Evaluation of Euphorbia Aphylla, Ziziphus Spina-Christi and Enterolobium Contortisiliquum as Molluscicidal Agents

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Abstract: The present work was carried out to evaluate the molluscicidal activities of ethanoic extract of three medicinal plant species namely Euphorbia aphylla, Ziziphus spina chriti, and Enterolobium contortisiliquum against Biomphalaria alexandrina and Lymnaea cailliaudi (natalensis) snails the immediate intermediate hosts of schistosomiasis and fascioliasis respectively. The experiments were conducted in accordance with WHO guidelines. Probit analysis was used to determine the LC90 and LC90 after 24 hours exposure. The highest molluscicidal potency was recorded for E. aphylla. It exhibited significant molluscicidal activity on both snails species. The LC95 and LC90 of this extract against Lymnaea cailliaudi were 0.66 and 0.88 ppm respectively and 87.6 and 142.5 ppm against B. alexandrina followed by Ziziphus spina-chriti which showed molluscicidal activity against L. cailliaudi with LC95 311 ppm and LC90 500 ppm and caused no mortality of B. alexandrina up to 1000 ppm. The least active was Enterolobium contortisiliquum which gave negative results against both snail species up to 1000 ppm. Further purification of active compounds present in Euphorbia aphylla and Ziziphus spina-chriti may eventually be of great value for the control of snails’ intermediate hosts of fascioliasis and schistosomiasis.


Key words: Plant molluscicides- Biomphalaria – Lymnaea- fascioliasis - schistosomiasis -Egypt.

1. Introduction

Schistosomiasis remains as one of the world’s most prevalent diseases (King and Dangerfield-Cha, 2008). It is estimated to infect 207 million people worldwide. Approximately tenth of the world population are living with the risk of infection (WHO, 2010). In Egypt, the disease is not only a prime health problem, but it is also an economic one, as it affects millions of farmers at the early age diminishing their productivity and exerting a serious socioeconomic problem (El-Baz et al., 2003). Biomphalaria alexandrina as specific intermediate host of Schistosoma mansoni is prevalent in both Upper and Lower Egypt (WHO, 2002).

Fascioliasis is a worldwide zoonotic disease caused by another trematode parasite of the genus Fasciola that infects over 17 million people causing significant morbidity and mortality (Mas-Coma et al., 2005; WHO, 2006). In Egypt, fascioliasis becomes hyperendemic and problematic where animal reservoir and snail vector are available (Rashed et al., 2008). Nearly 24 million Egyptians are at risk and about 800 000 suffering from fasciolosis (WHO, 1995, Haseeb et al., 2002). Human infection causes serious hepatic pathological consequences (Soliman, 2008). In addition, fascioliasis is responsible for economic losses estimated at around one billion Egyptian pounds per year (Haseeb et al., 2002). In general, El Shazly et al. (2002) found concomitant infection between fascioliasis and Schistosomiasis mansoni.

Treatment of Schistosoma and Fasciola infections remains highly problematic. In schistosomiasis, praziquantel is faced with failure to prevent reinfection as a result of development of drug resistance Schistosoma strain and serious side effects. Treatment of Fasciola requires high or multiple doses of drugs with frequent side effects (Ismail et al., 1999 and Abdul-Samie et al., 2010). Therefore snail control is considered not only complementary but essential in Schistosoma and Fasciola control. It is regarded as a rapid and efficient method for reducing or eliminating transmission and is among the methods of choice to bring these diseases under an adequate control through the breakage of the life cycle of the parasite (Mello-Silva et al., 2006; Jigyasu and Sing, 2010).

