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Electrophoretic profile of treated *Lymnaea natalensis* snails with *Pterocladia capillacea, Jania rubens* and *Ulva lactuca* algal extracts

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ABSTRACT

Marine algae have shown to be the most promising sources of new bioactive compounds against different snails transmitting parasites. In the present study three species of marine algae. *Ulva lactuca* (*U. lactuca*) , *Pterocladia capillacea* (*P. capillacea*) and *Jania rubens* (*J. rubens*) extracts having Lc_{50} values 121, 111.3 and 127 ppm respectively, have been evaluated to select the most potent one as molluscicides against *Lymnaea natalensis* (*L. natalensis*) snails for controlling fascioliasis. The protein content for *L. natalensis* snail tissues after treatment with *U. lactuca*, *P. capillacea* and *J. rubens* extracts was 243.6± 0.03. 196.6 ± 0.03 and 280.3 ± 0.05 µg/ml respectively and there was a significant decrease in protein contents of the treated snail tissues than controlled ones. The electrophoretic separation of snail tissues treated with mentioned algal extracts using Sodium Dodocyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), revealed several bands for each algal extract ranged from 21 to 205 kDa. The alteration in electrophoretic profile of treated snails includes appearance of new protein bands, disappearance of bands and change in the concentrations of shared bands with control snails. Based on these alterations, it could be concluded that the algal extracts have molluscicidal effect on *L. natalensis* snails.

INTRODUCTION

Aalgae , were shown early in the 20th century to contain a high number of so- called secondary metabolites, i.e. low molecular-weight organic compounds biosynthesized by the plant but not essential to its growth and survival but essential for its interaction with other organisms (Brimer, 2011). Marine organisms as red, green and brown algae have

shown to be one of the most promising sources of new bioactive compounds. (Blunt *et al.* 2014).

SDS-PAGE was used to separate tissue proteins of control and trematode-infected *B. alexandrina* snails. The separated profiles demonstrated the occasional appearance of protein fractions and the remarkable increase of concentration of certain molecular masses in infected snails at one week interval over four weeks post exposure to *Schistosoma mansoni* (El-Ansary *et al*., 2000). Electrophoresis of haemocyte homogenates of both non-infected and infected *B. alexandrina* snails with *S. mansoni* yielded a complex pattern of polypeptides (Sadaka *et al*. 2016).

Haemolymph protein concentrations were measured by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of infected and none infected *Lymnaea gedrosiana* with *xiphidiocercaria* larvae. Mean of protein concentration of haemolymph plasma showed an extra protein band (70 kDa). The results showed a significant difference between the amounts and the kinds of proteins in hemolymph of infected and none infected snails (Yaraghi *et al.* 2011). Treatment hemolymph of *B. alexandrina* snails with *Azadirachta indica* extract showed a decrease of protein contents in soft tissues when compared with the control group (Bakry *et al*. 2012). Hemolymph samples of the infected *L. stagnalis* with *Opisthioglyphe ranae* trematode or uninfected were collected during third and fourth weeks of rearing. The quantity of total proteins of the infected *L. natalensis* snails was near twice higher than in those uninfected. A higher share of 70 KDa proteins in infected than in uninfected (Dmochowska *et al*. 2013). The qualitative and quantitative effects of ethanol extracts from three local plants, namely *Euphorbia splendens* (Euphorbiaceae), *Ziziphus spina-christi* (Rhamnaceae) and *Ambrosia maritime* (Asteraceae) on the protein content of digestive gland of uninfected and infected vectors of schistosomiasis, *B. alexandrina* and *B. truncatus were evaluated*. The results of electrophoretic pattern of total protein showed differences in number and molecular weights of protein bands (Abdel-Haleem, 2013).

