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Evaluation of the Molluscicidal Activity of *Punica granatum*, *Calotropis procera*, *Solanum incanum* and *Citrullus colocynthis* Against *Biomphalaria arabica*

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Abstract: Snails control is considered the most effective method in reducing the transmission of bilharziasis. The molluscicidal activity of *Punica granatum,* commonly grown in Taif and three wild plants; *Solanum incanum, Calotropis procera* and *Citrullus colocynthis* was investigated against *Biomphalaria arabica*, the intermediate host of *Schistsoma mansoni,* in Saudi Arabia. Alcohol extracts of fruits, leaves, stems and roots of the tested plants were evaluated for their lethal activity against adult snails of *B. arabica* and embryos of egg-masses. All the four tested plants exerted toxic lethal effect against both adult snails and their embryos. The extract of the rind of *P. granatum* fruit was significantly ($p \leq 0.05$) more potent than other parts of the same plant and extracts of the other plants.The LC_{50} and LC_{90} of the fruit rind of *P. granatum* extract were 48 and 100 ppm respectively, while those of the other tested plants were 325-600 ppm and 575-950 ppm respectively. On the other hand, the LC_{50} and LC_{90} of *P. granatum* fruit against embryos of snail egg-mass were 65 and 150 ppm respectively and for the of the other tested extracts, they ranged between 425-1000 ppm and 650->1000 ppm respectively. It may be concluded that of the four tested plants, the extract of pomegranate rind could be considered as a candidate for control of *B. arabica* snail. *P. granatum* extract also has the advantage of being friendly to the environment as it is safe to fishes and animals.

Key words: Molluscicides · *Punica granatum* · *Calotropis procera* · *Solanum incanum* · *Citrullus colocynthis* · *Biomphalaria arabica* · Snails · *Schistosoma mansoni* · Molluscicide

INTRODUCTION

Schistosomiasis is a devastating parasitic disease of man kind second behind malaria in its deleterious effect in tropical and subtropical areas [1-3]. It has been estimated that this disease affects more than 200 million people and other 600 million are at risk of infection in more than 70 countries in the tropics [4]. Estimates suggest that about 85% of all schistosomiasis cases now occur in the Sub-Saharan African countries [5, 6]. Mortality due to schistosomiasis was estimated to be 15,000 deaths per year, which does not include the indirect mortality due to schistosomiasis infection sequelae such as liver disease, portal hypertension, haematemesis, non-functioning kidney, cervical and squamous cell bladder carcinoma. If these sequelae are taken into account, the overall estimate of deaths due to schistosomiasis can reach 200,000 per year [7, 8].

The life cycle of schistosoma involves the infection of some species of molluscs. Therefore, control of mollusks via molluscicides can play an important role in an integrated approach aiming to control the disease. Therefore, cheap and environmentally safe molluscicides would result in an important complementary tool for the

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control of schistosomiasis [9, 10]. The potential use of plants as molluscicides has recently received considerable attention and local plant species have been studied in different countries [11-20]. Plants with molluscicidal activity would not only control the vector snail but they would also have the advantage of easy availability at low cost, biodegradability and greater acceptance amongst users [21, 22].

In Saudi Arabia, the fresh water snail *B. arabica* is the intermediate hosts for *Schistosoma mansoni* which causes intestinal schistosomiasis. The prevalence of schistosomiasis in Saudi Arabia was reported to be 2.9/ 100,000 persons [23]. The highest prevalence was reported in Jazan, Bishah, Aseer, Al-Bahah and Taif [23, 24], where the life cycle of the parasite is presumably completed through rodents, baboon monkeys and infected humans [24-26].

The aim of this study was to conduct a laboratory evaluation of the toxicity of alcoholic extracts of fruits, stems, leaves and roots of *P. granatum, Cal. procera, S. incanum and Cit. colocynthis*, against *B. arabica* snails.

