*Rev. Inst. Med. Trop. Sao Paulo*
*56(6):505-510, November-December, 2014*
*doi: 10.1590/S0036-46652014000600009*

# EVALUATION OF THE MOLLUSCICIDAL POTENTIAL OF HYDROALCOHOLIC EXTRACTS OF *Jatropha gossypiifolia* Linnaeus, 1753 ON *Biomphalaria glabrata* (Say, 1818)

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## SUMMARY

The action of extracts from the stem, leaves, and fruit of *Jatropha gossypiifolia* on *Biomphalaria glabrata* was studied by analyzing survival, feeding capacity and oviposition ability. The extracts were obtained by macerating the plant parts in 92% ethanol, which were then evaporated until a dry residue was obtained and phytochemically studied. The molluscicidal activity on *B. glabrata* was investigated using the procedures recommended by WHO (1965). The amount of food ingested and oviposition were measured during each experiment. The extract of leaves from *J. gossypiifolia* was shown to be a strong molluscicidal agent, causing 100% mortality of *B. glabrata*, even in the lowest concentration tested, of 25 ppm. Regarding the fruit extract, there was variation in the mortality, depending on the concentration used (100, 75, 50 and 25 ppm). The snails that were in contact with the fruit extract had significant reduction in feeding and number of embryos in comparison to the control. The stem extract did not present molluscicidal activity nor had any influence on the feeding and oviposition abilities of *B. glabrata*, in the concentrations tested. In conclusion, the extracts of leaves and fruits of *J. gossypiifolia* investigated in this work show molluscicidal effect and may be sources of useful compounds for the schistosomiasis control.

**KEYWORDS:** Schistosomiasis; Snails; *Biomphalaria glabrata; Jatropha gossypiifolia*.

## INTRODUCTION

Schistosomiasis is a chronic disease of great importance for public health in Brazil, due to its high prevalence, severity and clinical complications. Thus, it constitutes an important source of morbidity and mortality, especially in developing countries[18, 24].

In Brazil, *Biomphalaria glabrata* (Say, 1818), *Biomphalaria tenagophila* (d'Orbigny, 1835), *Biomphalaria straminea* (Dunker, 1848) are the species involved in transmission of schistosomiasis[6]. In the epidemiological chain of this parasitosis, *B. glabrata* is recognized as the best adapted intermediate host for transmission (*Schistosoma mansoni* Sambon, 1907), due to its wide geographical distribution and transmission efficiency[21].

In areas of high prevalence, prevention strategies incorporate public health measures to improve sewage management and mass treatment programs. Travelers to endemic areas should be warned against fresh water exposure; however, this can be often difficult to avoid. Any individual with evidence of infection should be treated. The drug of choice is praziquantel given at a dose of 40 mg/kg [*S. mansoni* and *Schistosoma haematobium* (Bilhartz, 1852)] or 60 mg/kg (*Schistosoma japonicum* Katsurada, 1904) in two divided doses[12].

To control schistosomiasis, besides the treatment of infected patients, it is very important to control snail populations as a way of reducing the risk of disease transmission[20]. Among molluscicides produced in laboratory, niclosamide (Bayluscide®) is recognized as one of the agents most used in schistosomiasis control programs, with the aim of combating snails[5]. However, effective concentrations of this molluscicide concomitantly kill fish, which becomes a problem when it is used in areas where fishing is an important income and source of food for the population[15]. Moreover, this product is expensive and poorly biodegradable[3,10].

The Euphorbiaceae family is known for presenting species, such as *Euphorbia milii var. splendens* (Bojer ex Hook.) Ursch & Leandri 1955 (Christ thorn) that not only have proven molluscicidal activity[26], but they also do not have cytological action when used up to concentrations of 200 ppm[2]. The species *Jatropha gossypiifolia* Linnaeus, 1753, commonly known as bellyache bush, belongs to this family and is native from tropical regions of the Americas[1,27]. Many parts of this plant have been used in folk medicine to treat various diseases, such as peptic ulcers, diabetes, cancer and diarrhea, and as a scar healing and diuretic agent[14,22].