In Egypt, *Lymnaea natalensis* (*L. natalensis*) snail, has received a great attention because of its role in transmission of fasciolosis in man and animals. The snail control is considered not only complementary but essential in breakage *Fasciola* life cycle (Shaapan *et al.* 2015). Saad *et al.* (2019) made preliminary experiments on aqueous and ethanol extracts of four algal species of marine algae; *Pterocladia capillacaea* (*P. capillacaea*), *Jania rubens* (*J. rubens*), *Ulva lactuca* (*U. lactuca*), and *Enteromorpha intestinalis* (*E. intestinalis*) were tested for their lethal effect on *Lymnaea natalensis* (*L. natalensis*) snail, the intermediate host of the trematode parasite; *Fasciola gigantica,* (*F. gigantica*) . They decided the promising molluscicidal activity on *L. natalensis* snail. *P. capillacaea* and *J. rubens* aqueous extracts and *U. lactuca* ethanolic extract are the most potent and highly significant ones in the net reproductive rate and reduction.

The present study aims to determine the protein characterization of the potent marine algae extracts (*Ulva lactuca*, *Pterocladia capillacea* and Jania *rubes*; green and

red ones against *L. natalensis* snails for controlling fascioliasis. The work extends to include its protein contents and its pattern either quantitatively or qualitatively proved by SDS-PAGE.

MATERIALS AND METHODS

Snails

Lymnaea natalensis snails were collected from Abou Rawash, Giza and kept in aquaria (35 x 25 x 10 cm) filled with 0.5 L dechlorinated tap water and maintained according to Saad *et al*. (2019). The experiments were done in Parasitology and Animal Disease laboratory, National Research Centre, Giza .Egypt.

Potent algal extracts

Three different species of marine algae; one ethanolic extract species belongs to family Chlorophyceae (green algae); *Ulva lactuca* have Lc_{50} value 121 ppm and two aqueous extracts species; *Pterocladia capillacea* and *Jania rubens* have Lc_{50} value 111.3 and 127 ppm respectively and belong to family Rhodophyceae (red algae); were used, the LC_{50} for each aqueous and ethanol algal extract were calculated daily till the end of the experiment according to method adopted by Dahms and Hellio (2009). The period of experiment for snail metabolites established for 1 to 10 days.

Preparation of snail extracts for protein content calculation and electrophoretic studies

Preparation of homogenates

Snails were gently crashed, and their soft parts were dissected out under the microscope. Soft parts of snails were weighed and homogenized individually by glass homogenizer in 1:3 w/v of 0.1 M Tris/HCL buffer (pH=74 with 0.001 M EDTA and 0.001 m.B-mercaptoethanol. The resulting suspension was centrifuged at 14000 r.p.m. for half an hour at cooling temperature giving a clear supernatant which was diluted 1:3 of sample buffer containing the tracking dye (Bromophenol blue) (Timanova *et al*., 2003). Protein content was determined by the method of (Bradford, 1976) using Cary Series UV-Vis Spectrophotometer Apparatus and kept frozen until use as crude extracts.

Sodium Dodocyle Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed in 10% polyacrylamide gels according to Laemmli (1970). Crude extracts were separately mixed with sample buffer containing 2-mercaptoethanol under reducing condition before loading to the gel. After separation, slab gel was stained with commassie brilliant blue dye. Relative molecular weights of bands were calculated using marker supplied by Sigma-Aldrich. Molecular weights and bands intensity were determined using BIO RAD Gel Doc XR+ Apparatus (Toaleb *et al*., 2013).

RESULTS

Protein contents of snail tissues non-trated and treated with aqueous and ethanol algal extracts

The total protein contents in tissues of non-treated and treated *L. natalensis* snails with two aqueous and one ethanol algal extracts were shown in Table 1. Concerning the non-treated snails (control one), the protein content was 670.75±010 µg/ml .The mean value of protein content of snail tissues was 196.6 ± 0.03 and 280.3 ± 0.05 µg/ml after treatment with 300 ppm *P. capillacaea* and *J. ruben* aqueous extracts respectively .The mean value of protein content of snail tissues treated with 300 ppm *U. lactuca* ethanol extract was 243.6± 0.03 µg/ml. The results indicated that there was a significant decrease in protein contents of treated snail tissues compared to controlled ones.

