Evaluation of Schistosoma mansoni cercaricidal activity of Solamargine a steroid glycoalkaloid from Solanum syzybrilifolium

Jean-Jacques Bagalwa, Laurence Voutquenne-Nazabadioko, Charlotte Sayagh, Augustin Bashwira, Jean-Pierre B Baluku

▶ **To cite this version:**

Jean-Jacques Bagalwa, Laurence Voutquenne-Nazabadioko, Charlotte Sayagh, Augustin Bashwira, Jean-Pierre B Baluku. Evaluation of Schistosoma mansoni cercaricidal activity of Solamargine a steroid glycoalkaloid from Solanum syzybrilifolium. International Journal of Engineering Research and General Science, 2014. hal-02385491

HAL Id: hal-02385491
https://hal.univ-reims.fr/hal-02385491

Submitted on 2 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Evaluation of Schistosoma mansoni cercaricidal activity of Solamargine a steroid glycoalkaloid from Solanum syzybrilifolium

Jean-Jacques M. Bagalwa[1], Laurence Voutquenne-Nazabadioko[2], Charlotte Sayagh[2], Augustin S. Bashwira[1], Jean-Piere B. Baluku[1]

[1]Laboratory of Malacology, Department of Biology, Centre de Recherche en Sciences Naturelles (CRSN) at Lwiro, Democratic Republic of Congo. C/o. Petit Séminaire de Mugéri, P.O.Box 02, Cyangugu, Rwanda

[2]Groupe Isolement et Structure, Institut de Chimie Moléculaire de Reims (ICMR), CNRS UMR 6229, Bat.18, BP 1039, 51687 Reims Cedex 2, France

ABSTRACT - The cercaricidal activity of a mixed solamargine (1) and β-solamarine (2) solution were directly tested against *Schistosoma mansoni* cercariae and a time-concentration relationship was observed; the concentrations needed to kill all cercariae (LC_{100}) within 10 min of exposure were 0.01 mg/mL. Mixed solamargine (1) and β-solamarine (2) solution have a high level of cercaricidal activity against free swimming cercariae and it seems to be ecologically safe, since it is known to have very low toxicity to fish. The possible use of such sublethal concentrations in schistosomiasis transmission sites as an oriented promising technique to control this parasite and to minimize or prevent water pollution with pesticides.

Introduction

Schistosomiasis, a disease of various mammals including man, is caused by blood flukes of the genus *Schistosoma* (Brown, 1980). It is still a major helminth infection in the world at the beginning of the 21st century and an important public health problem in many tropical and bubtropical countries. Schistosomiasis is one of the major communicable diseases and it is second after the malaria in socio-economic and health importance in the developing countries (Bergquist and Colley, 1998; Larhsini *et al.*, 2010). It is endemic in 74 countries in the world (WHO, 1985), affects 200 million people, expose 800 million to the risk of the infection (Gryseels, 1991; Capron and Capron, 2002; Bilia *et al.*, 2000).

Controlling of the snail intermediate hosts of this disease by molluscicides (synthetic and/or of natural origin) is still one of the most promising means (WHO, 2009; Bagalwa *et al.*, 2010; Mahmoud *et al.*, 2011; Adewumi *et al.*, 2013). There are different kinds of schistosomiasis, but in all cases, the life cycle involves in the aquatic snails. The parasite multiplies into hundreds of cercariae in the snail and that can penetrate the intact human skin who is exposed to infected waters after leaving the snails (Marston and Hostettman, 1993). Chemotherapy is a general strategy for schistosomiasis control; but another more interesting strategy is to interrupt the disease vital cycle by snail's or cercariae's elimination.

For the control of snail-borne diseases, several synthetic compounds were developed such as copper sulfate, sodium pentachlorophenate, sulphonated hydrocarbons, tributylin fluoride. Only niclosamide (marketed as Bayluscide; Andrews *et al*, 1983) is widely used in control programs (Perrett and Whitfield, 1996). On the other hand, there is probability that some resistance to niclosamide can be induced under extreme conditions of genetic selection of the snails (Sulivan *et al*, 1984). Therefore, the potential use of plants for the biological control of the intermediate hosts of human schistosomiasis and other snail - transmitted parasitic infections has received a considerable attention (Medina and Woudbury, 1979; Kloos and McCullough, 1985; and Kloos *et al.*, 1985

and Perrett and Whitfield, 1996). The use of plants with molluscicidal properties is simple, inexpensive, and appropriate for the local control of the snail vector (Marston and Hostettman, 1993).

