In vitro Tests for Efficacy of Tannins Extracted from Pomegranate (*Punica granatum)* Against *Schistosoma mansoni* Miracidia

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Abstract: The objective of the present study was planned to investigate the *in vitro* efficancy of *Punica granatum* tannins of stem and root bark and fruit rind and placenta on miracidia of *Schistosoma mansoni* using different serial double concentrations. Results showed that a concentration as low as 0.39 ppm of the investigated tannins was enough to kill 100% of miracidia after 50-150 min and to kill 50% of miracidia within 25.1-48.3 min. At 50 ppm, the lethal time for 100% of miracidia ranged between 5 and 15 min and the lethal time for 50% miracidia ranged between 0.5 and 6 min. Placenta tannins were the most potent biocide for miracidia amongst the four tannins of pomegranate ($P \leq 0.05$). Fifty ppm pomegranate root and stem bark tannins killed 100% miracidia after 0.6 and 50 min, respectively, and 0.39 ppm killed 50% miracidia after 0.6 and 21.6 min respectively. The results concluded that the four investigated tannins of *P. granatum* were found to be completely lethal to *Schistosoma mansoni* miracidia and can be used as biocide.

Key words: medicinal plant, lethal time, Kingdom of Saudi Arabia, schistosomiasis

Introduction

Schistosomiasis is considered one of the most fatal and drastic disease of humans. It is the second most important parasitic disease after malaria in terms of overall morbidity and mortality. It is estimated that 200 million people are infected with schistosoma, of whom 120 million are symptomatic and 20 million have severe disease. Six hundred million people are at risk of infection (Chitsulo, *et al.*, 2000; Mostafa and Gad, 1997; Steinauer, *et al*., 2009; WHO, 2010). In some Arab countries like, Sudan, Egypt, Saudi Arabia, Yemen and Iraq, schistosomiasis is a major public health (Ahat, 1988; Ahmed, et al., 2009, Ghandour, 1988; Sebai, 1988). It affects millions of farmers at the early age, diminishing their productivity and exerting a serious socio-economic problem (Yousif, *et al.*, 1998). The most effective method of reducing the transmission of schistosoma is through the interruption of the life cycle of the parasite, which includes, snails, miracidia, cercariae, and adult worms (WHO, 2009). Though, several organic and inorganic chemical compounds are lethal to both cercariae and miracidia. These chemicals however, are toxic and have adverse effects on the environment (Fenwick and Webster, 2006). Consequently, there is an

increasing interest to find alternative cercaricides, miracidiacides and schistosomicides of plant origin which could be cheaper, safer and less hazardous to the environment (Adewunmi, *et al.*,1993; Allam, 2009; Antônio and Crotti, 2011; De Melo**,** *et al.*, 2011; Magalhães, *et al*., 2010; Moraes, *et al*., 2011; Mostafa, *et al*., 2011; Viyanant, *et al.,* 1982). Pomegranate has anti-protozoal activity and it is used in folk medicine for treatment of dysentery (Calzada, *et al.,* 2006; Wang, et al.,2010). Both molluscicidal and cercaricidal activities of extracts of pomegranate were demonstrated (Tripathi and Singh, 2000;Abo-Zeid, 2009). Rind methanol and water extracts were lethal to 100% of cercariae at concentrations of 25 and 30 ppm, respectively, after 24 h (Abo-Zeid, 2009). Towards the search for new product with highly efficacious and low environmental pollutant, cheap and safe. This connection had been planned to investigate the efficancy of different concentrations of *Punica granatum* tannins extracted from root and stem bark tannins as well as placenta and rind were tested for their lethal effect on miracidia of *Schistosoma mansoni in vitro* .

Materials and Methods

Separation of tannins

Powdered rind, placenta, stem and root barks were percolated in water over night in a shaking incubator. XAD-16 resin was packaged in a glass column, washed with methanol, and equilibrated with water for 12hrs. Vacuum was applied to remove water from the resin and the extract of powder was applied to the column and was eluted with copious amounts of water until the eluate was clear in colour. Water was removed from the column by vacuum aspiration. Adsorbed tannins were eluted several times by methanol and the dark brown solution collected was evaporated at 50 ºC in a rotary vacuum evaporator. Column was regenerated by washing with water and the procedure was repeated with another portion of extract (Seeram, *et al.,* 2005).