MATERIALS AND METHODS

Plants: Different parts of *P. granatum*, *Cal. procera, S. incanum and Cit. colocynthis,* were collected from Taif Governorate and were air dried at room temperature.

Extraction: Five grams of powdered dry leaves, stems, roots and fruits of *Cal. procera, S incanum and Cit. colocynthis* were extracted three times with methanol, 24 hours each. In case of *P. granatum* fruit rind and not the whole fruit was extracted. Solvents of each part of a plant were combined, filtered and evaporated at 40°C at a reduced pressure. Dried extracts were stored in air-tight tubes at -20°C until used.

Snails: The fresh water harmful snail *B. arabica* were collected from fresh water in the area of Hada, near Taif City. Prior to experiment snails were allowed to acclimate to laboratory conditions for 72 h.

Toxicity of Extracts to Snails: Toxicity experiments were performed by the method of Singh and Agarwal [27]. Briefly, 10 snails were kept in glass aquaria containing 1000 ml de-chlorinated tap water with or without the treatment. Each set of experiments, was replicated three times. Mortality was recorded after 24h. Mortality was recorded when there was lack of response to gentle prodding with a blunt needle.

Toxicity of Extracts to Embryos of Egg-Masses: One day age egg-mass of *B. arabica*, laid on transparent nylon sheets were immersed in beakers (1L capacity) containing different concentrations of extracts for 24 h. Recovery was done by immersion of the treated nylon sheet in another beaker containing 1 L of de-chlorinated tap water for another 24 h. Embryos were examined under the microscope for their viability [39].

Statistical Methods: Student's' test was applied to determine the significant differences between treated and control animals.

RESULTS

Methanol extracts of fruits, leaves, stems and roots of *P. granatum*, *Cal. procera, S. incanum and Cit. colocynthis* were evaluated for their molluscicidal activity after 24 h. The extracts of each part of each plant were tested comparatively against both *B. arabica* adult snails and embryos of their egg-masses (Fig. 1).

The relationship between the percent of mortality of snails or their embryos and the concentration of alcoholic extracts of fruit, stem, leaf and root of the tested plants were similar, but not identical and all had a sigmoid shape (Fig. 1). Stems of *S. incanum* and *Cit. colocynthis* and roots of *Cal. procera* were relatively more active compared to other parts of the same tested plant. On other hand, fruit rind of *P. granatum* was significantly ($P \geq 0.05$) more active than the other parts of the tested plant (Fig. 1). From Figure 1 it is noticed that snails were more sensitive to the lethal effect of extracts than their embryos in egg-masses (Fig. 1).

Comparison between the LC_{50} and LC_{90} of different parts of each plant against adult snails are shown in Figure 2. *P. granatum* fruit extract was significantly more active as a molluscicide than other parts of pomegranate and leaf extract was the least active. While the LC_{50} and LC_{90} of fruit extract were 48 and 100 ppm respectively, those of pomegranate leaf were 325 and 575 ppm respectively.

The fruit was the least active part and the root was the most active part in both *Cal. Procera* and *S. incanum.* On other hand, the most active part of *Cit. colocynthis* was the fruit and the least active was the stem.