The phytochemical investigation of *Solanum szybrilifolium* (Solanaceae), species plants known to synthesize steroidal alkaloids and spirostane derivatives and some of which has shown the molluscicidal activity of plant (Wanyonyi *et al.*, 2003). But its cercaricidal activity is not known until now. In our study, the methanolic extract of the fruit of *Solanum szybrilifolium* was fractionated and the biological activity of one fraction (B) was tested against cercaria produced by snails infested by *Schistosoma mansoni*. Two known compounds, solamargine (1), a chacotriose solasodine, and β-solamarine (2), a chacotriose tomatidenol, isolated from the active fraction B were reported for the first time in *Solanum szybrilifolium* (Bagalwa *et al.*, 2010). This fraction B has been presented as potential molluscicide in the future snail control programs with a careful monitoring on the environmental damaging effects, especially when indigenous populations use this plant for fishing during the dry season (Bagalwa *et al.*, 2010).
In the present work, we describe the cercaricidal activity of the fraction B isolated in *Solanum szybrilifolium* collected in East part of the Democratic Republic of Congo. The result of such study may provide cheap, locally produced, biodegradable and effective control agents against schistosomiasis in rural areas of developing countries where this disease is endemic.

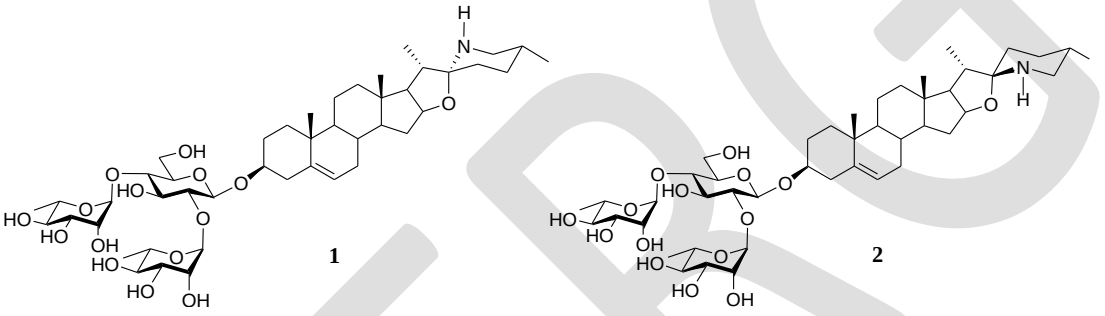

Figure 1. Structure of solamargine (1) and β-solamarine (2)

Material and Methods

Cercariae obtain

Schistosoma mansoni cercariae were obtain from snail *Biomphalaria pfeifferi* collected in the streams located at Lwiro (Kalengo and Birunga) using plankton net and kept in the laboratory of Malacology of the Centre de Recherche en Sciences Naturelles (Bagalwa and Baluku, 1998). In fact, snails were put in container with deionised water and exposed to artificial light for 3 h and the cercariae that released from snails in this period were concentrated by their phototropism on the top of a container made black on the base. The cercariae that were collected from the container by graduated wells (0.3 mL) and had the same emergence age (Helmy *et al.*, 2007; Medina *et al.*, 2009).

Extraction and isolation of the steroidal alkaloids Saponin

The dried and powdered fruit (300 g) was extracted with 96% methanol. The MeOH extract was concentrated and precipitated with acetone. Then, the crude saponin precipitate was dialyzed to give a saponin rich extract (2.69 g). This extract (2.5 g) was fractionated by VLC on reversed phase C_{18} (MeOH–H_2O, 60:40, 70:30, 80:20 and 100:0, each 300 ml) to give fraction A (1.52 g), B (680 mg), C (277 mg) and D (58 mg), respectively. Fraction A was repurified by RP-18 VLC (MeOH–H_2O, 40:60, 60:40, 80:20 and 100:0, each

200 ml) to give fraction A1 (1.08 g), A2 (64 mg), A3 (116 mg) and A4 (212 mg). A part of fraction B (100 mg) was chromatographed on silicagel CC (4 g) eluted with a gradient of $CHCl_3$–MeOH–H_2O (70:30:1 to 70:30:2) to give 1 (20 mg). A part of fraction C (70 mg) was purified by silicagel CC using a gradient of $CHCl_3$–MeOH (9:1 to 7:3) followed by preparative TLC on silicagel with $CHCl_3$–MeOH–H_2O (70:30:5) as eluant, to give solamargine (1) (13 mg) and β-solamarine (2) (5.8 mg).