Today, mollusciciding is regarded as an important aggressive strategy in the control of the snail hosts of these diseases (Giovanelli et al., 2001, Mello-Silva et al., 2006). Unlike the use of synthetic drugs, the uses of molluscicides prevent reinfection of people after treatment (WHO, 1993). Copper sulfate and niclosamide were used in Egypt within a program developed by Bayer AG however, due to their hazardous enviromental effects, their toxicities to non-target organisms and even man, they were stopped (WHO, 2002; Abdelrazek et al., 2007). Therefore the search for alternative molluscicides is still ongoing. During recent years
much attention has been drawn for the use of molluscicides of plant origin. The use of plants with molluscicidal properties appears to be a simple, inexpensive and safe alternative (Singh and Singh, 2010; Al-Daihan, 2010). Also, there is a continuous need to search for new plant species with ideal molluscicidal properties (Tantawy et al., 2004; Bakry and Hamdi, 2007). In Egypt, several local plant species screened and proved to have molluscicidal properties against different snail species e.g. Ambrosia maritīme (Abou Basha et al., 1994), Solanum species (Tantawy et al., 2000), Commiphora molmol (Abd-Allah et al., 2009), Guayacum officinalis, Calatropis procera and Euphorbia splendens (Bakry, 2009).

Euphorbia is the largest genus of flowering plants in the Egyptian flora (El-Karemy, 2008). Over the past twenty years, they have received considerable phytochemical and biological attention (Wu et al., 2009). According to Mwine (2011) a good number of Euphorbia species are actually potent as medicinal plants and their extracts have been isolated and patented as modern drugs. They have a variety of uses, such as for the treatment of intestinal parasites (Appendino and Szallasi, 1997 and Shi et al., 2008). They also possess antiameobic (Tona et al., 2000); anti-plasmodial (Tona et al., 2004) and anti-leishmanial activity (Ahmed et al., 2006). Earlier studies indicated that the euphorbiae have molluscicidal activity (Tantawy et al., 2004; Sermsart et al., 2005; Bakry 2009; Singh and Singh, 2010). Alkaloids and saponin are reported among active compounds of several Euphorbia species (Siddiqui et al., 2009).

Ziziphus spina-christi is one of the plants most commonly used in Egyptian folk for treatment of different diseases and is traditionally used in Arab countries as a medicinal plant (Rigal et al., 2006 and Nawash and Al-Horani, 2011). In field of parasitology, the ethanolic extract of Ziziphus spina-christi root showed anti-schistosomal activity (Aly et al., 2006, El - Rigal et al., 2006). Anti-leishmanial activities of ethanolic and aqueous extracts of the leaves have also been reported (Tonkal et al., 2005). The phytochemical composition of Ziziphus spina-christi reported the presence of four saponin glycosides and alkaloids (Shahat et al., 2001 and Anthony, 2005).

Enterolobium contortisiliquum is an important species of the family Fabaceae. The essential oil of Enterolobium contortisiliquum seeds has been reported to have an antimicrobial activity (Shahat et al., 2008). The plant was reported to be rich in saponin (Mimaki et al., 2004), a substance responsible for molluscicidal activity (Hostettmann et al., 1982, Osman et al., 2007).

It is now well established that in many plants the molluscicidal activity is due to the presence of saponin contents (Rawi et al., 1996, Osman et al., 2007 and Singh and Singh 2010) and alkaloid components (Melendez and Capriles, 2002, El-Ansary et al., 2003, Ahmed and Rifaat 2005, Silva et al., 2005 and Singh et al., 2010). According to these authors, plants containing one or more of these compounds are among the most promising for controlling schistosomiasis and fascioliasis.

Based on these facts and since Euphorbia aphylla (Euphorbiaceae), Ziziphus spina-christi (Rhamnaceae) and Enterolobium contortisiliquum (Fabaceae) have been described as plants rich in saponin and/or alkaloids. The present study is aimed to evaluate the molluscicidal activity of the ethanolic extracts of these plants against Biomphalaria alexandrina and Lymnaea caulliaudi (natalensis) the snails' intermediate hosts of Schistosoma mansoni and Fasciola species respectively in a trial to open new areas of application of extracts of these plants as eco-friend molluscicides.

2. Materials and Methods:

- Snails: Laboratory bred uninfected adult Biomphalaria alexandrina snails (6-8mm in diameter) and Lymnaea caulliaudi (natalensis) (8-10mm in shell length) from the stock reared in Medical Malacology Department, Theodor Bilharz Research Institute (TBRI) were used.

