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Efficacy of Chromolaena odorata leaf extracts on Biomphalaria pfeifferi eggs in control of Schistosomiasis

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Efficacy of *Chromolaena odorata* leaf extracts on *Biomphalaria pfeifferi* eggs in control of Schistosomiasis

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Schistosomiasis is still a problem in many parts of the world, with 85% infection in Africa. There is a need to control the snail, which is essential to complete the transmission circle. The molluscicidal activities of aqueous and ethanolic leaf extracts of *Chromolaena odorata* against eggs of *Biomphalaria pfeifferi* were investigated. Thirty viable eggs of this snail were exposed for 48 h to a serial dilution of 0 (control), 8, 20, 28, 40, 60, 80, and 100 ppm of distilled water and ethanolic extracts of *C. odorata* leaves. The LC_{50} and LC_{90} values of an aqueous extract were 65.75 and 139.54 ppm, respectively, while LC_{50} and LC_{90} of an ethanolic extract were 44.03 and 119.3 ppm, respectively. The two plant extracts showed a significant difference ($p < 0.05$) in the mortality rates of *B. pfeifferi* eggs. The results deserve further studies in order to identify and characterize the molluscicidal components of *C. odorata* leaves.

Daugelyje pasaulio šalių šistosomatozės problemos neišspręstos, o 85% šių susirgimų užregistruojama Afrikoje. Labai svarbi sraigių – pagrindinės šistosomų gyvybinio ciklo grandies – kontrolė. Ištirtas *Chromolaena odorata* lapų vandeninio ekstrakto ir etanolio ekstrakto moliuskocidinis poveikis sraigės *Biomphalaria pfeifferi* kiaušinėliams. Po trisdešimt šios rūšies sraigių kiaušinėlių 48 valandas buvo laikomi *C. odorata* lapų distiliuoto vandens ir etanolio ekstraktuose, kurių koncentracija buvo 0 (kontrolė), 8, 20, 28, 40, 60, 80 ir 100 milijoninių dalių. Vandeninio ekstrakto LC_{50} ir LC_{90} koncentracijos buvo, atitinkamai, 65,75 ir 139,54 milijoninių dalių, o etanolio ekstrakto LC_{50} ir LC_{90} koncentracijos – 44,03 ir 119,3 milijoninių dalių. Po 48 valandų trukmės poveikio buvo nustatyta, kad vandeninio *C. odorata* lapų ekstrakto įtaka *B. pfeifferi* kiaušinėlių mirtingumui buvo didesnė, negu etanolio ekstrakto ($p < 0,05$). Rezultatai rodo tolesnių tyrimų poreikį, siekiant identifikuoti moliuskocidinius *C. odorata* lapų komponentus.

Keywords: *Biomphalaria pfeifferi* eggs; *Chromolaena odorata*; leaf extract; molluscicide candidate

Introduction

Schistosomiasis is endemic in about 74 countries, and more than 207 million people are infected worldwide, of which 85% of them live in Africa (WHO 2011). In Nigeria, 22 million people are infected, which include more than 16 million children (Carter Centre 2010).

The transmission cycle of *Schistosoma* species requires a specific freshwater snail vector, *Biomphalaria pfeifferi* as an obligate intermediate host (Engels et al. 2002; Zhou et al. 2008). The importance of snail control should not be overlooked. Despite greatly improved chemotherapy by a single-dose oral treatment with praziquantel, there are logistical problems in mass treatment, the threat of reinfection remains (Wilkins 1989), and vaccination is still a distant prospect (Butterworth 1992). The control of vector snails is, therefore, relevant to the control of this parasitic disease. There is a great interest in the use of molluscicidal plants by local communities in a self-supporting system of a rural schistosomiasis control program (Taylor 1986). Plant molluscicides can prove to be an ideal source of low cost, safe, and effective molluscicides (Marston and Hostettmann 1985; Sigh K, Sigh A, and Sigh DK 1996).

