performed in order to evaluate the toxic effects (Schall et al. 1991) and molluscicide properties regarding temporal and seasonal evolution and geographical stability (Schall et al. 1992). From the latex of *Euphorbia milii* var. *hislopii* for example eight milliamines were isolated including five new substances (Zani et al. 1993).

Although several plants have been assayed in the search for molluscicidal activity, no reports have been published so far on *Physalis angulata* L.

The genus *Physalis,* which belongs to the family Solanaceae, includes about 120 species with herbal characteristics and perennial habits (Kissmann & Groth 1995), distributed around the temperate and tropical zones of the world and some of them are used as food as well as in popular medicine. Physalins are steroidal constituents of *Physalis* sp. and are characterized by their modified ergostane-type framework, being 16,24-cyclo-13,14-secosteroids (Vasina et al. 1986, Glotter 1991, Makino et al. 1995a, b, Tomassini et al. 2000).

P. angulata L. grows in the North and Northeast regions of the country where it is known as Camapu, Bucho de Rã, Joá or Juá de Capote and Mata-Fome (Branch & Silva 1983). In Pará the fruit is eaten and the roots are used to treat hepatitis and malaria. Such uses are widespread due to the abundance of the plant and easy cultivation which contributes to a large scale use.

To date no phytopharmaceutical is known to control schistosomiasis and there is no reference in the literature concerning the molluscicidal activity of this plant. Considering the widespread use of Camapu throughout the country we decided to study the plant as a mean to control the snail vectors of schistosomiasis.

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MATERIALS AND METHODS

The Chemistry Laboratory of Natural Products, PN_2 of Farmanguinhos, prepared the extracts, fractions and pool of physalins and the Department of Biology, Instituto Oswaldo Cruz-Fiocruz, performed the assays with snails.

Plant material - P. angulata was collected in October 1995 in Belém, State of Pará, Brazil. The identification and the classification was performed by Dr Lúcia d'A Freire Carvalho, from Jardim Botânico, Rio de Janeiro and an authentic sample stored at the herbarium of the Universidade Federal do Rio de Janeiro, Botany Departament under the reference number of RFA23907/8. Specimens were also cultivated in the Fundação Oswaldo Cruz's campus, Rio de Janeiro.

Snails studied - Specimens of *Biomphalaria tenagophila* from Jacarepaguá, Rio de Janeiro, RJ, were supplied by the Department of Malacology, Instituto Oswaldo Cruz-Fiocruz and kept in a 277 l culture tank filled with chlorine-free water, containing sterilized substratum consisting of red earth, calcium carbonate and oyster shell flour. The culture was mantained in the Department of Biology according to Paraense and Corrêa (1988), and the snails fed with fresh lettuce (*Lactuca sativa* L.).

Extracts and fractions of the plants employed - The whole plant and its parts, such as roots, stems, leaves, and fruits were dried at room temperature, ground and measured with ethanol, methanol, ethyl acetate, dichoromethane, chloroform, and hexane following a general procedure. Each extract was submitted to Mabry modified technique just to obtain the pool of physalins from which it is possible to get pure physalins such as withasteroid derivatives, that is seco-ergostanes having 28 carbon atoms, a ketone at C_{15}, two lactones (furan and pyran type) attached at C_{18} and C_{20} positions respectively. A total of 11 extracts and 2 fractions of *Physalis angulata* L. (Table I) were obtained and tested.

Preparation of the solutions to be tested - Each extract, fraction and pool of physalins was dissolved into 10 ml of dimethyl sulphoxide (DMSO) and diluted to 1,000 ml with distilled water. Solutions were obtained in the following concentrations: 0.1 mg/l, 1 mg/l, 10 mg/l, 20 mg/l, 30 mg/l, 40 mg/l, 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l, 300 mg/l, 400 mg/l and 500 mg/l.

TABLE I

Types of extracts and plant parts of *Physalis angulata* L. used in the experiments

Extract and fraction	Part used
Ethanol	Leaf
Ethanol	Root
Ethanol	Whole plant
Ethanol	Stem
Ethyl acetate	Whole plant
Acetone	Whole plant
Dichloromethane	Whole plant
Aqueous	Stem
Aqueous	Capsule of the fruit
Methanol	Whole plant
Methanol	Fruit
Pool of physalins	Leaf
Pool of physalins	Stem

Bio-assays

Exposure period - Following the method described by WHO (1965), snails, with a shell diameter of 6-10 mm, were assayed side by side with a control (with distilled water) and an experimental group (with the solutions) for 24 h.

Table II reports the different types of extracts, fractions, pool origins, and concentrations assayed as a first stage experimental technique. For each extract, fraction, pool, and control (distilled water) two beakers 1,000 ml with 15 mollusks each were assayed in a total of 690 snails.

TABLE II

Mortality of *Biomphalaria tenagophila*, when submitted to different concentrations of extracts and fractions of *Physalis angulata* L. First trial

Extract or fraction	Concentration (mg/l)	Dead	Mortality (%)
Controle	0	0	0
Ethanol extract - leaves	500	1	3.3
Aqueous extract - stems	500	2	6.7
Acetone extract - whole plant	500	22	73.2
Ethanol extract - roots	500	27	90
Pool physalins - stems	200	22	73.3
Pool physalins - leaves	200	28	93.3
Ethyl acetate fraction - whole plant	100	22	73.3
Pool physalins (leaves)	100	27	90

Number of snails: 30

Table III describes the second stage experimental run with the solutions that showed molluscicidal activity in the first test. The assay was made with 315 animals, at temperature of 26 ± 1ºC. The snails were not fed during this period and all the groups were kept in 500 ml of the respective solution.