- Plant Material:

  The plants used in this study were Euphorbia aphylla (Family Euphorbiaceae), Ziziphus spina-christi (Family Rhamnaceae) and Enterolobium contortisiliquum (Family Fabaceae). The plant materials were collected locally from Faculty of Agriculture, Assiut University. Plant species was kindly identified and extracted by Prof. Dr. Zedan Z. Ibrahim, Pharmacognacy Department, Faculty of Pharmacy, Assiut University. Voucher specimens of each plant were kept in the Museum of Pharmacognacy Department, Faculty of Pharmacy.

- Preparation of Plant Extracts:

  The aerial parts of Euphorbia aphylla, Ziziphus spina-christi and the mature ripe fruit of Enterolobium contortisiliquum were cleaned, cut into small pieces and dried in shade then ground using blender. About 250 g of air dried powdered plant material was extracted with ethanol (70%), filtered and distilled off under vacuum at temperature not exceeding 50 °C and the residues were stored in dry glass bottles (Bakry, 2009).

- Preparation of Molluscicide Solutions: (According to Singab et al., 2006)
Stock solutions of 1000 ppm were freshly prepared by dissolving 1 g of each ethanolic extract in the minimal amount of dimethylsulfoxide (DMSO), and made up to 1000 ml by adding dechlorinated water. A series of concentrations (0.25 – 1000 ppm) were prepared from the stock solution of Euphorbia aphylla and double serial concentrations (100-200-400 etc.) were prepared from the stock solutions of Ziziphus spina-christi and Enterolobium contortisiliquum.

Determination of molluscicidal activity:

WHO, 1965 guideline was followed for evaluation of the molluscicidal activity of the extracts. A series of exploratory experiments were conducted using the previously prepared concentrations to determine the toxicity range of the plant extracts against the tested snails. Once the extent of the toxicity range was determined, several intermediate concentrations were prepared from the stock solutions (diluted with dechlorinated water) to give mortalities between 0-100% according to Osman et al., 2007.

For each experimental concentration three replicates were prepared, each of 10 snails/ L. Another three replicates were prepared in dechlorinated water as control. Snails were exposed to the molluscicide suspension for 24 hours at room temperature (exposure period). The tested snails were left in water for another 24 hour and examined to assess mortality (Recovery period). Snails were considered dead if they probed and remained motionless or if the shell looked discoloured. Mortality rates were recorded. Probit regression analysis (SPSS version 7) aimed to determine the LC\textsubscript{50} and LC\textsubscript{90} values as well as their 95% confidence limits were carried out according to Finney (1971).

3. Results

Molluscicidal activity of Euphorbia aphylla:

The effect of various concentrations of ethanol extract of the aerial portion of Euphorbia aphylla on adults of Biomphalaria alexandrina and Lymnaea cailliaudi (nalterensis) after 24 hour exposure are listed in tables 1 and 2.

The LC\textsubscript{50} and LC\textsubscript{90} of this extract against Biomphalaria alexandrina after 24 hour exposure were 87.6 and 142.5 ppm respectively. While the LC\textsubscript{50} and LC\textsubscript{90} of the same extract against Lymnaea cailliaudi (nalterensis) after 24 hour exposure were 0.66 and 0.88 ppm respectively.

There was a significant difference between molluscicidal activities of ethanol extract of Euphorbia aphylla against both snails. Lymnaea cailliaudi (nalterensis) were more sensitive to Euphorbia aphylla extract than Biomphalaria alexandrina adults.

The probit mortality showed that the response of the two snail species illustrated a linear relationship with the concentrations (dose ppm) of the ethanol extract of Euphorbia aphylla as revealed in figure 1, 2. The exposed snail species responded differently to different concentrations of the studied plant extract.

Molluscicidal activity of Ziziphus spina-christi:

Molluscicidal effect of ethanol extract of the aerial portion of Ziziphus spina-christi on B. alexandrina gave negative results up to 1000 ppm.