Chromolaena odorata (L.) King & H. Rob. of the family Asteraceae (Compositae) is used as an antibacterial, antiplasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypentensive, antiinflammatory, astringent, deuretic, and hepatotropic agent (Phan et al. 2001; Akinmoladun, Ibukun, and Dan-Ologe 2007). It is also applied topically as an antidote against the sting from the spine of the common sea catfish. An aqueous decoction of the roots is used as an antipyretic and analgesic remedy, and a leaf extract with salt is used as a gargle for sore throats and colds. *C. odorata* was first introduced into Nigeria through contaminated seed lots of *Gmefina arborea* around 1937, and by 1960, it occupied the southeastern states of Nigeria. This plant species is now widespread in the southern parts of Nigeria, being present in more than 20 of 36 states (Parker and Fryer 1975). No work was actually found on the molluscicidal potency of *C. odorata* on any stage of *Biomphalaria pfeifferi* in Nigeria. This work evaluates

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the potency of aqueous and ethanol extracts of *C. odorata* as molluscicide against egg masses of *B. pfeifferi* in the laboratory.

Material and methods

Field work

Collection of snail samples

Adult *B. pfeifferi* snails were collected from Lake Awba bank, very close to the dam (latitude 7°26′–7°28′ North and longitude 3°53′–3°54′ East) on the first week of November 2010. Collection was done between 8.00 am and 12.00 noon, using a flat dip-net scoop as described by Richie, Radke, and Ferguson (1962) and Demian and Kamel (1972). The scoop was used starting at the edge of the water, dragged through the surface of the site substratum to a distance of about one meter towards the middle of the water body without picking up too much bottom mud which made the finding of snails impossible, and then lifted upwards vertically. The content of the scoop was washed gently in the lake water, and collected snails were gathered in a plastic bucket containing some dam water with some sterile cotton wool. The samples were taken to the Parasitology Research Laboratory, Department of Zoology, University of Ibadan, for identification, maintenance, and laboratory studies. Snails were identified to the species level using the identification key proposed by WHO (1971).

Collection of plant samples

The leaves of *C. odorata* were collected in a wet sac from the Botanical Garden, University of Ibadan, in the morning (7–9 am). A wet sack was used to avoid direct exposure to sunlight which may lead to dehydration. Leaves were rinsed to remove dust, sand, and unwanted materials. Identification and authentication of plant species were done in the Department of Botany, University of Ibadan.

Laboratory work

Maintenance of snails

In the laboratory, infected snails were identified using the shedding method described by Frandsen and Christensen (1984) by placing each snail in a beaker half-filled with dechlorinated tap water. The beakers were exposed to the day light and left for 1 h or more to allow cercariae to emerge. The snails that shed cercariae were gathered in one circular glass trough half-filled with dechlorinated tap water. Healthy snails were maintained in the aquaria of circular glass troughs (12 cm depth and 30 cm diameter). Each trough (stock aquarium) was interiorly covered with polythene bags before being filled with dechlorinated tap water, a layer of clay, and some gravel sterilized by heating on an electric cooker for at least 1 h. The tap water used had been strongly aerated for about three days to allow evaporation of chlorine and then the troughs were filled two-thirds full with such dechlorinated water.

Green *Lactuca sativa* (salad or prickly lettuce) leaves were immersed in boiling water for about one minute and then cooled in tap water. After the removal of the midrib, the leaves were dried and powdered and offered as food to the snails three times a week. Soft parts of the green leaves were preferred by snails over the rest. Studies had shown that snails fed on dry ribs of the leaves cannot survive for long. The aquaria were maintained at a temperature between 25 and 30 °C. Water was being changed once a week or when necessary.

Collection and preparation of egg masses

The laboratory-fed snails laid eggs. The polythene sheets were checked for egg masses after 24 h, then the snails were transferred to a new plastic trough and the same was repeated. The parts of the polythene sheet, which contained egg masses, were cut out to be used in different treatments after their transfer to containers containing 200 ml of dechlorinated tap water till eggs hatched into juveniles. One-week-old juveniles were used in the experiment.

Preparation of extracts

The stock solution of an aqueous extract was prepared by mixing 10 g of powdered leaves soaked in 450 ml (22,222 ppm) of distilled water for 24 h with occasional vigorous shaking. Then, the suspension was filtered using filter paper (Whatman No 1 filter paper). The marc was washed with several portions of distilled water to adjust the volume of the solution to 500 ml (20,000 ppm) by using volumetric flasks. The plant extracts were used immediately after extraction to ensure their freshness. The same was applicable to 70% ethanol to obtain an ethanolic extract. After the extraction, the solvent was removed by evaporation.