Various studies have been conducted in Northeastern Brazil, based

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on the folk knowledge on the use of different plants, in order to validate their effect on a specific disease[11]. In view of the need to search for natural products with molluscicidal activity and low operational cost, the objective of the present study was to analyze the chemical components and evaluate the molluscicidal effect of hydroalcoholic extracts of the stem, leaves and fruit of *J. gossypiifolia*, taking into consideration the survival, feeding and oviposition ability of *B. glabrata*, in contact with these extracts.

## MATERIAL AND METHODS

**Assay on the molluscicidal activity of the plant extracts:** The snails used in the tests were of the species *Biomphalaria glabrata* that had been collected in neighborhoods on the outskirts of the city of São Luís (S 2° 31' 48", W 44° 18' 10") and were selected after quarantining, to ensure that they were negative for *S. mansoni.* The evaluation of the molluscicidal activity of the extracts was determined in accordance with the procedures previously described[28]. Snails with shells of 10-18 mm in diameter were exposed to the extracts for 24 h. After exposure, the snails were rinsed and kept in dechlorinated water and were observed for four days. A control group was kept in dechlorinated water under the same experimental conditions. Four different concentrations were used for the extracts from each part of *J. gossypiifolia*: 100, 75, 50 and 25 ppm. For each concentration, ten snails were used with three repetitions. The snails were handled in accordance with the principles of animal welfare in scientific experiments. Dead snails were those considered as showing discoloration of shells, immobility, exposure of the visceral mass, releasing hemolymph and lack of heartbeat, with the use of a stereoscopic microscope.

**Plant Material:** The plant material (leaves, fruit and stem) of *Jatropha gossypiifolia* L. was sampled in the neighborhood of Cohatrac I, in the municipality of São Luís, state of Maranhão, at 6:30 a.m., in September 2010. One sample was taken for preparation of exsiccate and sent to the Ático Seabra herbarium of the Federal University of Maranhão, where it is found under registration number 1293.

**Preparation of the plant extract:** Leaves, fruit and stem without impurities were selected and cut into pieces with scissors. These were then stored in large glass flasks separately for each plant part, and 92% ethanol was added. The material was then macerated using a glass rod. The ratio of plant material to solvent was 1:4 for the fruit, 1:7 for the leaves and 1:4 for the stem. After fifteen days of maceration, the material was filtered in a simple funnel system using filter paper, and the hydroalcoholic extracts were concentrated in a rotary evaporator, to obtain the dry extracts.

**Table 1**
IC50 from extracts of different parts of the *Jatropha gossypiifolia* to *Biomphalaria glabrata*

| Extract | IC50 |
|---|---|
| Fruits | 53.60ppm |
| Leaves | > 100ppm |
| Steam | <25ppm |
| Std. error logIC50 0.019 | |

**Phytochemical analysis:** The phytochemical analysis on *Jatropha gossypiifolia* was performed by researching the following secondary metabolites: tannins, saponins, flavonoids and coumarins, which present high polarity; and steroids and triterpenoids, which present low polarity. The sequential route for prospecting for chemical components in the hydroalcoholic extract of *J. gossypiifolia* is in Table 2. The concentrations of the compounds are described using crosses, where (+++) means strongly positive, (++) moderately positive, (+) weakly positive, (-) traces and (0) undetected, in accordance with the methodology described by MATOS (2009)[13].

**Table 2**
Methodology of prospecting the chemical constituents of hydroalcoholic extracts of *Jatropha gossypiifolia*

| Type Metabolite | Quantity of extract used (mL) | Methodology Matos (2009) |
|---|---|---|
| Taninns | 4 | Reaction with ferric chloride |
| Saponins | 80 | Foam test and precipitation |
| Alkaloids | 80 | Reacion with Hager, Mayer and Dragendorff |
| Flavonoids | 15 | Test of ΔpH with NaOH and $H_2SO_4$ |
| Coumarins | 3 | Under fluorescent light UV |
| Steroids and triterpenoids | 80 | Test of Liberman-Buchard |
| Steroidal aglycones and triterpenoids | 80 | Hydrolysis of saponins with subsequent testing Liberman-Buchard |