Table 1**:** protein contents of *L. natalensis* after treatment with *P. capillacaea, J. rubens,* and *U. lactuca* algal extracts

Algal extract	LC_{50} **values**	**Mean Number of protein contents (µg/ml) ± SE**
P. capillacea	111.295 ppm	196.6 ± 0.03
aqueous extracts		**
J. rubens	127.128 ppm	280.3 ± 0.05
aqueous extracts		**
U. lactuca	121.273 ppm	243.6 ± 0.03
Ethanol extracts		**
Control	-------	670.93 ± .010
(Non-treated snails)		

** High Significant P ≤0.01

Electrophoretic profile of snail tissues treated with aqueous and ethanoic extracts

The electrophoretic separation of soluble protein from tissues of treated snails with aqueous and ethanoic extracts of the studied algae was displayed in figure 1. The alteration in electrophoretic profile includes appearance of new protein bands, disappearance of bands and change in the concentrations of shared bands with control

snails. Separation of tissue proteins of treated snails with *P. capillacea* aqueous extract revealed 10 bands for 300 ppm and their molecular weights ranged from 21 to 205 kDa. The number of shared bands between treated snails and control was 3 of molecular weights 205,131and 55 kDa .Analysis of tissue proteins of treated snails with *J. rubens* aqueous extract revealed 11 bands for 300 ppm and their molecular weights ranged from 23to 205 kDa.The number of shared bands between treated snails and control was 4 of molecular weights 205,71,55 and 49 kDa .SDS-PAGE analysis of tissue proteins of treated snails with *U. lactuca* ethanol extract revealed 11 bands for 300 ppm and their molecular weights ranged from 22 to 205 kDa .The number of shared bands between treated snails and control was 4 of molecular weights 205, 139.4,58.3 and 45 .

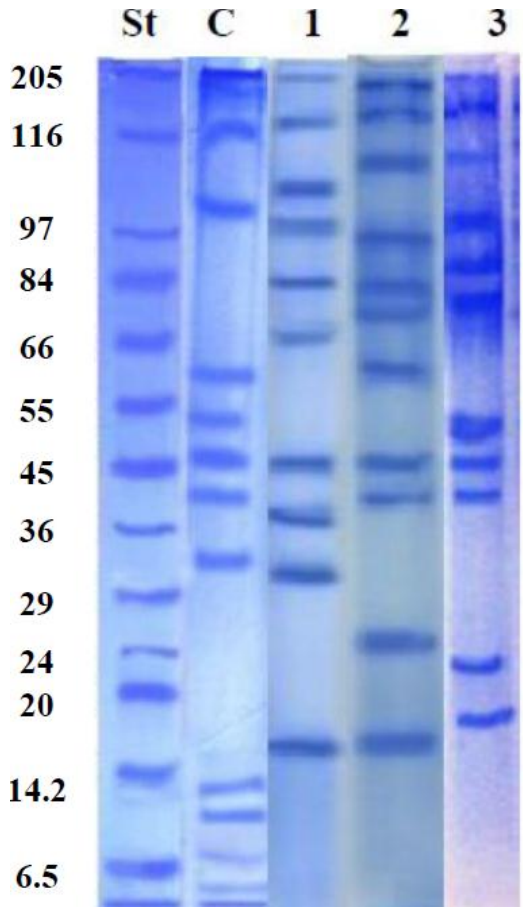

Figure (1): SDS-PAGE showing total protein bands of *L. natalensis* tissues. St: Marker. C: Untreated (Control). Lane 1: Treated with *P. capillacae*. Lane 2: Treated with *J. rubens*, Lane 3: Treated with *U. lactuca*.

DISCUSSION

In the present study there was a significant decrease in protein contents of *L. natalensis* snail tissues treated with different concentrations of *P. capillacaea, J. rubens, U. lactuca* aqueous and ethanol extracts. This result could be one of the modes of action