Preparation of dilution and Cercaricidal activity

A series of concentrations of mixed solamargine solution was prepared on basis of weight/volume using deionised water. Therefore, 0.6 mg of mixed solamargine was dissolved in 10 mL of deionised water. Six concentrations were made by dilution with deionised water (0.06; 0.03; 0.01; 0.001; 0.0001 and 0.00001 mg/mL). A standard was made with 0.1 mg of digitonine in 100 mL of distilled water (0.0001 mg/mL) (Bagalwa *et al.*, 2010). Approximately 20-30 freshly emitted cercariae were placed in each well and three wells for each concentration were tested (using different gradual concentrations) and it is the same for the positive (digitonine) and negative (distilled water) control. During the exposure period, the cercariae were observed under a binocular loupe after 10, 20, 30, 40, 50 and 60 minutes. The observation was made on the cercarial movement and mortality recorded at successive intervals of time. 50 ml of deionised water containing 20-30 fresh cercariae were used as negative control (Ritchie *et al.*, 1974; Medina *et al.*, 2009) and another group of cercariae was exposed to digitonin as a positive control. Results were expressed in percentage in terms of destruction of the cercariae. The effectiveness of the tested isolated extract on *Schistosoma mansoni* cercariae was determined (Litchfield et Wilcoxon, 1949). The collected data was computerized to give LC_{00}, LC_{50}, and LC_{100} values determined by probit analysis.

Results

Compound **1** and **2** (Figure 1) at the concentration going from 0.01 mg/mL to 0.06 mg/mL, presents a high activity against *Schistosoma mansoni* cercariae, killing the totality of the cercariae (100 %) in 10 minutes (Table 1).

Table 1. Cercaricidal activity of mixed solamargine (1) and β-solamarine (2) solution isolated from *Solanum syzybrilifolium* according to the time of exposure

Extrait	Concentration tested (mg/mL)	Mortality rate (%) of cercariae after exposure period (minute)					
		10	20	30	40	50	60
Solarmagine and β-solamarine	0.06*	100	100	100	100	100	100
	0.03	100	100	100	100	100	100
	0.01	100	100	100	100	100	100
	0.001**	19±13	31±5	50±0	61±5	61±5	86±5
	0.0001	0	0	0	11±5	11±5	14±5
Control deionised water		0	0	0	0	0	0

Control digitonin	0.001	100	100	100	100	100	100
	0.0001	0	0	0	0	0	0

Legend: * Lethal concentration of fish (LC_{100})
** No effect to fish (LC_{00})

The mortality rate of cercariae varied with time and concentration of the solamargine rate. From concentration 0.06 mg/mL to 0.01 mg/mL the mortality rate is 100 % equivalent to the concentration of 0.001 for the digitonin. At the concentration of 0.001 mg/mL the mortality rate increased with the time of exposure as shown in figure 2.