**Verification of the effect of the hydroalcoholic extracts of *Jatropha gossypiifolia* on the feeding behavior of *Biomphalaria glabrata:*** The surviving snails of each container (capacity of 2 L) were fed with 6 g of lettuce, on a daily basis, after 24 hours of exposure to the extract. In order to assess the amount of lettuce that was consumed by the snails, the amount of food remaining in each 24 hours of the experiment was weighed using an analytical scale and was subtracted from the initial amount of food. It was found that if the lettuce were left under observation during four days, the lettuce would degrade and it would not be possible to measure the remaining quantity. The problem was solved by measuring the amount of water in the lettuce every 24 hours and renewing the amount withdrawn with a new lettuce, with the same amount as the previous one. Thus, the final amount of lettuce evaluated within four days was the sum of determining the quantity of lettuce evaluated every 24 hours.

**Verification of the effect of the hydroalcoholic extracts of *Jatropha gossypiifolia* on oviposition by *Biomphalaria glabrata:*** Before exposure, three polystyrene plates (3 x 4 cm) were placed in each flask to investigate the number of eggs laid by the snails. At the end of the test, the polystyrene plates and the egg masses on the walls of the

flask were removed using a spatula. The embryos were counted using a stereomicroscope by concentration and extract plant part.

**Statistical analysis:** In order to observe the effect of the extracts on the oviposition and feeding of *B. glabrata*, a descriptive statistical analysis was performed using means ± standard deviations. The results relating to molluscicidal activity of the parts of the plant and tested concentrations were evaluated using the ANOVA Main Effect test, with a significance level of $\alpha = 0.05$, to observe whether there was any difference between the types of extract analyzed and the type of concentration. The test was performed using the Statistic 7.0 software, StatSoft, Inc. Tulsa, USA. The IC50 values were calculated by nonlinear regression using the software GraphPad Prism 5.0. Significance was set to $p < 0.05$.

## RESULTS

**Phytochemical analysis:** The phytochemical study on *J. gossypiifolia* showed that, among the plant parts analyzed, the leaves presented the greatest variety of secondary metabolites. Catechins were present at low concentrations; saponins, triterpenoids and alkaloids were present at moderate concentrations; and tannins and steroids were present at high concentrations (Table 3).

**Table 3**
Secondary metabolites found in hydroalcoholic leaves, fruit and stems of *Jatropha gossypiifolia*

| Secondary metabolites | Leaves | Fruits | Stem |
|---|---|---|---|
| Saponins | + + | + | 0 |
| Taninns | + + + | ++ | - |
| Steroids | + + + | ++ | + |
| Triterpenoids | + + | + | 0 |
| Alkaloids | + + | ++ | 0 |
| Flavonoids | 0 | + | 0 |
| Coumarins | 0 | 0 | 0 |
| Catechins | + | 0 | 0 |

L: +++ = Strongly positive; ++ = Moderaly positive; + = Weakly positive; - = Traces; 0 = undetected.

Conversely, the stem had lower amount of metabolites, presenting only steroids at low concentrations, and only traces of tannins. The leaves and fruit presented similar compositions of secondary metabolites, although the leaves showed some metabolites at higher concentrations. The fruit was the only part among the three plant parts analyzed that presented flavonoids. Details regarding the metabolites found and their concentrations, according to the part of the *J. gossypiifolia* plant, are shown in Table 3.

Although the phytochemical study that was performed aimed to seek high polarity metabolites to test them for water dwelling organisms like *B. glabrata*, information such as whether low polarity metabolites, like steroids and triterpenoids, are present or absent is essential, because it provides an idea of the type of saponin that exists in the extracts of the plant studied.

The aglycone part of the saponins found in the leaves and fruit were identified as triterpenoid saponins. The fruit presented a smaller amount than in the leaves.