Molluscicidal potency tests of plant extracts on snail eggs

A different volume of 0.0 (control), 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, and 2.5 ml from the stock solution of both extracts of each plant were added to the equal volume of 500 ml of dechlorinated tap water in plastic trough containers (10 cm depth × 17 cm diameter) to have a working solution. Then, the concentration of each solution was calculated in ppm: 0.0, 8, 20, 28, 40, 60, 80, and 100 ppm, respectively. Thirty snail eggs were immersed in each trough containing the solution. After 48 h of exposure to the plant extracts, the eggs were transferred to fresh dechlorinated water and maintained there for another 24 h. Molluscicidal tests with each plant extract dose were repeated twice. Thereafter, mortality counts were done under a microscope and recorded.

Dilution procedure

Different concentrations of aqueous extracts tested in the experiments were prepared from the stock solutions using the following dilution procedure.

$$V_1 \times C_1 = V_2 \times C_2,$$

where V_1 = volume taken by a pipette from stock solution, V_2 = final volume (500 ml of dechlorinated tap water in a trough), C_1 = concentration of stock solution, and C_2 = concentration needed for testing.

Statistical analysis

The raw data were subjected to probit analysis, BioStat 2007 Professional version 3.2, to obtain LC_{50} (ppm) and LC_{90} (ppm), probit regression graph, and Chi-square (χ^2). Regression equations and R^2 were obtained from the same software using regression analysis. Weight was taken in an electrical balance.

Results

Experimental results

Table 1 presents the LC_{50} and LC_{90} values of *C. odorata* aqueous and ethanolic extracts on *B. pfeifferi* egg masses. The LC_{50} and LC_{90} values of the aqueous extract were 65.75 and 139.54 ppm, respectively. Statistically, the aqueous extract was potent against eggs of *B. pfeifferi* at 48 h ($x^2 = 20.58$, $df = 6$; $p < 0.05$). The LC_{50} and LC_{90} values of the ethanolic extract on the eggs of *B. pfeifferi* were 44.03 and 119.03 ppm, respectively. Statistically, the aqueous extract was more potent than the ethanolic extract at 48 h ($x^2 = 9$, $df = 6$; $p < 0.05$).

The slope values of the regression relationship between egg mortality and treatment at different concentrations of the two *C. odorata* extracts showed a linear increase in mortality of *B. pfeifferi* eggs from low to higher concentrations (Figure 1). The χ^2 analysis showed that the molluscicidal potency of *C. odorata* extracts was significant at different concentrations for a 48 h exposure period (Figure 2).

Bench side observations

The egg masses that were susceptible to different extracts became pale after 48 h of exposure. The embryos of the dead eggs remained motionless in their cells when observed under a microscope and showed no signs of development contrary to observations recorded in the control.

Table 1. Toxicity of *Chromolaena odorata* leaf extracts on the egg stage of *Biomphalaria pfeifferi* snails.

	Aqueous extract	Ethanol extract
Regression equation	$y = 21{,}683 + 0.0444$[a]	$y = 3007 + 0.0388$[a]
χ^2 ($p < 0.05$)	20.58	9.00
LC_{50} (ppm)[a]	65.75	44.03
LC_{90} (ppm)[a]	139.54	119.03
R^2	0.8447	0.9567

[a]Mean lethal concentration (ppm).

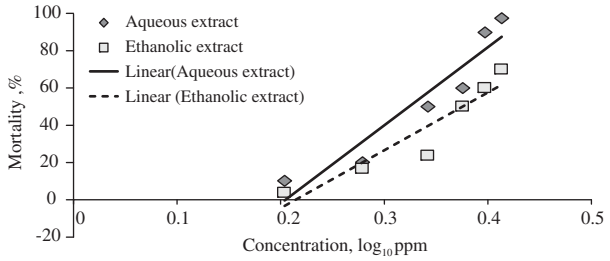

Figure 1. *Chromolaena odorata* aqueous and ethanolic extracts on eggs of *Biomphalaria pfeifferi* after a 48 h exposure.