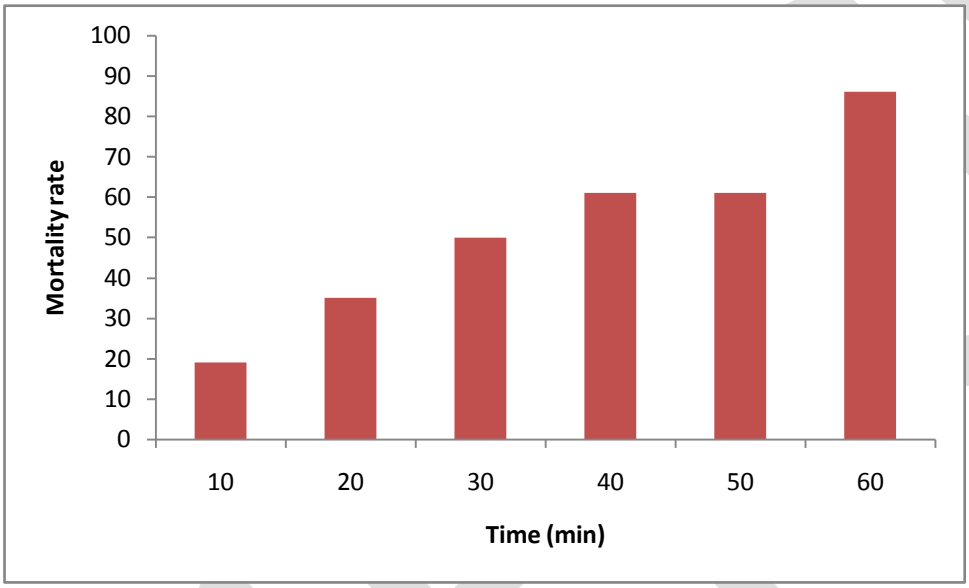

Figure 2. Mortality rate (%) of cercariae at the concentration of 0.001 mg/mL of solamargine (1) and β-solamarine (2) extract from *Solanum syzybrilifolium* according to the time of exposure (min)

The LC_{00}, LC_{50} and LC_{100} of the cercaricidal activity at different time of exposure is presented in table 2.

Table 2. Cercaricidal activity (mg/mL) at different time of exposure

Lethal Concentration	Concentration (mg/mL) after exposure period (minute)					
	10	20	30	40	50	60
LC_{00}	0.01	0.01	0.01	0.01	0.01	0.01
LC_{50}	0.0047	0.0048	0.0037	0.0033	0.0031	0.0025

LC_{100}	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

The LC_{50} of the solamargine decreased with the time of exposure. But the LC_{100} and LC_{00} doest not varied in time.

The relationship between the mortality rate and the time of exposure is present in the figure 2.

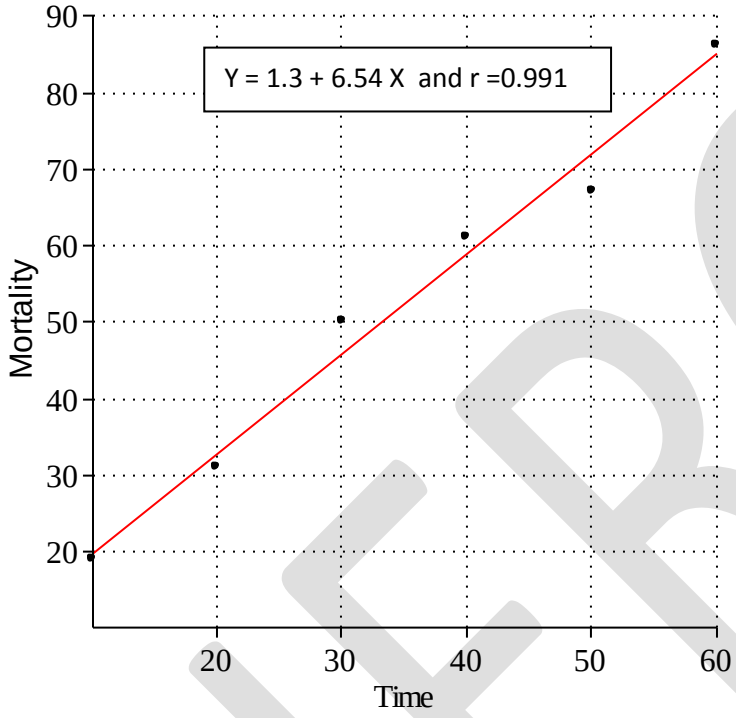

Figure 2. Correlation relationship between the mortality rate (%) and the exposure time (minute) of cercariae again extract at 0.001 mg/L.

This figure shows a positive correlation between mortality rate and the time of exposure of cercariae at the concentration of 0.001 mg/mL.

Discussion

Biomphalaria pfeifferi, known as intermediate host of *Schistosoma mansoni* is widely distributed in tropical region. *Biomphalaria pfeifferi* larvae lethality assay is considered to be the most useful for the preliminary assessment of general activity, and the toxicity bioassays have show correlation with some cytotoxic and pesticide activities (Harborne and Dey, 1991; Shoeb *et al.*, 2007; Subhan *et al.*, 2008). In this work, the bioassays were performed to evaluate the toxicity of Solamargine extract in *Solanum szybrilifolium* against *Schistosoma mansoni* cercariae in vitro. All *Solanum szybrilifolium* extracts and the isolated compound, solarmagine, exhibited high