Preparation of *S. mansoni* miracidia and eggs

S. mansoni eggs were recovered from stools of patients, admitted to (Edwani Hospital in Taif, Kingdom of Saudi Arabia) were emulsified in 10 volumes of 10% sodium chloride and the sediment was washed with cold saline and stored over night in the a refrigerator. The mixture was diluted by tap water and exposed to bright light to allow the ova to hatch.

Effect of tannins on miracidia

Tissue culture plates were used as test chambers to observe the viability and death of miracidia under a dissecting microscope (Techounwou, *et al.*,1991). Twenty miracidia were placed in 1 ml dechlorinated water in each well. Serial double concentrations of tannins were added to each experimental well as follow: 0.39, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50 ppm. Three replicates were prepared for each tested concentration.

Calculation of lethal time% (LT50 and LT100) from regression curves

The effect of different tannins of *P. granatum* on the mortality of *miracidia* followed a sigmoid pattern. Linear regre-

ssion analysis was preferred to a semi-logarithmic analysis and the equation: Y= a + b X, was applied (Levesque**,** 2007) and found to be more appropriate with high correlations (r^2< 0.7- 0.99), and significance (p< 0.01-0.3).

Results

Good correlations were observed between the mortality rates of miracidia (LT50%) and concentrations (ppm) of tannins, with r^2 values exceeding 0.6, except for some odd cases. The pattern of the correlation of LT50% increased reciprocally with conce-ntration. The mean death time for untreated control miracidia was 42 ± 1.58h, however, when miracidia were exposed to different concentrations of tannins, they died in less than 150 min. The results of the present study showed that the lethal times required to kill 50% of *S.mansoni* miracidia (Figs1-4) at the lowest concentration 0.39ppm were 37.5 ,48.3, 25.1and 26.4 min for the root, stem, placenta, and rind tannins extracted from *P. granatum* respectively (25.1-48.3min) while the highest conce-ntration 50 ppm were 0.69,6,0.62 and 0.5 min respectively (0.5 -6min). Also the results showed that the time required to kill 100% of miracidia(Figs 5-8) exposed to the lowest concentration 0.39 ppm tannins of root ,stem ,placenta and rind were 120, 150, 50, and 90 min respectively(50-150min). However, the highest concentration 50 ppm were5, 15,15and 5 min respectively (5-15 min). . Placenta tannins were the most potent miracidiacide amongst the four investigated tannins (P≤ 0.05).

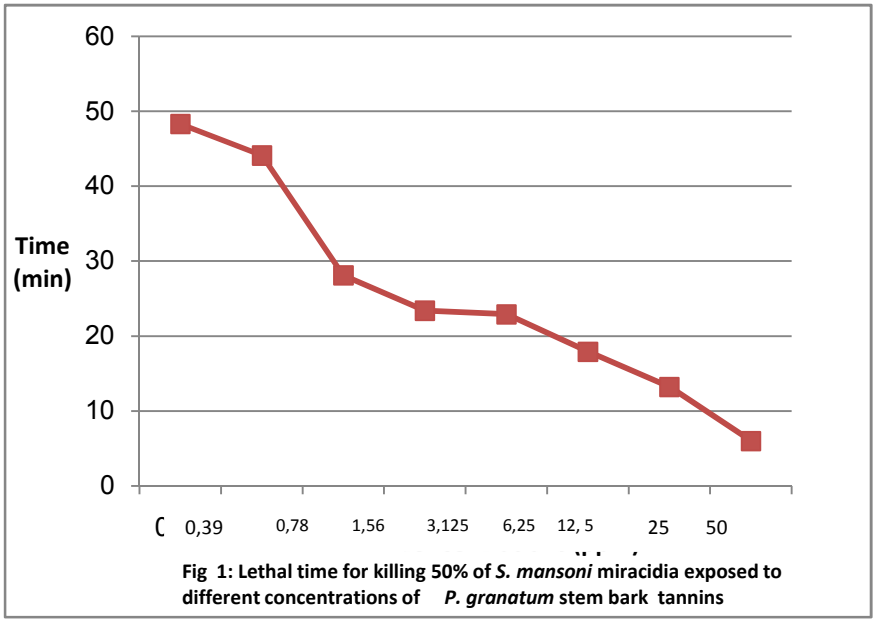

Fig 1: Lethal time for killing 50% of *S. mansoni* miracidia exposed to different concentrations of *P. granatum* stem bark tannins

