Effect of some Plants Extracts as a Molluscicides Against the Snail Lymnaea auricularia L. in Basrah

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Abstract
This study showed the ability to use the aqueous extracts of fruit peel of Punica granatum and leaves of Myrtus communis in the biological control of Lymnaea auricularia, the intermediate host of Fasciola gigantica which are collected from Garmit-Ali river in Basrah during the period from September-October 2009. The study results showed the high effects of two plants on the survival rate, but The aqueous extract of Punica granatum showed high activity against snails than aqueous extract of Myrtus communis. The Lc50 through (24, 48, 72, 96) hr. were (1.75, 1.5, 0.5, < 0.5 mg/ml) for Punica granatum and (> 2, 2, 1.25, 1 mg/ml) for Myrtus communis.

Introduction
Some aquatic snails act as intermediate hosts for the larvae of trematode Fasciola gigantica, which causes fascioliasis (Singh et. al., 2009). The WHO has tested several hundred of synthetic compounds for the control of the snail host. Although effective, these molluscicides have so far not proved themselves to be entirely satisfactory (Aladesanmi, 2007). Control measures for these diseases may be achieved by destroying the intermediate host. Many plant species have been tested as molluscicides all over the world, as indicated by (Kloos & Mc-Cullough, 1987) and (Jurberg et. al., 1989). Snail can be controlled by utilizing molluscicide based on inorganic-organic compounds plant molluscicides have been used to reduce populations of snail hosts of Fasciola spp. (Singh et. al., 2009).

The role of Lymnaeid snails in the transmission of Fasciola gigantica in Iraq has been established by Al-mashhadani (Al-Mashhadani, 1970). The snails belong to the family Lymnaeidae are known to act as intermediate hosts of both human and animal fascioliasis (Aladesanmi, 2007). The WHO recommended that safety testing should parallel developmental stages of plant molluscicide (Awe et. al., 1995). It was recommended by WHO to find out the source of cheap, effective and environmental acceptable non-chemical molluscicide that target snail in the endemic area with fascioliasis. This study deals the activity of some plant extracts to control of snail Lymnaea auricularia as the intermediate host of Fasciola gigantica without of any damage in aquatic environmental such as fish and bird.
Materials and Methods
Snails sample collection
Snails were collected from Garmat-Ali river in Basrah during the period from September-October 2009. The collection method done by using small nets and then bringing to the Lab. with some water of river.

Plant sources
Two types of plants were used in this study Myrtus communis L. (Myrtaceae) and Punic granatum L. (Punicaceae). Fruit peel of Punica granatum and Leaves of Myrtus communis were collected from Basrah market and house garden respectively in September. Plants classification was done in Biology Department, College of Basrah University.

Aqueous Plants extracts procedure
Fruit peel of Punica granatum and Leaves of Myrtus communis were collected, then washed with water and left under room temperature and good air draft to drying, then the drying fruit peel was ground by mill and stored in plastic bags until used. 100 grams for each ground (fruit peel and leaves (powder)) were mixed with 250ml of distilled water. The mixture mixed by magnetic stirrer for 24 hr. at room temperature, then put in centrifuged (3000/r.p.m.) for 15 min., the supernatant was taken and filtrated through filter paper (Whatman No.1) then dried by freeze drier (Harborne, 1984; WHO, 1998). After prepared the plants extracts, we preparing the stock solution by dissolving 500 mg in 100 ml distilled water, (half gram in 100 ml tap water = 5 mg / 1ml) for each plant extract, then the following concentrations were prepared from stock solution: 10% (0.5 mg/ml), 20% (1 mg/ml), 30% (1.5 mg/ml) and 40% (2 mg/ml).

Experimental procedure:
1- Snails from Garmit-Ali river in Basrah were collected, and let in Lab. for three days to ensure and know that there is no infection with cercaria parasite, then snails were classified.
2- The snails were exposed to concentrations. Each concentration was done in triplicate beside the control. Ten snails but in each triplicate within 100 ml.
3- Biological test experiments on snails were done including (survival behavior and appearance changes) such as no response and dead. The needle was used to know the mobility and biology of snail. The dead snails were calculated every day after 24 hour. Then 50% lethal dose Le50 values were calculated by linear regression analysis (FAO, 1989).

Statistical analysis
One way analysis and prevalence were used in this study (Hill, 1988).