molluscicidal activity and low lethality against non-target species (fish and macroinvertebrate) (Bagalwa *et al.*, 2010). The lethal concentration of solamargine (1) and β-solamarine (2) solution again fish with 100 % of lethality was 0.06 mg/L (100 %) in laboratory conditions. At this concentration this concentration the mortality of cercariae was also 100 %. Using the extract concentrations that kill 100 % of fish, total death of *Schistosoma mansoni* cercariae was recorded. 50 % death of cercariae was remarked after 1/2 hour of exposure to concentrations of 0.001 mg/mL. Moreover, cercariae exposed to extract concentrations 0.01 mg/mL were completely killed after 10 minutes. This lethal concentration (0.01 mg/mL) is better than the lethality of the extracts of *Croton floribundus* (0.4 mg/mL) observed in laboratory experiment (Medina *et al.*, 2009; Kamel *et al.*, 2010) and for *Calendula micrantha* witch killed 100 % of cercariae by 100 ppm dry powder within 2 and 24 h of exposure (El-Emam *et al.*, 1986). The same result was observed when cercarial are tested against *Furcraea selloa marginata* and *Bacillus thuringiensis kurstaki* (Osman *et al.*, 2011). The high activity of the mixed solamargine (1) and β-solamarine (2) solution are due to the possible synergism within the two compounds and between different glycoalkaloid types under natural conditions as proved by others authors (Wanyonyi *et al.*, 2002; Roddick *et al.*, 2001; Al Chami *et al.*, 2003; Ikeda *et al.*, 2003). For extracts of the Arabian or Somali gum *Commiphora molmol* (family: Burseraceae), Masoud *et al.*, (2000) found that the oleo - resin extract showed a more pronounced cercaricidal potency than the oil. Total death of cercariae was remarked after 1/4 h of exposure to 10.5 and 2.5 ppm. This concentration is high than the concentration of solamargine obtains in this study. At the concentration of 0.001 mg/mL the mortality rate increased with the time of exposure (Figure 2). The LC_{50} of the cercaricidal activity decreases with the time of exposure (Table 2).

The relationship between the mortality rate and the time of exposure (Figure 2) shown a positive linear correlation ($Y = 1.3 + 6.54 X$ and $r = 0.991$). Ahmed and Ramzy, (1997) observed that the cercaricidal properties of water extract of the leaves of *Solanum nigrum* were directly tested against *Schistosoma haematobium*, *Schistosoma mansoni* and *Fasciola gigantica* cercariae and a time-concentration relationship was observed; the concentrations needed to kill all cercariae (LC_{100}) within 10 min of exposure were 0.01 mg/mL for *Schistosoma mansoni* cerariae and 0,001 mg/mL for digitonin.

Mixed solamargine (1) and β-solamarine (2) solution have a high level of cercaricidal activity against free swimming cercariae. Any cercaria which is not killed by the application may be so attenuated that it becomes either unable to infect humans or fail to mature and cause significant pathology in those who they do infect as observed in others works (Hilal *et al.*, 1989; Perrett *et al.*, 1994; Ahmed and Ramzy, 1997). On the other land, it seems to be ecologically safe, since it is known to have very low toxicity to fish (Bagalwa *et al.*, 2010). Saponins affect surface tension due to their froth-forming ability. The haemolytic activity of saponins is attributed to their formation of complexes with cholesterol in red blood cell membranes, which causes a collapse of the cell and the release of haemglobin (Hostettmann and Marston, 1987; Adewumi *et al.*, 2013). The molluscicidal activities of saponins have been shown to vary with the position of the glycoside chain (mono and bidesmosidic differences), nature of the sugar chains, the sequence of the sugars, the interglycosidic linkages and the substitution patterns of the aglycone (Hostettmann and Marston, 1987). Than saponins seems to hold the greater promise for the control of snail vectors schistosomiasis and cercariae.

Conclusion

It is concluded that cercarial sublethal concentrations from molluscicides (synthetic or of plant origin) that may be present in water bodies during schistosomiasis control operations, could be of great value for attenuation of cercarial infectivity to the final host. This study throws light, also, upon the possible use of such sublethal concentrations in schistosomiasis transmission sites as an oriented promising technique to control this parasite and to minimize or prevent water pollution with pesticides.