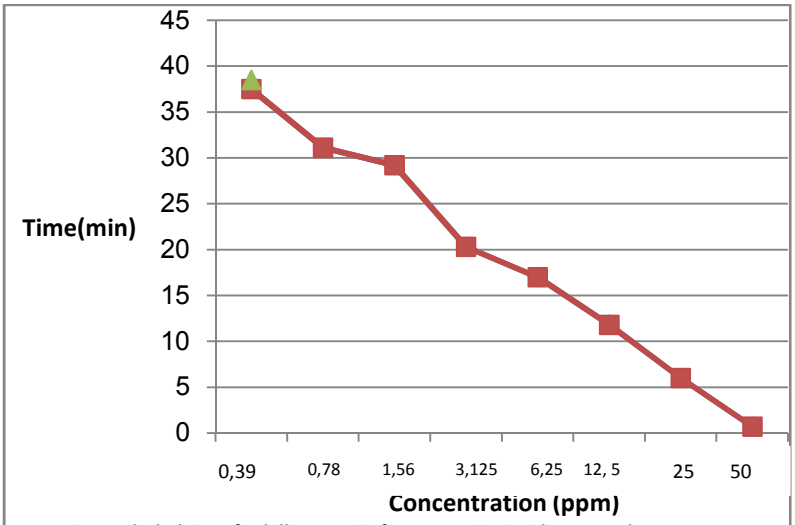

Fig 2. : lethal time for killing 50 % of *S.mansoni* miacidia expoed to different concentrations of *P.granatum* root bark tannins

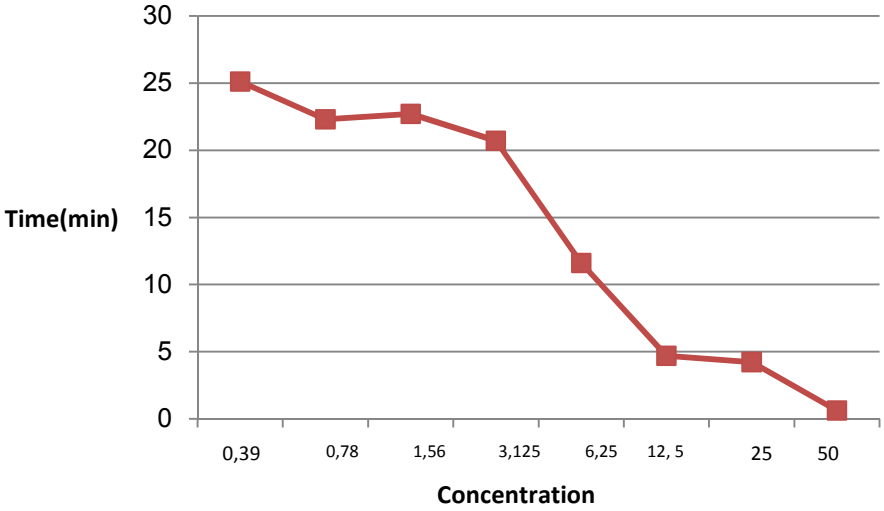

Fig 3: Lethal time for 50% killing o of *S.mansoni* miracidia exposed to different concentrations of *P.granatum* fruit placenta tannins