of molluscicidal effect of active principles of tested algae which is mainly saponins and alkaloids. Reduction of protein levels may be due to direct interference of plant extract molluscicides with the protein synthesis (Singh and Tiwari, 2013). The synthesis of protein in any tissue can be affect in two ways by a chemical, (i) it either affect the RNA synthesis at the transcription stage or (ii) it somehow affects the uptake of amino acid in the polypeptide chain. In this case the RNA synthesis would be inhibited resulting in reduced RNA as well as protein content and only the protein content would be affected (Singh *et al*. 2010). The current results are supported by Abdel Megeed (1999), who recorded reduction in the total protein content in *L. natalensis* tissues treated with *Calendula micrantha*. In contrary to the current results, Hassan *et al*. (2010), revealed that total protein contents increased in both haemolymph and tissues of *L. natalensis* and *B. alexandrina* treated with butanol fractions of *Meryta denhamii*. Mello-Silva *et al.* (2007) recorded increase in protein content of *B. glabrata* treated with sub-lethal concentration of *Euphorbia splendens* latex attributed the increase to the acceleration in gluconeogenesis process. The increase in protein content was attributed to alterations in most of biochemical parameters. These alterations could lead to cell lysis, resulting in the release of a large quantity of protein. On the other hand, Abdel-Haleem (2013), revealed that ethanol extracts from three local plants, namely *Euphorbia splendens*, *Ziziphus spinachristi* and *Ambrosia maritima* failed to reveal any molluscicidal effects on the total protein contents of the *B. alexandrina* and *B. truncatus* snails.

Total proteins concentration of haemolymph of both species, *Physa* and *Lymnaea* indicated confused results, where in *physa,* its concentration remained constant after exposure to Tributyletin for two weeks but it highly increased after four weeks of exposure. *Lymnea* snails exhibited different reactions then increased to the same concentration as control after four weeks of exposure (El-Feky *et al.* 2009). This conflict could be attributed to the unique response of each snail species to each type and concentration of particular active principle of biological molluscicides. On the other hand, Ghosh and Chatter Jee (1989) observed an increase of total protein concentration due to stress. They suggested that the increase in protein was most probably resulting from increase in lypolyses damage to cellular organization which gives false indication coming from the damaged tissues within the snails. Also, the decreased protein content may be attributed to the destruction/necrosis of cells and consequent impairment in protein synthesis machinery (Yaraghi *et al.* 2011).

In the current study, the molluscicidal effects of algal extracts on *L. natanelsis*, only didn't restrict on decrease in its protein contents but extend to include change in the protein pattern either quantitatively or qualitatively. The most potent studied 3 algal species; *P. capillacaea*, *J. ruben* and *U. lactuca* having 111.3,127 and 121 ppm respectively resulted in little differences in number of shared protein bands of treated and control snails. The obtained bands in *J. ruben*, *U. lactuca* and *P. capillacea* are nearly similar and ranged between (21-205 kDa). These results could be explained to the same

mode of action of algal molluscicidal compounds which may results in dissociation of large polypeptide into smaller ones or aggregation or recombination of multiple polypeptides to larger ones.

Other explanation includes the immune response of the snail to algal extract particles or molecules. The involvement of immune system includes increase in lectins concentrations which are proteins and increase in number of hemocytes. As a result of snail – algal extracts interaction some hemocytes lysed. These events may result in alterations in protein pattern of snails. This speculation is supported by the explanation of El-Ansary *et al*. (2000) who calculated that changes in the protein profile of a parasitized molluscan host may be due to an immunological response of the host to the parasite and also mention that snails and other invertebrates have much simple type of immune response, called "innate immunity." This response lacks specificity and it is similar to any foreign particle or organism and the response changes only quantitatively.

The impact of molluscicidal activity of any biological product on snails protein pattern was previously investigated (Rawy *et al*. 1993; Aly *et al*. 2004 and El-Sayed, 2006). Recently, this notion is further supported by the protein electrophoretic pattern of two target snails; *B. alexandrina* and *B. trauncatus* exposed to ethanol extracts from three local plants, namely *Euphorbia splendens*, *Ziziphus spinachristi* and *Ambrosia maritime* which revealed differences in the number and molecular weight of protein bands compared to control snails (Abdel-Haleem, 2013).

CONCLUSION

This study it could be concluded that *P. capillacea*, *J. ruben* aqueous and *U. lactuca* ethanoic extracts are the most potent molluscicides against *L. natanelsis* snails and have molluscicidal effect on proteins of treated snails and greatly going for obtaining the specific purified fraction against studied snail.

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