Recovery period - At the end of each experiment, the snails were removed from the experimental solutions, rinsed in distilled water and kept in flasks of distilled water for 24 h. The experimental temperature was maintained constant.

Identification of dead snails - After the recovery period, the identification of dead snails was made based on the snails' immobility and the odor of the soft parts.

Statistical analysis - Lethal doses (LD_{50} and LD_{90}), were evaluated by means of probit analysis (Finney 1971). The plot probit of kill against log of concentration (mg/l) provides a simple graphic representation of the doses response ratio.

RESULTS

The results of the first and second stage tests are summarized in Tables II and III, respectively. The other concentrations showed no significant mortality.

Table III shows the percentage of mortality during the lethal doses (LD) determination and Table IV the probit analysis data.

TABLE III

Mortality of *Biomphalaria tenagophila*, when submitted to different extracts and fractions doses of *Physalis angulata* L. Second trial

Extracts and fractions	Concentration (mg/l)	Dead	Mortality (%)
CG	0	0	0
Ethyl acetate - whole plant	20	1	6.7
	30	3	20
	40	5	33.3
	50	4	26.7
	100	13	86.7
	150	13	86.7
Pool of physalins - leaves	40	2	13.3
	50	4	26.7
	100	10	66.7
	150	12	80
Acetone extract - whole plant	200	9	60
	250	3	20
	300	13	86.7
	400	15	100
Pool of physalins - stems	150	5	33.3
Ethanol extract - roots	300	10	66.7

CG: control group; number of snails: 15

TABLE IV

Lethal doses (LD_{50} and LD_{90}) for *Biomphalaria tenagophila*, obtained with solutions of the extracts and fractions of *Physalis angulata* L.

Extracts and fractions	N	LD_{50} (mg/l)	LD_{90} (mg/l)
Ethyl acetate - whole plant	105	55.3	139.4
Acetone extract - whole plant	75	178.2	321.3
Pool of physalins - leaves	90	82.5	201.2

N: number of snails

DISCUSSION

We agree with the analysis by Rouquayrol et al. (1980), who indicate that studies on the molluscicidal activity of plants, in planorbids, using different extracts (aqueous, alcohol and organic solvents) have provided partially promising results. However, we believe that the use against schistosomiasis should be postponed until further research in view of the existence of other trematodiosis of medical and veterinary importance.

According to Mott (1987) for a the plant to be considered as molluscicide, it should be registered in concentrations up to 100 mg/l and be able to kill 90% of the snails, 24 h after contact. On the other hand, Farnsworth et al. (1987) agreed to a maximum concentration tested of up to 100 mg/l. However, they use percentage scales that allow the discrimination of the molluscicidal activity as weakly active and active, depending on the number of dead snails. Therefore, we decided to adopt of Farnsworth's et al. (1987) methodology. According to the records, the results obtained from several plant parts of *P. angulata* with solvents of different polarities showed that solutions prepared from some extracts (ethanolic from the leaves and roots, aqueous from the stem, acetone from the whole plant) were weakly active, at a concentration of 500 mg/l; however the pool of physalins (stems) was weakly active at a concentration of 200 mg/l, while in the ethyl acetate extract from the whole plant and the pool fraction of physalins from the leaves were considered active, at the concentration of 100 mg/l. Ethanolic extracts from the stem, methanolic, dichloromethane and ethanolic from the whole plant, aqueous from the capsule of the fruit, and methanolic from the fruit were inactive in the first assay (Table II), contrary to Farnsworth's et al. (1987) report. However, the reported methodology is unclear and leaves many gaps. The present results, using the described methodology allow the determination of LD_{50} and LD_{90} in the solutions.

Rouquayrol et al. (1973), working with leaves, fruit, stem, root, and seeds of *Pithecelobium multiflorum* Bent (canafistula), using the crude aqueous and ethanolic raw extracts, measured the molluscicidal activity; these results were also observed with hexane extracts, as indicated by Mendes et al. (1984). However, we did not use the methodology of Rouquayrol et al. (1980), in which the solution in distilled water was neutralized by the addition of dilute aqueous hydrochloric acid or aqueous sodium hydroxide, although the authors registered positive results. In the assays with *P. angulata*, pH adjustment of the tested solutions was avoided, because, according to Fox et al. (1963) and Pereira and Souza (1974), any pH alteration in the solutions to be tested, is capable of influencing the result. Regarding the used parts of the plant, several authors worked with raw extracts (Mendes et al. 1984) and fractions (Pereira et al. 1978), a methodology which we followed and with which we obtained positive results also confirming the differences among parts used. Thus ethanolic extracts from the leaves and roots of *P. angulata* are molluscicidal, ethanolic extracts from the stem and the whole plant are inactive.

Analyzing these results, from the 13 different forms and extracts tested in the laboratory 5 (ethyl acetate and in acetone from the whole plant, ethanolic from the root, pool of physalins from the stem and the leaves) showed molluscicidal activity. For 3 of them (ethyl acetate and acetone extracts from the whole plant and pool of physalins from the leaves) LD_{50} and LD_{90} could be determined.

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