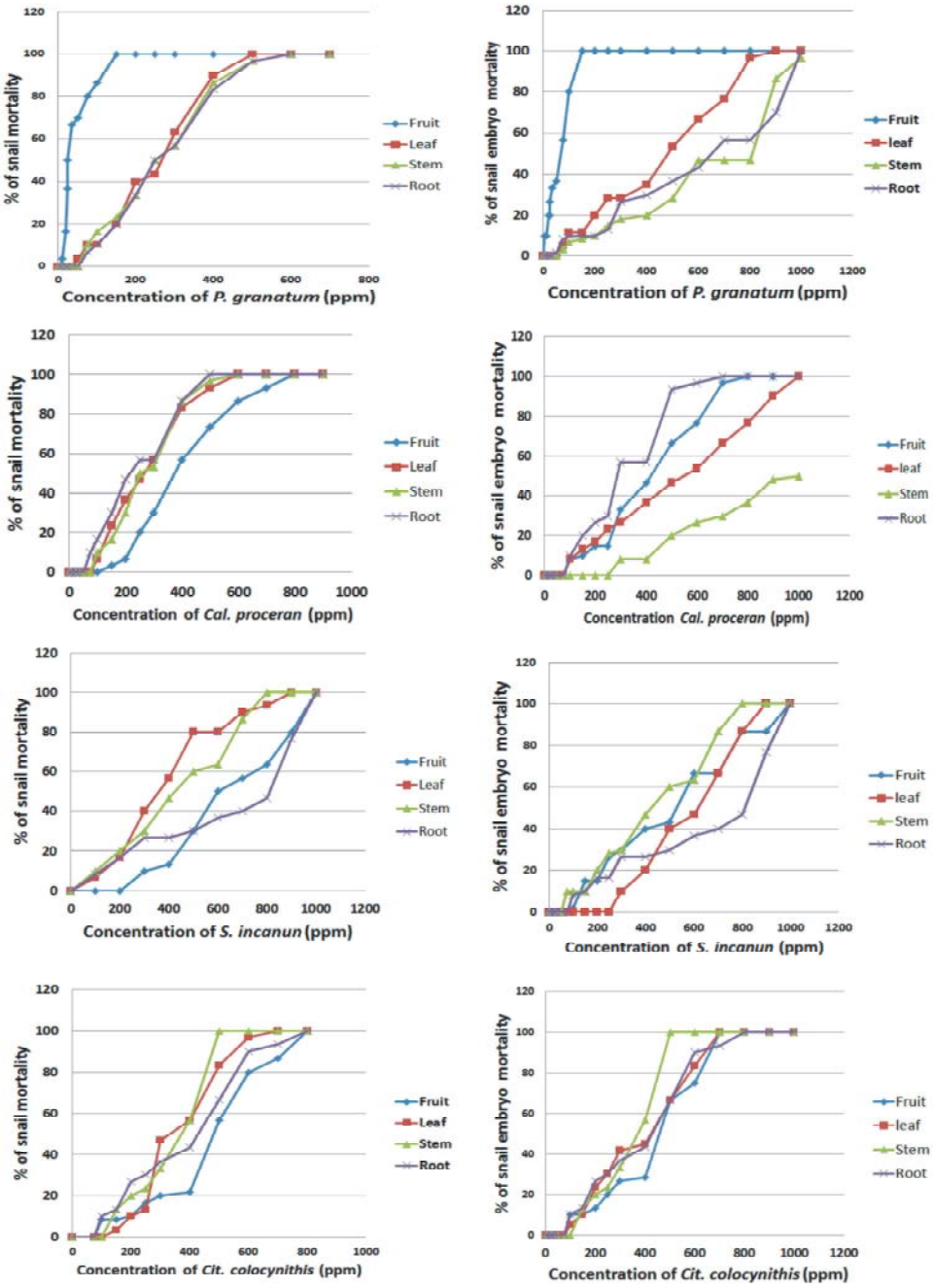

Fig. 1: Percent of mortality of *B. arabica* snails (left) and embryos (right) after exposure for 24h to extracts of fruits, leaves, stems and roots of the four tested plants

The LC_{50} of parts of *Cal. procera, Cit. colocynthis* and *S. incanum* ranged between 350 and 600 ppm and the LC_{90} ranged between 575 and 950 ppm (Fig. 2).

Fig. 3 shows the LC_{50} an $LC_{90}$of the different parts of the tested plants against embryos of *B. arabica.* Generally, speaking embryos were less susceptible to the lethal effect of extracts than snails. As in the case of snails, the most active part of *P. granatum* was the fruit and the least active was the root. The fruit was significantly ($P \geq 0.05$) more active against embryos than other parts. On the other hand, the most active parts of the other tested plants were root, stem and leaf in cases of *Cal. Procera, S. incanum* and *Cit. colocynthis* respectively (Fig. 3).