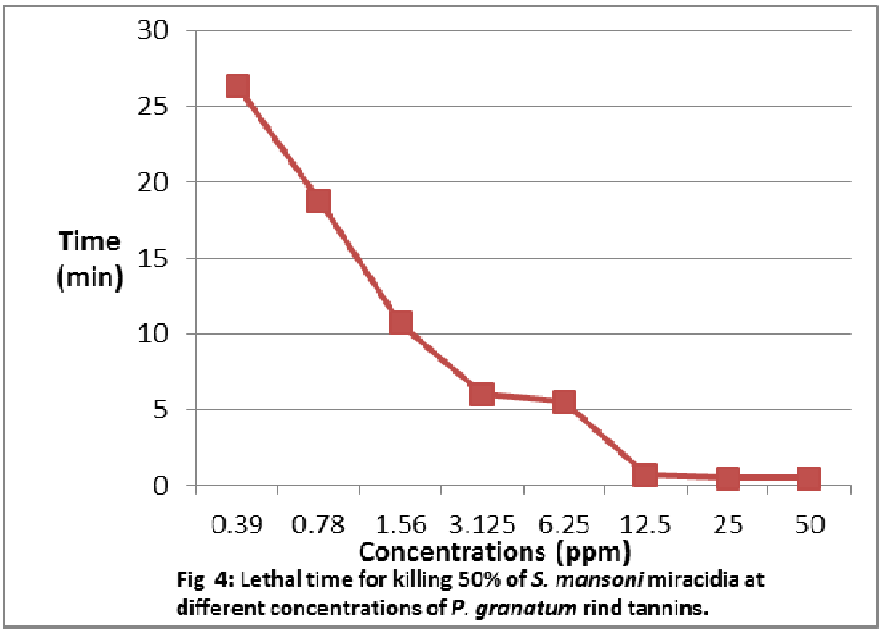

Fig 4: Lethal time for killing 50% of *S. mansoni* miracidia at different concentrations of *P. granatum* rind tannins.

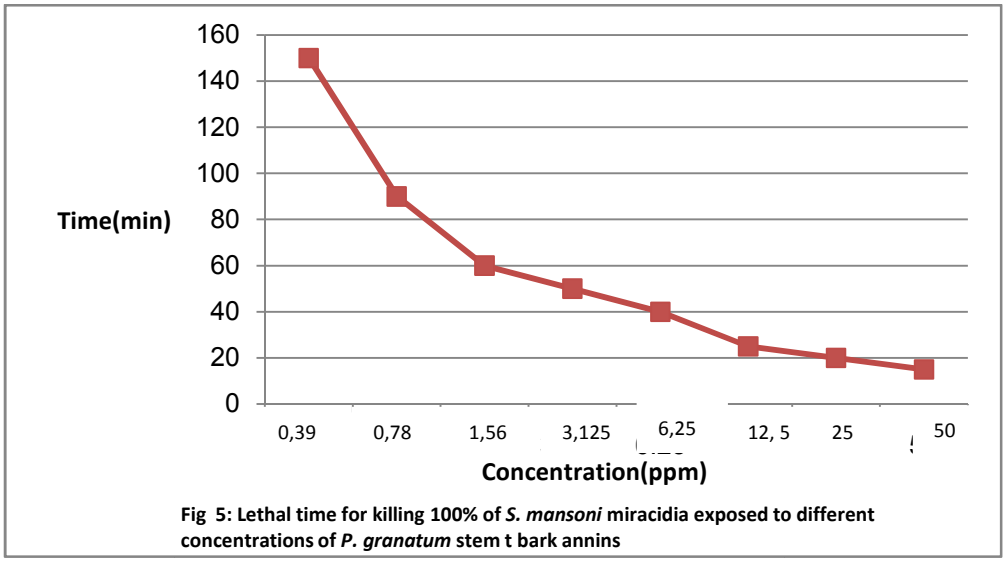

Fig 5: Lethal time for killing 100% of *S. mansoni* miracidia exposed to different concentrations of *P. granatum* stem t bark annins

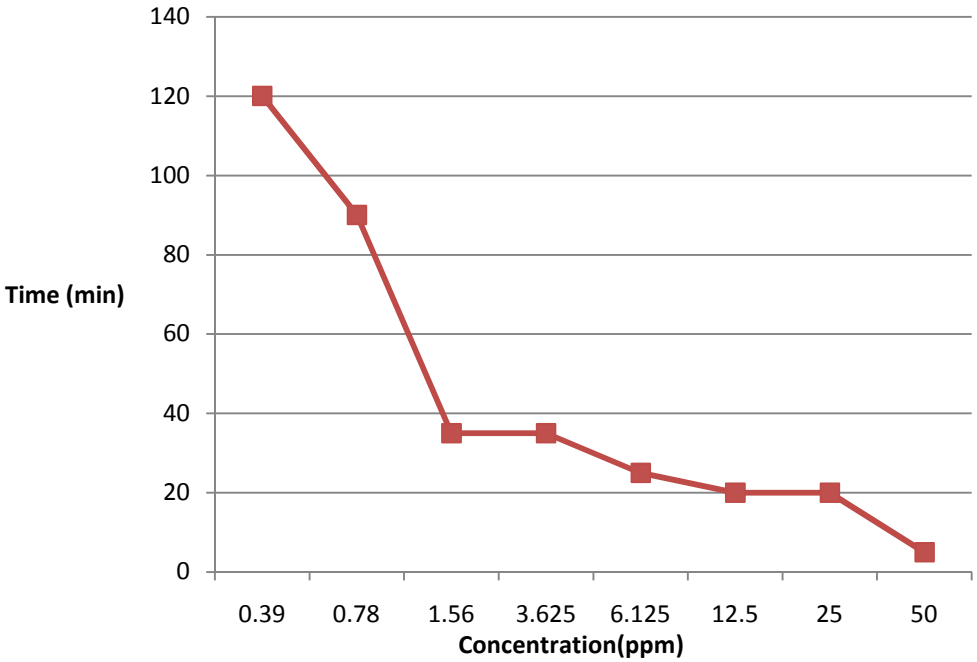

fig 6: Lethal time for killiing 100% of *S. mansoni miracidia exposed to different concentrations of P. granatum* root bark tannin