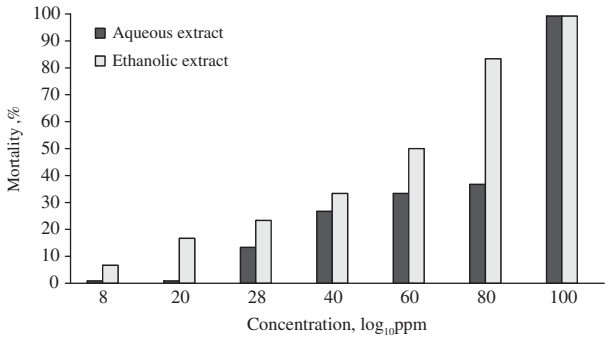

Figure 2. Percentage mortality of *Biomphalaria pfeifferi* eggs recorded on different *Chromolaena odorata* extract concentration.

Discussion

In the present study, *C. odorata* was screened for its molluscicidal activity. *B. pfeifferi*'s eggs were susceptible to *C. odorata* extracts at different concentrations. The ethanolic extracts of this plant leaves that killed 50 (LC_{50}) and 90% (LC_{90}) of the eggs were 65.75 ppm and 139.54 ppm, respectively. The potency of an ethanolic extract on the eggs was found to be higher when compared to other plant extracts used in other works. Adenusi and Odaibo (2008) reported that the ethanolic extract of *Dalbergia sissoo* leaves on *B. pfeifferi* egg masses had LC_{50} and LC_{90} as 236.12 and 604.91 ppm, respectively. However, *C. odorata* aqueous extract had lower molluscicidal effects. The eggs of most freshwater snails are more resistant to attacks from other developmental stages (juvenile and adult). This may be due to the protective covering of capsular jelly-like material which covers and protects the eggs from the external environment (Parashar et al. 1995).

The results showed that the ethanolic extract of *C. odorata* was effective against the embryos within the egg masses as reported by Salawu and Odaibo (2011). Parashar et al. (1995) reported a similar trend in the ovicidal action of niclosamide against the eggs of *Lymnaea auricularia*. Sukumaran, Parashar, and Rao

(1994, 1995, 2002) reported the potency of butanolic extracts of plants like *Sapindus trifoliatus, Agave americana, Balanites aegyptica, Jatropha gossypifolia*, and *Vaccaria pyramidata* against freshly laid eggs of *Lymnaea luteola*.

Following the WHO guidelines for the study of plant molluscicides, it is recommended that a useful molluscicidal plant must not be a source of prejudice (fear or superstition) (Mott 1987). There is no known superstition which would preclude the use of *C. odorata*. Generally, the herbs *C. odorata* appear to be promising plants for the control of schistosomiasis because they conform to the most of the criteria of an ideal plant molluscicide found in it.

Traditionally, fresh leaves or a decoction of *C. odorata* have been used throughout Vietnam for many years as well as in other tropical countries for the treatment of leech bite, soft tissue wounds, burn wounds, skin infection, and dento-alveolitis (Phan et al. 2001).

Conclusion

The screening of plant species for their molluscicidal potency began more than 70 years ago, and the search for a good molluscicide will ever continue. A necessary research in the field of plant molluscicides, especially plants of a great medicinal value, should be encouraged. Therefore, molluscicide can be an effective means of reducing snail populations, at least temporarily, and will play an important part in schistosomiasis control in third world endemic countries.

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References

Adenusi, A.A., and A.B. Odaibo. 2008. Preliminary laboratory assessment of the crude aqueous and ethanolic extracts of *Dalbergia sissoo* plant parts for molluscicidal, ovicidal and cercaricidal activities. *Travel Medicine and Infectious Diseases* 6: 219–27.

Akinmoladun, A.C., E.O. Ibukun, and I.A. Dan-Ologe. 2007. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Scientific Research and Essays* 2: 191–4.

Butterworth, A.E. 1992. Vaccines against schistosomiasis: Where do we stand? *Transactions of the Royal Society of Tropical Medicine and Hygiene* 86: 1–2.