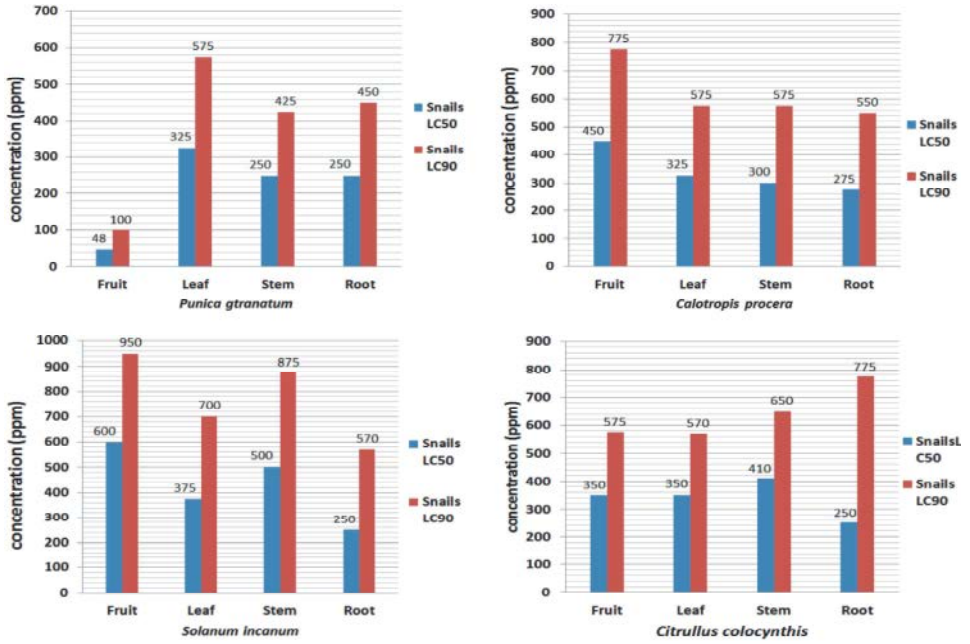

Fig. 2: LC_{50} and LC_{90} of extracts of fruit, leaf, stem and root of the tested plants against *B. arabica* snails after exposure to extracts for 24h