Acknowledgements

The authors are grateful to the laboratory staff of the Department of Biology of Lwiro (DRCongo) and Pharmacognosy of UMR 6229 CNRS (France) for respectively collecting material and fractionation of material.

REFERENCES:

1. Adewumi A.A.J., Aina V.O., Zhang C.S., He Z., 2013. Assessment of the Molluscicidal Activities of Sasanqua Saponin. Current Research Journal of Biological Sciences, 5, 1, 1-4.
2. Ahmed, A. H., Ramzy, R. M. R., 1997. Laboratory assessment of the molluscicidal and cercaricidal activities of the Egyptian weed, *Solanum nigrum* L. Ana Trop. Med. Parasit, 91, 8, 931-937.
3. Al Chami L., Mensez R., Chataing B., O'Callaghan J., Usubillaga A., LaCruz L., 2003. Toxicological effects of α-solamargine in experimental animals. Phytotherapy Res , 17, 254–258.
4. Andrew P., Thyssen J., Lorke D., 1983. The biology and toxicology of molluscicides, Bayluscide. Pharmacol and Therap., 19, 245-295.
5. Bagalwa M., Baluku B., 1998. Variations mensuelles du taux d'infestation et du potentiel de transmission de *Biomphalaria pfeifferi* (Gasteropode, Planorbidae) dans deux systèmes aquatiques de la République Démocratique du Congo. Méd Trop. 58, 372 – 374.
6. Bagalwa M., Voutquenne-Nazabadioko L., Sayagh C.; Bashwira S., 2010. Evaluation of the biological activity of the molluscicidal fraction of *Solanum sisymbriifolium* against non target organisms, Fitoterapia, 81, 767 - 771.
7. Bergquist N.R., Colley D.G., 1998. Schistosomiasis vaccines: research to development, Parasitol. Today 14, 99–104.
8. Bilia A.R., Braca A., Mendez J., Morelli I., 2000. Molluscicidal and piscicidal activities of Venezuelan Chrysobalanaceae plants. Pharmacol Lett 66, 53-59.
9. Brown DS. 1980. Freshwater snails in Africa and their medical importance. Taylor and Francis Ltd., London.
10. Capron A, Capron M., 2002. Vaccine development against schistosomiasis from concepts to clinical trials. Br. Med Bull., 62, 139-148.
11. El-Emam M.A., Shoeb H.A., Ebid F.A., Refai L.A., 1986. Snail control by *Calendula micrantha*. J. Egyp. Soc. Parasitol. 16, 2, 563–571.
12. Gryseels B., 1991. The epidemiology of schistosomiasis in Burindi and its consequences for control. Trans. R. Soc. Trop. Med. Hyg, 85, 626 – 633.
13. Harborne J.B., Dey P.M., 1991. Methods in plant biochemistry. Assays for bioactivity. Academic press, London, 6, 8-10.
14. Hilal S. H., Aboutabl E. A., Yousif F., 1989. Molluscicidal and cercaricidal properties of *Anagallis arvensis* L. ssp arvensis. Egypt. J. Bilh., 70, 1, 1-9.
15. Ikeda T., Tsumagari H., Honbu T., Nohara T., 2003. Cytotoxicity of steroidal glycosides from Solanum plants. Biol Pharm Bull., 26, 1198–1201.
16. Kamel E.G., El-Emam M.A., Mahmoud S.S.M., Fouda F.M., Bayaumy F.E., 2010. Attenuation of *Schistosoma mansoni* cercarial infectivity to albino mice by methanol extract of some plant species. Pesticide Biochemistry and Physiology 98, 342–348.
17. KIoos H., McCuIlough F. S., 1985. Ethiopian plants with proven and suspected molluscicidal activity: a new approach in plant evaluation J. Trop. Med. Hyg., 88, 189-196.