Carter Centre. 2010. Schistosomiasis Control Program. http://www.cartercentre.org/health/schistosomaisis/index.html (accessed December 10, 2010).

Demian, E.S., and E.G. Kamel. 1972. Growth and population dynamics of *Bulinus truncatus* under semi-field conditions in Egypt. *Proceedings of Egyptian Academy of Science* 25: 37–60.

Engels, D., L. Chitsulo, A. Montresor, and L. Savioli. 2002. The global epidemiological situations of schistosomiasis and new approach to control and research. *Acta Tropica* 82: 139–46.

Frandsen, F., and N.O. Christensen. 1984. An introductory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. *Acta Tropica* 41: 181–202.

Marston, A., and K. Hostettmann. 1985. Plant molluscicides. *Phytochemistry* 2: 639–52.

Mott, K.E. 1987. *Plant Molluscicides*. Chichester/Geneva: John Wiley and Sons/UNDP/World Bank/WHO.

Parashar, B.D., M.P. Kaushik, A.K. Gupta, R.V. Swamy, and K.M. Rao. 1995. Toxicity of some molluscicides to freshwater snail *Lymnaea auricularia* the vector of animal fasciolasis and to non-target organisms. *Proceedings of the Academy of Environmental Biology* 4: 183–7.

Parker, C., and J.D. Fryer. 1975. Weed control problem causing major reductions in world food supplies. *FAO Plant Protection Bulletin* 23: 83–95.

Phan, T.T., L. Wang, P. See, R.J. Grayer, S.Y. Chan, and S.T. Lee. 2001. Phenolic compounds of *Chromolaena odorata* protect cultured skin cells from oxidative damage: Implication for cutaneous wound healing. *Biological and Pharmaceutical Bulletin* 24: 1373–9.

Richie, L.S., M.E. Radke, and F.F. Ferguson. 1962. Population dynamics of *Australorbis glabratus* in Puerto Rico. *Bulletin of the World Health Organization* 27: 171–81.

Salawu, O.T., and A.B. Odaibo. 2011. The molluscicidal effects of *Hyptis suaveolens* on different stages of *Bulinus globosus* in the laboratory. *African Journal of Biotechnology* 10, no. 50: 10241–7.

Sigh, K., A. Sigh, and D.K. Sigh. 1996. Molluscicidal activity of Neem (*Azadirachta indica*). *Ethnopharmacology* 52: 35–40.

Sukumaran, D., B.D. Parashar, and K.M. Rao. 1994. Molluscicidal properties of *Agave americana and Balanites aegyptica*. *International Journal of Pharmacology* 32: 232–8.

Sukumaran, D., B.D. Parashar, and K.M. Rao. 1995. Toxicity of extract of *Jatropha gossypifolia and Saponaria saponaria* as molluscicide against freshwater snails *Lymnaea luteola* and *Indoplanorbis exustus*, the vectors of animal schistosomiasis. *Fitoterapia* 56: 393–8.

Sukumaran, D., B.D. Parashar, and K.M. Rao. 2002. Evaluation of some plant molluscicides against a freshwater snail *Lymnaea luteola*, the vector of animal schistosomiasis. *Pharmaceutical Biology* 40: 450–5.

Taylor, P. 1986. Water supply and sanitation programmes to support schistosomiasis control in Zimbabwe. *Tropical Medicine Parasitology* 37: 188–94.

Wilkins, H.A. 1989. Reinfection after treatment of schistosome infections. *Parasitology Today* 5, no. 3: 83–8.

World Health Organization [WHO] 1971. Meeting of directors of collaborating laboratories on molluscicide testing and evaluation: Document No. WHO/SCHISTO/17–6. *Geneva* 21: 34–30.

World Health Organization [WHO] 2011. Schistosomiasis: Number of people treated worldwide in 2009. *Weekly Epidemiological Record* 86: 73–80.

Zhou, X.N.M., G.J. Yang, K. Yank, X.H. Wang, and Q.B. Hong. 2008. Potential impact of climate change on schistosomiasis transmission in China. *American Journal of Tropical Medicine and Hygiene* 78: 188–94.