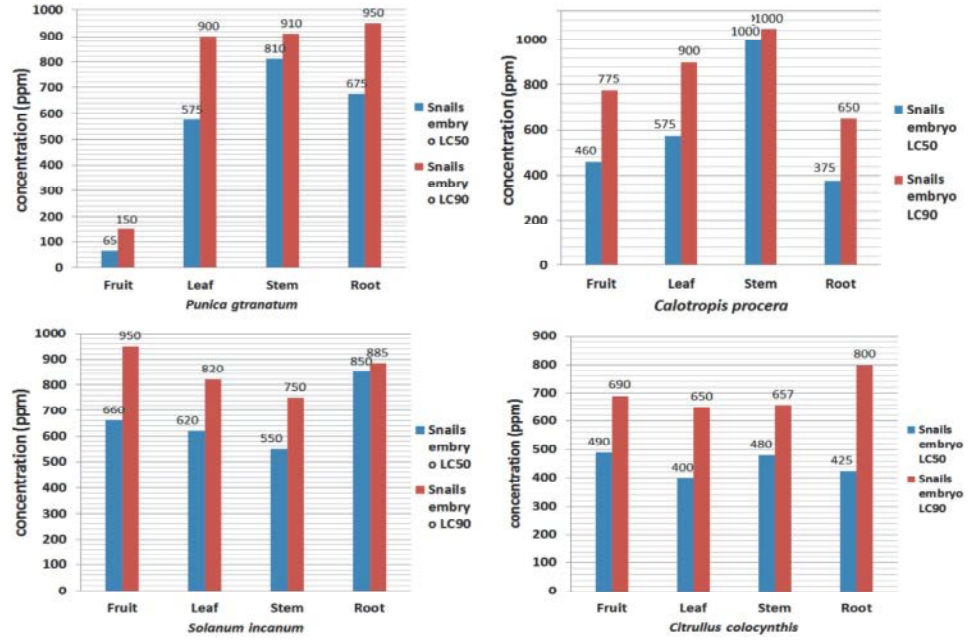

Fig. 3: LC_{50} and LC_{90} of extracts of fruit, leaf, stem and root of the tested plants against *B. arabica* embryos for 24h

A part from *P. granatum* fruit which had a LC_{50} and LC_{90} of 65 and 150 ppm respectively, the LC_{50} of the other ranged between 425 and 1000 ppm and the LC90 ranged between 650 and >1000 ppm. The least lethal tested part of tested plants was the stem of *Cal. procera* which had LC_{50} and LC_{90} of ≥1000 ppm (Fig. 3).

As previously mentioned, the effect of extracts of different parts of the tested plants on the mortality of snails and embryos of egg-mass followed a sigmoid pattern. Therefore, linear regression analysis was performed.The equation: Y= a + b X, was applied and the LC_{50s} were calculated precisely from the regression equations.

Table 1: LC_{50} of different parts of the tested plants against adult snails and embryos of egg-masses of *B. arabica*

Plant part	Plant	Adult snails	Embryos
Fruit	*Punica granatum*	47.800	65.66
	Calotropis procera	533.50	616.25
	Solanum incanum	653.40	660.50
	Citrullus colocynthis	368.30	427.80
Leaf	*Punica granatum*	203.91	462.39
	Calotropis procera	398.78	528.90
	Solanum incanum	399.90	580.77
	Citrullus colocynthis	378.80	387.70
Stem	*Punica granatum*	276.26	641.60
	Calotropis procera	359.60	975.80
	Solanum incanum	513.96	434.80
	Citrullus colocynthis	451.60	335.30
Root	*Punica granatum*	290.62	612.42
	Calotropis procera	310.15	319.29
	Solanum incanum	284.69	657.00
	Citrullus colocynthis	426.70	381.70

Based on the $LC_{50}$of snails, the most active fruit, leaf and stem were for *P. granatum* (47.80, 203.91 and 276.26 ppm respectively) and the most active root was for *S. insane* (284.69ppm). On the other hand, with regard to the lethal effect of extracts of different parts of the tested plants against embryos of egg-mass, the most active fruit was for *P. granatum* (65.66 ppm), the most active leaf was for *Cit. colocynthis* (387.7 ppm), the most active root was for *Cal. procera* (319.29 ppm) and the most active stem was for *Cit. colocynthis* (335.30 ppm) as shown in Table 1.

DISCUSSION

Interruption the life cycle of schistosoma through snails control is considered the most effective method in reducing the transmission of Bilharziasis. Therefore application of molluscicides plays an important role in controlling the disease [28]. As chemical molluscicides impose complex effects on man, domestic animals, fish and aquatic vegetation, there has been an increasing interest in alternative plant molluscicides [29, 30]. The aim of this study was to investigate the molluscicidal activity of *P. granatum* which is commonly grown in Taif and three wild plants which grow wildly. These plants were *S. insane* were *Cal*. procera and *C. colocynthis*. Extracts of the investigated plants were tested against adult snail of *B. arabica* and their egg-mass embryos.

Comparison between the efficiency of extracts of different parts of the investigated plants against adult snails, demonstrated that extracts of fruit rind, stem and leaf of *P. granatum* were more potent than the corresponding extracts of other investigated plants. However this was only significant in case of fruit extracts ($p \leq 0.05$). In the case of root extracts, the LC_{50} *P. granatum* extracts were comparable to that of *S. incanum* extracts.