18. Kloos H., Urn, K. C., Heyneman D. and McCuIlough F. S., 1985. Preliminary screening of some Egyptian and Ethiopian plants for molluscicidal activity and observations on a possible method for natural snail control. J. ParasitoL, 71, 228-231.
19. Larhsini M., Sebbane R., Kchakech H., Markouk M., Bekkouche K., Abbad A., El Abdouni khayari M., Sterner O., 2010. Screening of some Moroccan plant extracts for molluscicidal Activity. Asian J. Exp. Biol., 1, 4, 964-967.
20. Litchfield J.T., Wilcoxon E., 1949. A simplified method of evaluating Dose-effect experiments, J. Pharmacol. Exp. Therap., 96, 99–113.
21. Mahmoud M. B., Ibrahim W. L., Abou-El-Nour B. M.,. El-Emam M. A, Youssef A. A., 2011. Biological and biochemical parameters of *Biomphalaria alexandrina* snails exposed to the plants *Datura stramonium* and *Sesbania sesban* as water suspensions of their dry powder. Pesticide Biochemistry and Physiology, 99, 96–104.
22. Marston A., Hostettmann K., 1993. Search for antifugal, molluscicidal and larvicidal compounds from African medicinal plants. J Ethnopharmacol., 38, 215-223.
23. Masoud A. M., Fawzy S. M., Salama O. M., 2000. Laboratory studies on the molluscicidal and cercaricidal activities of *Commiphora molmol*. Egypt J. Aquat Biol A Fish., 4, 2, 251-26
24. Medina F. R., Woodbury R., 1979. Terrestrial plants molluscicidal to Iymnaeid hosts of *Fascioliasis hepatica*. in Puerto Rico. J. Agrig. Univ. of Puerto Rico, 63, 366-376.
25. Medina J. M., Peixoto J. L. B., Silva A. A., Haraguchi S. K., Falavigna D. L. M., Zamuner M. L. M., Sarragiotto M. H., Vidotti G. J., 2009. Evaluation of the molluscicidal and *Schistosoma mansoni* cercariae activity of *Croton floribundus* extracts and kaurenoic acid. Brazilian Journal of Pharmacognosy, 19, 1B, 207-211.
26. Osman G. Y., Mohamed A. M., Kader A. A., Mohamed A. A., 2011. Biological studies on *Biomphalaria alexandrina* snails treated with *Furcraea selloa marginata* plant (family: Agavaceae) and *Bacillus thuringiensis kurstaki* (Dipel-2x). Journal of Applied Pharmaceutical Science, 1, 10, 47-55.
27. Perrett S., Whitfield P. J., 1996. Currently available molluscicides. Parasitology Today, 12, 156-159.
28. Perrett S., Whitfield P. J., Bartlett A., Sanderson L., 1994. Attenuation of *Schistosoma mansom* cercariae with a molluscicide derived from *Millettia thonningu.* Parasitology, 109, 559-563.
29. Ritchie L.S., Lopez V.A., Cora J.M., 1974. Prolonged application of an organation against *B. glabrata* and *S. mansoni* in molluscicides in Schistosomiasis control, Edt. Cheng T.C, Academic Pres-, New York and London, 77–88.
30. Roddick J.G.,Weissenberg M., Leonard A.L., 2001. Membrane disruption and enzyme inhibition by naturally-occuring and modified chacotriose-containing Solanum steroidal glycoalkaloids. Phytochemistry, 56, 603–610.
31. Shoeb M., MacManus S.M., Jaspars M., Kong-Thoo-Lin P., Nahar L., Celik S., Sarker S.D., 2007. Bioactivity of two Turkish endemic *Centaurea* species, and their major constituents. Rev Bras Farmacogn., 17, 155-159.
32. Subhan N., Alam M.A., Ahmed F., Shahid I.J., Nahar L., Sarker S.D., 2008. Bioactivity of *Excoecaria agallocha*. Rev Bras Farmacogn., 18, 521-526.
33. Sulivan J. T., Cheng T. C., Rowland K. H. 1984. Milotic responses of the anterior pericardial wail of *Biomphalaria glabrata* (Mollusca) subjected to challenge. J. Inv. Path., 44, 114- 116.
34. Wanyonyi A.W., Chhabra S.C., Mkoji G., Eilert U., NjueW., 2002. Bioactive steroidal alkaloid glycosides from *Solanum aculeastrum.* Phytochemistry, 59, 79–84.
35. Wanyonyi A.W., Chhabra S.C., Mkoji G., Njue W., Tarus P.K., 2003. Molluscicidal and antimicrobial activity of *Solanum aculeastrum.* Fitoterapia, 74, 298–301.
36. WHO, 1985. The control of schistosomiasis. WHO Technical Report, Series No. 728, Geneva.

37. WHO, 2009. The control of schistosomiasis, Technical Report Series No. 922, Geneva.

IJERGS