Molluscicidal effect of ethanol extracts of Ziziphus spina-christi on L. cailliaudi showed LC\textsubscript{50} 311 ppm and LC\textsubscript{90} 500 ppm. The probit mortality showed that the response of L. cailliaudi illustrated a linear relationship with the concentrations (dose ppm) of the ethanol extract of Ziziphus spina-christi as revealed in figure 3.

Molluscicidal activity of Enterolobium contortisiliquum:

Ethanol extract of the fruit of Enterolobium contortisiliquum (Family Fabaceae) gave negative results against both snail species up to 1000 ppm.

Table (1): Mortality rates, LC\textsubscript{50} and LC\textsubscript{90} of ethanolic extract of Euphorbia aphylla against Biomphalaria alexandrina

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
<th>LC\textsubscript{50}</th>
<th>LC\textsubscript{90}</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>87.6(39.99–332.5)</td>
<td>142.5(98.3–1092.3)</td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>12</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>9</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>30</td>
<td>6</td>
<td>20</td>
<td></td>
<td></td>
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<tr>
<td>20</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td></td>
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</tbody>
</table>

Probit Transformed Responses
Figure 1. Dose/probit regression line of Euphorbia aphylla on Biomphalaria alexandrina

Table (2): Mortality rates, LC$_{50}$ and LC$_{90}$ of ethanolic extract of Euphorbia aphylla against Lymnaea cailliaudi (nalatensis).

<table>
<thead>
<tr>
<th>Conc.(ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>0.66(0.62</td>
<td>0.88(0.821-0.966)</td>
</tr>
<tr>
<td>0.80</td>
<td>30</td>
<td>24</td>
<td>80</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>0.75</td>
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<td>18</td>
<td>60</td>
<td></td>
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<tr>
<td>0.60</td>
<td>30</td>
<td>12</td>
<td>40</td>
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<tr>
<td>0.50</td>
<td>30</td>
<td>6</td>
<td>20</td>
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<td></td>
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<tr>
<td>0.25</td>
<td>30</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
</table>

Probit Transformed Responses

Figure 2. Dose/probit regression line of Euphorbia aphylla on Lymnaea cailliaudi (nalatensis)
Table (3): Mortality rates, LC$_{50}$ and LC$_{90}$ of ethanolic extract of Ziziphus spina-christi against Lymnaea cailliaudi (nalanatensis)

<table>
<thead>
<tr>
<th>Conc. (ppm)</th>
<th>Number of tested snails</th>
<th>Number of dead snails</th>
<th>Mortality rates (%)</th>
<th>LC$_{50}$</th>
<th>LC$_{90}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>30</td>
<td>30</td>
<td>100</td>
<td>311(163.83-465.68)</td>
<td>500(384.002-1089.676)</td>
</tr>
<tr>
<td>600</td>
<td>30</td>
<td>27</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>30</td>
<td>24</td>
<td>80</td>
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<tr>
<td>300</td>
<td>30</td>
<td>20</td>
<td>66.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>5</td>
<td>16.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Dose/probit regression line of Ziziphus spina-christi on Lymnaea cailliaudi (nalanatensis).

4. Discussion:
Schistosomiasis and fascioliasis are worldwide parasitic diseases infecting 207 and 17 million people respectively causing significant morbidity and mortality (WHO, 2006, 2010).

In Egypt, positive association between liver fluke infection and schistosomiasis was detected in several governorates. It was explained by the co-existence of both parasites intermediate hosts inhabiting the same type of water bodies (Haseeb et al., 2002). Once, these snails intermediate host destroyed, the life cycle will be disrupted (Hamed, 2010; Jigyasu and Sing, 2010). To achieve this goal, different synthetic molluscicidal compounds were used (Essawy et al., 2009; Kristoff et al., 2010).