**Evaluation of the molluscicidal potential of the extracts from the stem, leaves and fruit of *Jatropha gossypiifolia:*** The hydroalcoholic extracts of *J. gossypiifolia* leaves were lethal for *Biomphalaria glabrata* in all the concentrations tested. The 100% mortality rate was reached after 24 hours of exposure, for the concentrations of 100 and 75 ppm, and after 72 hours, for the concentrations of 50 and 25 ppm. Snail mortality was not observed in the control group or in the group treated with hydroalcoholic extract from the stem of *J. gossypiifolia* even in the highest concentration, 100 ppm. Regarding the group of animals in contact with hydroalcoholic extract of the fruits of *J. gossypiifolia*, there was variation in mortality, with different percentages, 100, 86, 40 and 26.6% to the concentrations of 100, 75, 50 and 25 ppm, respectively (Fig. 1). This data showed that there were differences regarding the efficiency of the molluscicidal activities of the extracts of the three plant parts [$F = 73.165$, $p < 0.05$] (Table 1).

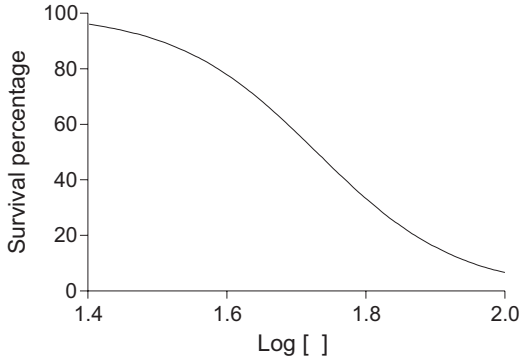


**Fig. 1 -** Regression analysis of the concentrations of the *Jatropha gossypiifolia* fruit extracts versus the percentage of survival of *Biomphalaria glabrata*.

The snails, when subjected to the hydroalcoholic extract of the leaves of *J. gossypiifolia* at the concentrations of 100, 75, 50 and 25 ppm, presented retraction of their cephalopodal mass, from which some groups were seen to release hemolymph. Concerning the hydroalcoholic extract from the fruits of *J. gossypiifolia,* the animals presented retraction of the cephalopodal mass without releasing hemolymph. With the hydroalcoholic extract of the stem of *J. gossypiifolia,* no activity on the cephalopodal mass was observed. In the control group, the snails remained alive.

**Evaluation of the effect of the extracts from the stem, leaves and fruit of *Jatropha gossypiifolia* on the feeding ability of *Biomphalaria glabrata:*** The feeding ability of the snails exposed to the hydroalcoholic extract of the leaves of *J. gossypiifolia* was completely suppressed, differently from the snails that were in contact with the hydroalcoholic extract from the stem, and also differently from the snail treated with the hydroalcoholic extracts from the fruits. The detailed results on the amounts of lettuce ingested by *B. glabrata* are shown in Table 4.

**Evaluation of the effect of the extracts from the stem, leaves and fruit of *Jatropha gossypiifolia* on oviposition by *Biomphalaria glabrata:*** The snails in contact with the solutions of the extract of leaves

**Table 4**
Feeding capacity of *Biomphalaria glabrata* under different conditions

| Part of plant | Concentration (ppm) | Quantity of ingested Lettuce (g) [Mean and standard deviation] |
|---|---|---|
| Fruits | 100 | 0.0 ± 0.0 |
| | 75 | 0.7 ± 0.1 |
| | 50 | 4.0 ± 0.2 |
| | 25 | 4.3 ± 0.32 |
| Stem | 100 | 5.83 ± 0.05 |
| | 75 | 6.0 ± 0.0 |
| | 50 | 5.73 ± 0.05 |
| | 25 | 5.96 ± 0.05 |

showed a high mortality rate, which had an influence on the impossibility of laying eggs. Moreover, even snails that showed no immediate mortality (during an exposure of 24 h) did not perform the oviposition activity. Moreover, the snails in contact with the hydroalcoholic extract of the fruit showed a variation according to the concentration. There was no influence on the oviposition by *B. glabrata* when in contact with solutions of the extract of the stem. The mean and standard deviation of embryos posed by *B. glabrata* is shown in detail in Table 5, by concentration and the plant extract.