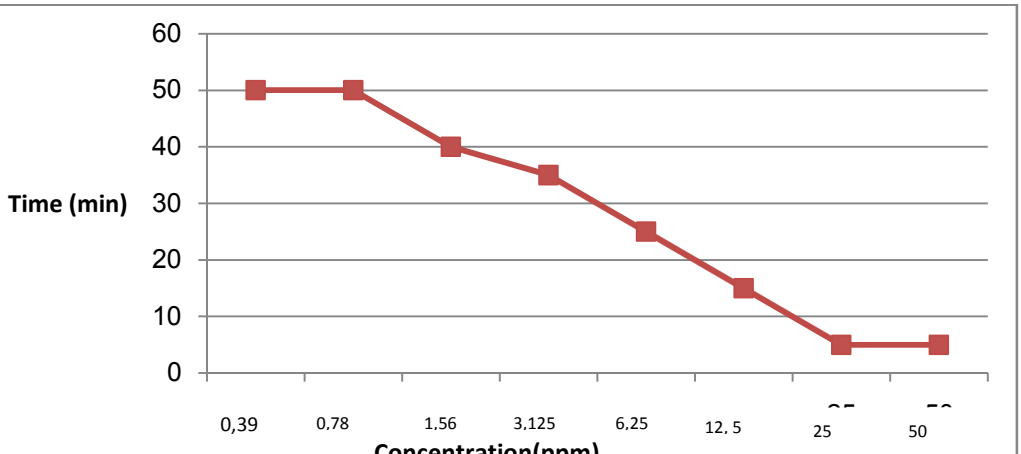

Fig 7 Lethal time for killing 100% of *S. mansoni* miracidia exposed to different concentrations of *P. granatum* Placenta tannins

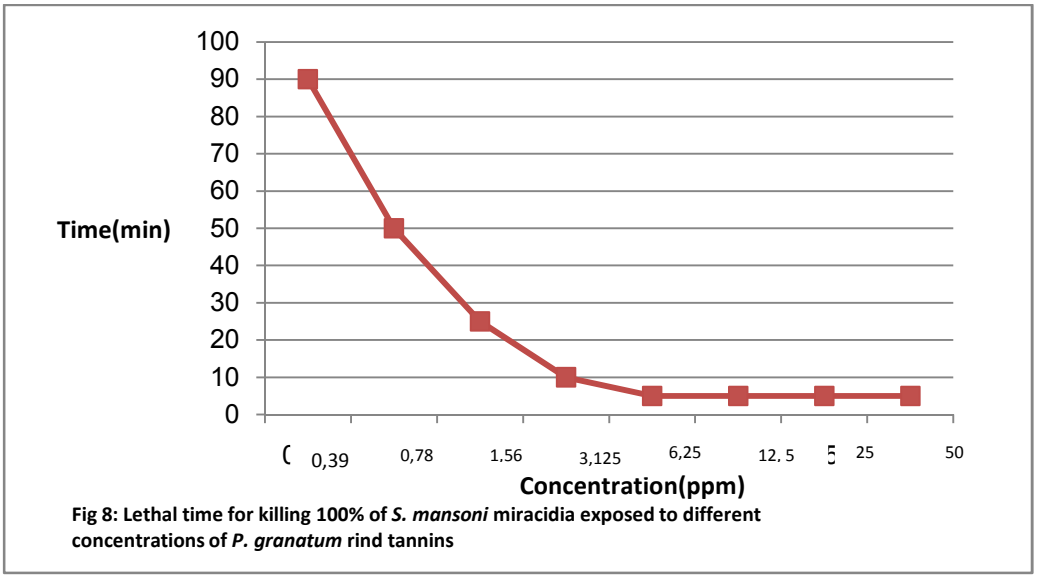

Fig 8: Lethal time for killing 100% of *S. mansoni* miracidia exposed to different concentrations of *P. granatum* rind tannins