The high costs of synthetic molluscicides, their toxicities to non-target organisms and even man as well as the complex organization required in their application, are a major setback to their continued use in schistosomiasis and fascioliasis control programmes. A potential cost effective alternative is the use of compounds from plant origin (WHO, 1993, 2003). Many plants have been screened for their intrinsic molluscicidal properties in an attempt to find an alternative to synthetic ones. Plants containing alkaloids and saponin are among the most promising plants for controlling schistosomiasis (El-Ansary et al. 2003, Silva et al. 2006; Singh and Singh, 2009; Singh et al., 2010).

Based on these facts and in view of extending problem of schistosomiasis and fascioliasis in terms of morbidity, mortality, treatment cost, it was decided to study three medicinal plant species namely: Euphorbia aphylla, Ziziphus spina-christi, and Enterolobium contortisiliquum for their molluscicidal activity against Biomphalaria alexandrina and L. cailliaudi (nalanatensis) according to WHO, 1965 guidelines.

1) Molluscicidal activity of Euphorbia aphylla:
Euphorbiaceae is one of the largest families of...
flowering plants. Members are widely distributed all around the world and some of which are yet to be identified (Sing and Sing, 2010). Molluscicidal activity is widespread in the family Euphorbiaceae, although activity varies greatly from species to species (Al-Zanbagi, 2005, Sharma et al., 2009).

The present study demonstrated that the ethanol extract of Euphorbia aphylla possesses molluscicidal activity. These results are in harmony with Mello-Silva et al. (2006), Bakry (2009) and Sharma et al. (2009) who revealed the molluscicidal activity of different Euphorbia species with varying degrees of potency.

In the present study, The LC_{90} and LC_{50} values of Euphorbia aphylla against B. alexandrina are promising in comparison with some previously studied related plants as Euphorbia gymnoclada which did not show a molluscicidal effect against B. glabrata (Silva et al., 1971). Euphorbia schimperiana and Euphorbia helioscopia caused no mortality up to 100 ppm. on Biomphalaria pfeifferi (Al-Zanbagi 1999). Aqueous extract from Jatropha curcas L. (Euphorbiaceae) performed poorly against snails transmitting Schistosoma mansoni as 500 ppm caused 50% mortality (Rug and Ruppel, 2000).

Also this activity is better than Atriplex stylosa, Guayacum officinalis and Calatropis procera with LC_{50}, ranging from 180 to 360 ppm. against Egyptian B. alexandrina. On the other hand this activity is lower than that E. splendens (LC_{90} 73 and LC_{50} 40 ppm) (Bakry 2009). These differences in potency can be attributed to several factors including the locality of the plant species, time of collection of the plant sample, part used, storage conditions, method of extraction and solvents type (Brackenbury and Appleton, 1997 and Hassan et al., 2010).

The current study was extended to prove the molluscicidal effect of ethanol extract of Euphorbia aphylla on L. cailliaudi after 24 hours exposure. The LC_{50} and LC_{90} were 0.66 ppm and 0.88ppm respectively. This activity is higher than the latex of E. hirta against Lymnaea acuminata (LC_{50} 1.29 ppm) (Yadav and Singh, 2011). Also this activity is much higher than that of Commiphora molmol oil (LC_{50} and LC_{90} 50 and 85 ppm respectively) (Allam et al. 2001), Phytolacca dodecandra (Endod) (LC_{50} 2.8 ppm) (Yohannes et al., 1979) and Meryta denhamii (LC_{50} 26.4 and LC_{90} 70.8 ppm) (Hassan et al., 2010) against Lymnaea cailliaudi.

It worth mention that Commiphora molmol (Myrrh) is a plant recommended as safe molluscicides(Massoud et al.,2004 , Al mathal and Fouad.,2006) and has been licensed for medical use in Egypt and several countries as a fasciolicidal and schistosomicidal drug with high efficacy and safety(Aly and Aly,2006 and Abdul-Samie et al., 2010). Also Phytolacca dodecandra is the best studied plant molluscicide (Esser et al., 2003).