Results
The results of this study showed that the life span and the activity of the snails decreased with increasing concentration of aqueous plant extracts of Punica granatum and Myrtus communis. There was an increase in the percentage of dead snails with increasing the time exposure for plant extracts. Table (1,2)
The Lc₅₀ through (24, 48, 72, 96) hr. were (1.75, 1.5, 0.5, < 0.5 mg/ml) for *Punica granatum* and (> 2, 2, 1.25, 1 mg/ml) for *Myrtus communis*. Table (3). Statistically, there were significant differences among different concentrations of plant extracts (p<0.01).

Table (1) The number and percentage of dead snails after their exposure to various concentrations of aqueous extract of *Punica granatum*

<table>
<thead>
<tr>
<th>Exposure times (hours)</th>
<th>10 % (0.5 mg/ml)</th>
<th>20 % (1 mg/ml)</th>
<th>30 % (1.5 mg/ml)</th>
<th>40 % (2 mg/ml)</th>
<th>control</th>
</tr>
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<tbody>
<tr>
<td>24</td>
<td>2 (20)</td>
<td>2 (20)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>0</td>
</tr>
<tr>
<td>48</td>
<td>2 (20)</td>
<td>3 (30)</td>
<td>5 (50)</td>
<td>7 (70)</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>5 (50)</td>
<td>6 (60)</td>
<td>7 (70)</td>
<td>9 (90)</td>
<td>0</td>
</tr>
<tr>
<td>96</td>
<td>6 (60)</td>
<td>8 (80)</td>
<td>10 (100)</td>
<td>10 (100)</td>
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</tr>
</tbody>
</table>

Table (2) The number and percentage of dead snails after their exposure to various concentrations of aqueous extract of *Myrtus communis*

<table>
<thead>
<tr>
<th>Exposure times (hours)</th>
<th>10 % (0.5 mg/ml)</th>
<th>20 % (1 mg/ml)</th>
<th>30 % (1.5 mg/ml)</th>
<th>40 % (2 mg/ml)</th>
<th>control</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>0</td>
<td>1 (10)</td>
<td>2 (20)</td>
<td>2 (20)</td>
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Table (3) Lethal concentration Lc₅₀ after (24, 48, 72, 96) hr. for two plant extracts against snails
The results of the present study showed high effect of *Punica granatum* and *Myrtus communis* extracts on the survival rate and activity of snails at different concentrations when comparing with the control. This observation may be belong to the chemical effects of tannins and some phenolic substances of these plants. The results demonstrated a decrease survival and life span of snails with the increase of the concentration and time exposure, the Lc$_{50}$ value after 72 hr. experiment for aqueous extracts of *Punica granatum* and *Myrtus communis* were (0.5 mg/ml) and (1.25 mg/ml) respectively. The mechanisms of action of *Punica granatum* on snails is due to presence of tannins and phenolic substances were able to precipitate on the protein of cell membrane during its penetration (Bakir, 1997). These compounds form hydrogen bounds with nitrogen free and multihydroxyl-groups, causing inhibition of some enzymes which are very essential to the organism (Reed, 1995; Covington, 1997). Al-Mayah, (2002) reported high effect of aqueous extract of *Punica granatum* against *Fasciola gigantica* parasite and their larval stages such as miracidia and the redia which isolated from infected *Lymnaea auricularia*.

<table>
<thead>
<tr>
<th>Exposure times (hours)</th>
<th>Lc$_{50}$ of <em>P. granatum</em> (mg/ml)</th>
<th>Lc$_{50}$ of <em>M. communis</em> (mg/ml)</th>
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<tr>
<td>24</td>
<td>1.75</td>
<td>&gt; 2</td>
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<tr>
<td>48</td>
<td>1.5</td>
<td>2</td>
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<tr>
<td>72</td>
<td>0.5</td>
<td>1.25</td>
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<tr>
<td>96</td>
<td>&lt; 0.5</td>
<td>1</td>
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</table>

The high activity of the leaves extract of *Myrtus communis* may be due to its containing this plant on the many chemical compounds which have biological activity such as mytrol, tannins, bitter principle and phenol compound (uniphenol and manyphenol) and galic acid (Elisha *et. al.*, 1988; Kery, *et. al.*, 1985). The important of using the aqueous extract of some plants is due to the fact that the water has no effect on the chemical
compound in plant extracts and it dose not enter with it (Leven et al., 1979; Al-Hilli, 2000). The aqueous plant extract of *Nicotinana tobaccum* leaves (Solanaceae) is used as a molluscicide against the snail *Bulinus truncatus* the intermediate host of urinary *Schistosome haematobium* in Iraq (Hanoon et al., 2004). The present study might be a primary step in the use of biological control which needs more investigations in future. Attempts showed be focused on the purification and isolation of the active substance for plant extracts.

**References**