**Table 5**
Effects of hydroalcoholic extracts of *Jatropha gossypiifolia* on the oviposition ability of *Biomphalaria glabrata*

| Part of Plant | Concentration (ppm) | Quantity of embryos [Mean and standard deviation] |
|---|---|---|
| Fruits | 100 | 0.0 ± 0.0 |
| | 75 | 105.66 ± 5.68 |
| | 50 | 252.33 ± 3.21 |
| | 25 | 370 ± 2.64 |
| Stem | 100 | 556.66 ± 7.09 |
| | 75 | 581.33 ± 2.51 |
| | 50 | 504.33 ± 2.08 |
| | 25 | 524.66 ± 4.93 |

## DISCUSSION

In Brazil, the first studies on molluscicides of plant origin have demonstrated the activity of aqueous extracts from *Serjania* sp. Linnaeus stems and fruit from *Sapindus saponaria* Linnaeus, popularly known as soap, on *B. glabrata*. This action was attributed to the saponins present in these plants[16].

There are many species of tropical plants that have substances with molluscicidal activity, especially among the Asteraceae, Euphorbiaceae, Fabaceae and Phytolaccaceae families containing different types of substances of secondary plant metabolism that may have molluscicidal activity[19].

Of the molluscicidal activity of 23 medicinal plants from the Northeastern region of Brazil on the snail *B. glabrata*, the extract from the leaves of *Annona muricata* Linnaeus was prominent at the concentration of 100 ppm, showing efficiency and resulting in the mortality of 100% of the snails tested[11].

The action of extracts from 13 species in the Piperaceae family were studied on *B. glabrata*, including the following five species (*Piper aduncum* Linnaeus, *P. crassinervium* Kunth, *P. cuyabanum* C.DC, *P. diospyrifolium* Kunth and *P. hostmannianum* R.S), showing molluscicidal activity at concentrations ranging from 10 to 60 ppm with 100% mortality[17], which is a similar result among all concentrations of leaves of *J. gossypiifolia* in this study.

There are several reports on the efficiency of extracts of parts of *J. gossypiifolia* against various species of molluscs: *Biomphalaria glabrata*, *Biomphalaria pfeifferi* (Krauss, 1848), *Bulinus globosus* (Morelet, 1866) and *Lymnaea luteola* Lamarck, 1822. It demonstrated the ability of this plant to promote increased cardiac output on *B. glabrata* and *Archachatina marginata* (Swainson, 1821)[2,25].

In other snails, such as the *Lymnaea acuminata* Lamarck, 1822 species, plants of the Euphorbiaceae family (*J. gossypiifolia, Euphorbia royleana* Boiss*, Euphorbia antisyphilitica Zucc.* and *Euphorbia lactea cristata* Haw.) were studied. They were monitored for 24 h and showed LC50 with an average of $1.4 \times 10^{-6}$ [23].

The elucidation of the molluscicidal activity requires studies that reveal details regarding the phytochemical profile of the plant[5]. The phytochemical approach not only facilitates the material to be studied, but also allows researchers to direct its use for various purposes, such as the development of new drugs and possible antibiotics, and even for some purposes like that of the present study, in seeking molluscicidal substances from plants.

As a natural defense, the plant produces substances (secondary metabolites) in greater diversity and intensity in its leaves and fruit, which are target organs for predators, such as insects and other herbivores.

Among the metabolites synthesized by plants, saponins are of interest because they present molluscicidal activity. They are substances that have an amphipathic structure formed by hydrophilic sugar residues bonded to a hydrophobic aglycone. In general, these metabolites present the ability to interact with sterols, which are present in the plasmatic membrane of cells, thus allowing ions and water to enter the cell and resulting in cell rupture, which releases hemoglobin[8]. Although the hemolytic properties are an outstanding characteristic of saponins, it is not common to all saponins and this is why the structural definition of aglycone in these compounds is a way of determining this trait[9].

Saponins can be classified in two types: steroidal and triterpenic. The triterpenic saponins consist of a group of natural compounds that present a wide spectrum of biological and pharmacological activities, among them, the ability to promote hemolysis of cells[7]. Maybe this explains the fact that there was a release of hemolymph from snails in contact

with the extract from leaves, reaction caused by the interaction between the amount of triterpenic saponins present in leaves and cells of the *B. glabrata* snails. The hemolytic properties of these extracts from leaves, evidenced in a test against the hemolysis of human erythrocytes, obtained positive results for all concentrations tested. The data is unpublished.