In the present study, Lymnaea cailliaudi has been found to be more susceptible than Biomphalaria alexandrina to the toxic action of Euphorbia aphylla with the latter requiring high concentrations as lethal doses when compared with the first species. This observation is in accordance with the findings of other investigators using other molluscicides (Allam et al., 2001 and Hassan et al., 2010). The difference in susceptibility of the two snails to the lethal effect of the same extract could be attributed to the natural resistance of different snail’s genera and that the molluscicides may vary in their toxicological effects according to the species of the snails’ used (Bakry and Hamdi, 2007 and Osman et al., 2007).

Beside its remarkable molluscicidal potency, Euphorbia aphylla also presents some very interesting characteristics for an ideal plant molluscicide. It is cosmopolitan and perennial plant. It is not edible to animals and easily cultivable (its multiplication is done by means of asexual reproduction which does not require frequent watering or application of pesticides or fertilizer) (Baptista et al., 1997 and Brickell, 2008).

2) Molluscicidal activity of Ziziphus spina-christi:

In the present study, the LC_{50} and LC_{90} of ethanol extract of Ziziphus spina–christi against L. cailliaudi after 24 hours exposure were 311 and 500 ppm respectively. This activity is much higher than that reported for the Egyptian plant, Ambrósia maritima (damsissa) (LC_{90} 3000 ppm) against Egyptian Lymnaea cailliaudi (Abou Basha et al., 1994).

In the present study, based on the LC_{50} and LC_{90} values, Ziziphus spina-christi demonstrated less potent molluscicidal activity than Euphorbia aphylla against Lymnaea cailliaudi which can be attributed to the differences in each plant active ingredients, their mode of action and method of penetration of the snails (Rawi et al., 1996).

In the present study, Ziziphus spina–christi gave negative results up to 1000 ppm on B. alexandrina. These results reconfirmed that Lymnae cailliaudi is more sensitive. The possibility for the same plant extract to have molluscicidal activity against certain snail species and absence of activity against other species were recorded by Yasuraoka et al. (1980) who found that the seeds of Jatropha carcas (Family Euphorbiaceae) have a relatively high toxicity against Oncomelania while it showed no effect against Lymnaea snails.

3) Molluscicidal activity of Enterolobium contortisiliquum:
In the present study, Enterolobium contortisiliquum is selected due its richness in saponins (Mimaki et al., 2004). Saponins have haemolytic properties and toxic effect on most cold-blooded animals including snails and are proved to have molluscicidal activity (Herlt et al., 2002, Osman et al., 2007 and Singh and Singh, 2009).

In the present study, failure of Enterolobium contortisiliquum fruits to produce molluscicidal activity on both snail species up to 1000 ppm. could be attributed to the fact that saponins responsible for its activity are extracted in greater measures with more polar solvents. Supporting this explanation the results obtained by Hassan and Abdel-Rahman (2008) who found that the butanol fraction of Hedera canariensis (family Araliaceae) has molluscicidal activity against Biomphalaria alexandrina and Lymnaea caulliaudi. While ethyl acetate extract of the same plant was inactive. On the contrary, Hassan et al (2010) found that the butanol fraction of Meryta denhamii flowers which belongs to the same plant family was inactive and ethyl acetate was active against the same snail species.

Conclusion:

The use of Euphorbia aphylla may play vital role in controlling schistosomiasis and fascioliasis. The plant is commonly available, easy to collect and prepare for use. Therefore it is the most suitable for biological application which offers a potentially simple, readily available and inexpensive molluscicidal agent of plant origin. In future, more attention should be paid to the mechanism of action of Euphorbia aphylla on molluscs and application techniques for its use as plant molluscicides in rural communities. Phytochemical investigations to identify the bioactive ingredient(s) responsible for the molluscicidal potency are recommended. Toxilogical studies on man, fauna and flora of the fresh water are needed to conclude about the possible toxics properties of the ingredient(s).

Results of Ziziphus spina- christi suggests further laboratory tests to search for the presence of active component in the different parts o the plant. Such studies would increase their potential for future use as plant molluscicides.

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