In India the extract of stem bark of pomegranate was tested against, the intermediate hosts of *Fasciola hepatica, Lymnaea acuminata* snails [31]. *P. granatum* bark extract had a molluscicidal activity and at the same time was not toxic to fishes [31]. The 24 h LC_{50} of ethanol crude extract of *P. granatum* was 22.42 mg/l and the 24 h LC_{50} of column-purified bark extract was 4.39 mg/l (4.39 ppm). In our study the concentrations of stem extract of *P. granatum*, required to kill 50 % snails of *B. arabica* snails and their embryos, were 276.26 and 641.60 ppm respectively. The higher activity, found against *Lymnaea acuminata* snails may be attributed to the use of stem bark and the purification of the stem bark extract through column chromatography. Alternatively, *L. acuminata* snails may be more sensitive to pomegranate stem extracts than *B. arabica*.

In this study, we found that the fruit rind extracts of *P. granatum* was lethal to snails of *B. arabica* and their embryos at low concentrations. The LC_{50s} of the fruit rind against snails and embryos of egg-mass after 24h were 47.80 and 65.66 ppm respectively and the LC_{90s} were 100 and 150 ppm respectively. The ability of fruit peel extracts to kill embryos of egg-mass would further the ability of pomegranate to eradicate snails and control its population. The lower susceptibility of embryos of egg-mass may be attributed to a lower penetration of active phytoconstituents of extracts through the egg shell.

Cal. procera was reported to have a molluscicidal activity against the fresh water snail *L. acuminate* [32]. The LC_{50} of water extract of leaves was 14.09 mg/litre (14.09 ppm) and the ethanolic extract was 44.1 mg/litre [32]. On contrast to that in this study higher concentrations were obtained. The LC_{50} of *Cal. procera* ranged between 275 and 490 ppm and the LC_{90} ranged between 500 and 775 ppm against adult snails of *B. arabica*. The higher LC_{50} obtained in our study could be attributed to the environment of the plant which might affect its activity or the variation in susceptibility of different types of snails to the extracted plant.

Fakih [33] tested the latex of *Cal. procera* on *B. arabica* snails. The LC_{50} of the latex was equivalent to 100 ppm. The lower LC_{50} obtained by Fakih [33], compared to our higher LC_{50} obtained in this study, may be explained by the fact that in his study, the latex was used directly without extraction.

S. incanum was reported to contain solamargine, found in other species of *Solanum* with molluscicidal activity [34, 35]. Therefore, in this study, it was investigated for its possible molluscicidal activity against *B. arabica*. The most potent extracted part of the plant as a molluscicide was the root and the least effective extracted part was the fruit. The LC_{50} of the root in this study was 250 ppm and the LC_{90} was 570 ppm. Embryos of egg-mass were even less susceptible to *S. insane* extracts and their LC_{50} and LC_{90} were 250 and 570 ppm, respectively.

Hmamouchi, *et al.* [36], in Morocco, demonstrated the molluscicidal activity of alcoholic extracts of *Cit. colocynthis*, against *Bulinus truncatus*, the intermediate host of *Schistosoma haematobium*. The extract killed 50% and 90 % of the snails at 70 ppm and 122 ppm respectively Hmamouchi, *et al*. [36]. The higher lethal concentration found in this study might be attributed to the difference in the type of snail used.

In this study *P. granatum* alcoholic extract had LC_{90} of 100 ppm and 125 ppm for snails and egg-mass embryos, respectively, which is similar to the results obtained by Taher, *et al.* [37].

According to Mott [38] for a plant to be considered as a potent molluscicide, it should kill 90% of the snails, after 24 h contact at a concentration of $\leq$100 ppm. Therefore, data of this study suggest that of the plants tested, extract of *P. granatum* fruit rind could be considered as a promising efficient molluscicide.

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