Although the phytochemical analysis showed that triterpenic saponins were present in the fruit of *J. gossypiifolia*, the absence of hemolytic activity among the snails can be attributed to the small amount of this substance found in this part of the plant. A similar profile was observed in plants tested against *B. glabrata*, which showed molluscicidal activity without promoting the release of hemolymph in the snails[29].

Regarding the influence of the extracts on the feeding behavior of the snail, more studies are necessary, since previous studies conducted on plant extracts only aimed to ascertain the molluscicidal activity. The present study is the first on the feeding behavior. One possible explanation for the low food consumption of the snails in contact with extracts from leaves and fruit is that, besides the mortality caused by these extracts, locomotive ability was hindered, which in turn would have made it impossible for live snails to actively seek for their food.

Feeding is a fundamental factor for the development and proliferation of snails, and when it is affected, this can lead to losses with regard to the establishment of populations of *B. glabrata*. Thus, physiological mechanisms, such as the movement of planorbids in their constant search for food, are not only survival mechanisms, but they also influence the propagation and increase the population structure.

*Biomphalaria glabrata* snails are prolific, hermaphrodite animals that can either self-fertilize or crossbreed. They reach sexual maturity at 30 days of age, when they achieve the ability to lay eggs. The eggs are then kept in gelatinous masses, which may contain more than 100 eggs. Although they may be laid on any submersed solid structure and, almost on a daily basis[4], this study observed that the extract from the fruit of *J. gossypiifolia* did not present an increased mortality effect, such as the extract from the leaves of *J. gossypiifolia* did. However, it reduced the oviposition ability of *B. glabrata*, in comparison to the control group and the stem extract.

Based on the results obtained using extracts from parts of *J. gossypiifolia*, there is a need to isolate the bioactive compounds of the extract from the leaves for future tests. In addition, in order to use this extract in the field, it is necessary to perform toxicity tests on non-target organisms, such as fish.

## RESUMO

**Avaliação do potencial moluscicida de extratos hidroalcoólicos do caule, folhas e frutos de *Jatropha gossypiifolia* Linnaeus, 1753 em *Biomphalaria glabrata* (Say, 1818)**

Estudou-se a ação dos extratos do caule, folhas e frutos de *Jatropha gossypiifolia* (Pinhão-roxo) sobre *Biomphalaria glabrata* analisando a sobrevivência, capacidade alimentar e de oviposição. Os extratos foram obtidos pela maceração das partes do vegetal em álcool etílico 92%, evaporados até obter-se um resíduo seco e estudados fitoquimicamente. A atividade moluscicida em *B. glabrata* seguiu os procedimentos recomendados pela WHO (1965). A medida de quantidade de alimento ingerido e a oviposição foi realizada durante cada experimento. O extrato das folhas de *J. gossypiifolia* mostrou-se um forte agente moluscicida, promovendo 100% de mortalidade de *B. glabrata* mesmo na menor concentração testada, 25 ppm. Com o extrato dos frutos houve variação na mortalidade, de acordo com a concentração utilizada (100, 75, 50 e 25 ppm). Os caramujos em contato com o extrato dos frutos apresentaram quando comparado ao controle uma diminuição significativa nos comportamentos alimentares e de número de embriões. O extrato do caule não apresentou atividade moluscicida e nenhuma influência sobre a capacidade alimentar e de oviposição de *B. glabrata* nas concentrações testadas. Em conclusão, os extratos de folhas e frutos de *J. gossypiifolia* investigados neste trabalho apresentam efeito moluscicida e possivelmente podem ser fontes de compostos no controle da esquistossomose.

## ACKNOWLEDGEMENT

To Mr. Francisco Moraes, a technician at FUNASA, who helped identifying the snail breeding sites; to the Federal University of Maranhão (UFMA) and the Research Support Foundation of Maranhão (FAPEMA) for their financial support.

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Received: 25 April 2013
Accepted: 26 March 2014