Discussion

Some medicinal plants have been screened for their lethal effect to miracidia. Two of them are the most popular molluscicides, used to interrupt the life cycle of shistosoma, namely, *T. tetraptera* (Aridan) and *Phytola-ccadodecandra* (Endod). Aridan was found to be lethal to miracidia at 400 ppm after 30 min (Adewunmi, 2005). Endod extract, on the other hand, was found to be more active

than Aridan against *S. mansoni* miracidia. The LC_{50} of Endod was 8.2 ppm (Dhina and Shift, 1996). At 4 ppm aqueous extracts of Endod prevented infection of snails with miracidia (Birrie, *et al.*, 1998). Ethanol extract of *Iris pseudacorus* leaves exhibited time-concentration dependent miracidiacidal effect (Ahmed and El-Hamshary, 2005). The LC_{100} within 5 min, 30 min, and an hr of exposure were 2.7, 1.6 and 0.9 mg/l respectively.

Abdel Aziz, *et al.*, (2011), studied the activity of *Plectranthus tenuiflorus* on miracidia. The LC_{50} was 24.37 mg/100 ml in more than 2 h. The biocidal effects of *N. sativa* crushed seeds against miracidia was investigated by Mahmoud, *et al.*, (2005), who found that 4 ppm were lethal to miracidia after 1 min.

In the present study, tannins of pomegranate were found completely lethal to miracidia A concentration as low as 0.39 ppm of the investigated tannins was enough to kill 100% of miracidia after 50-150 min and to kill 50% of miracidia within 25.1-48.3 min. At 50 ppm, the lethal time for 100% of miracidia ranged between 5 and 15 min and the lethal time for 50% miracidia ranged

between 0.5 and 6 min. Placenta tannins were the most potent biocide for miracidia amongst the four tannins of pomegranate ($P \leq 0.05$). Fifty ppm pomegranate root and

stem bark tannins killed 100% miracidia after 0.6 and 50 min, respectively, and 0.39 ppm killed 50% miracidia after 0.6 and 21.6 min respectively.

The results concluded that the four investigated tannins of *P. granatum* were completely lethal to *Schistosoma mansoni* miracidia as low as 0.39 ppm within a period from50 to 150 min and can be used as biocide. Further studies are needed to find alternative cercaricides, miracidiacides and schistomicides of plant origin which could be cheaper, safer and less hazardous to the environment.

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تأثير تانينات الرمان المستخلصة من قلف الساق والجذر ومن قشرة ومشيمة الثمرة علي طور ميراسديا دودة البلهارسيا المعوية

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المستخلص

في هذة الدراسة تم اختبار التأثير القاتل للتانينات المستخلصة من قلف ساق وجذر الرمان وقشرة ومشيمة ثمرة الرمان على طور ميراسيديا دودة البلهارسيا المعوية. أثبتت الدراسة أن جميع التانينات المستخلصة ذات تأثيرقاتل للميراسيديا. فعند تراكيز منخفضة يصل لـــ 0.39 جزء في المليون ماتت كل الميراسيديا (100%) تحت تأثير التانينات المستخلصة في وقت تراوح بين 50 و150 دقيقة و ماتت 50% منها بعد 25–50 دقيقة. أما عند تركيز 50 جزء في المليون فأن الميراسيديا ماتت بنسبة 100% بعد 5 إلى 15 دقيقة وبنسبة 50% بعد 0.50 إلى 6 دقيقة. و عند مقارنة التأثير القاتل للتانينات المستخلصة المختلفة اتضح أن تانينات المشيمة كانت الأقوي في حين كانت تانينات الساق الأضعف و مع ذلك قتلت الأخيرة 50% من الميراسيديا بعد 48.3 و 6.0 عند تركيزي 0.39 و 50 جزء في المليون على التوالي. كما قتلت 100%من الميراسيديا بعد 15 و 150 عند تركيزي 0.39 و 50 جزء في المليون على التوالي. أما تانينات المشيمة فقد قتلت الميراسيديا عند نفس التركيزين بنسبة 100% بعد 15 و 50 دقيقة على التوالي وبنسبة 50% بعد 0.62 و 25.1 دقيقة على التوالي . وبالتالي يتضح من هذه الدراسة أن تانينات قشرة ومشيمة ثمرة الرمان وتانينات قلف الساق والجذر ذات كفاءة عالية في قتل ميراسيديا دودة البلهارسيا المعوية.