

# Laboratory Assessment of Molluscicidal Activity of *Glinus Oppositifolius* (L.) Aug. DC. (Aizoaceae)

Adama Dénou\*, Adiaratou Togola, Kari Tvette Inngjerdingen, Drissa Diallo, Berit Smestad Paulsen

**Abstract**— The negative impact of synthetic molluscicides on the environment and their high cost necessitated search for an alternative approach of using plant extracts for the control of schistosomiasis. The objective of this study was, therefore, to evaluate some crude extracts (butanol and ethyl acetate) and pure products of *Glinus oppositifolius* aerial part for their molluscicidal effect against schistosome snail intermediate hosts. Assessment of the molluscicidal activity against *Bulinus truncatus* and *Biomphalaria pfeifferi* was made by immersion method in accordance with WHO guideline. The results of mortality were statistically analyzed using excel sheets. After 24 hours exposure, BuOH extract has shown the lowest LC50: 64.3 and 91.7 ppm for *Bulinus truncatus* and *Biomphalaria pfeifferi* respectively. Moreover EtOAc extract gave a LC50: 86.2 ppm with *Bulinus tuncatus*. Although the crude extracts were more active, both crude extracts and pure products from *G. oppositifolius* revealed a dose-dependent activity on *Bulinus truncatus* and *Biomphalaria pfeifferi*. The results showed that *G. oppositifolius* has molluscicidal activity against *B. truncatus* and *B. pfeifferi* snails. Yet, further comprehensive evaluation is recommended for the possible use of *G. oppositifolius* against *B. truncatus* and *B. pfeifferi*.

**Index Terms**— *Biomphalaria Pfeifferi*, *Bulinus Truncatus*, *Glinus Oppositifolius*, Schistosomiasis, Snails.

## I. INTRODUCTION

Human schistosomiasis is an acute and chronic disease caused by the blood flukes (trematode worms) belonging to the genus *Schistosoma* [1]. People get infected by having contact with freshwater that harbors free-swimming larval forms of the parasite shed from freshwater snail intermediate hosts [2, 3]. Schistosomiasis is one of the most prevalent parasitic diseases, infecting over 206 millions of people all over the world [4]. The main disease causing schistosome species are *Schistosoma haematobium* (*S. haematobium*), *S. mansoni*, *S. japonicum*, *S. mekongi* and *S. intercalatum* [5].

Schistosomiasis remains a public health problem in several parts of the world, particularly in Africa where 92% of all the

people requiring preventive chemotherapy for schistosomiasis live and at least 224 million affected people live in sub-Saharan Africa [1]. It ranks second only to malaria as the most common parasitic disease, killing an estimated 280,000 people each year in the African region alone [6]. Schistosome infection is the second parasitic infection after malaria as a cause of morbidity and mortality [7, 8]. Estimates indicate that 85% of all schistosomiasis cases are in sub-Saharan Africa [9]. The infection is transmitted by *Biomphalaria pfeifferi* and *Bulinus truncatus* which are the intermediate hosts in the Farako channel.

Chemical control of the snail hosts using synthetic molluscicides is very expensive and toxic to fish and amphibians [10]. A logical alternative is to use plants with molluscicidal properties, especially as such substances may be efficient, cheap, environmentally safe, and will involve the local community more closely in schistosomiasis control. Many plant species have been proved to have molluscicidal properties against different snail species. The most potent plant that has been known to have molluscicidal activity against snail intermediate hosts of schistosome is *Phytolacca dodecandra* [11]. *Alternanthera sessilis* [12], *Balanites aegyptiaca* [13] and *Jatropha curcas* [14] also lie among plants with molluscicidal activities. *Glinus oppositifolius* has been reported by traditional healers for treating joint pains, inflammations, diarrhoea, intestinal parasites, fever, boils and skin disorders [15, 16]. A molluscicide effect has been obtained by methanol and dichloromethane extracts of *Glinus oppositifolius* on three types of snails, *Biomphalaria glabrata*, *Biomphalaria pfeifferi* and *Bulinus truncatus* [17].

The present study was therefore undertaken to evaluate the crude butanol and ethyl acetate extracts and pure products (GO1 and 12-Glucosyl-GO1) from *Glinus oppositifolius* for their molluscicidal activity in Mali.

## II. MATERIAL AND METHODS

### A. Study Site:

Field studies were carried out in Farako channel (Gomidjirambougou) while laboratory studies took place in the Department of Traditional Medicine, National Institute of Research in Public Health of Bamako. Snails used for the study were *Bulinus truncatus* and *Biomphalaria pfeifferi* collected during the months of november and december 2012 from the Farako channel at Gomidjirambougou. The collection site extends over a hundred meters and is located not far from the Samé road. At this level, two bridges are connecting the Samé road to Lazaré. The collection site is characterized by sandy rock substrates where water pH was

Adama Dénou, Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (Mali);

Adiaratou Togola, Department of Traditional Medicine, National Institute of Research in Public Health (Mali);

Kari Tvette Inngjerdingen, Faculty of Mathematics and Natural Sciences, School of Pharmacy, University of Oslo.

Drissa Diallo, Faculty of Pharmacy and Department of Traditional Medicine (Mali);

Berit Smestad Paulsen, Faculty of Mathematics and Natural Sciences, School of Pharmacy, University of Oslo.

\*Corresponding author: Adama Dénou, University of Sciences, Techniques and Technologies of Bamako, BP:,

varied between 6 and 7. The collection site is shaded with an average sunshine.

**B. Maintenance of Snails in the Laboratory:**

Snails brought from the field were took off channel water to well water and separated by species. Then each specie was put in a plastic bin containing 50mL of well water having a pH equal 5. These bins that contained the snails were kept at room temperature and the animals were fed with fresh lettuce leaves (Fig. 1 and Fig. 2). After two days the well water of bins was renew. After three days the snails were transferred to test solutions and medium (distilled water with 1% dimethyl sulfoxide) for molluscicidal activity.

**C. Molluscicidal Activity**

Preparation of test solutions: These solutions are prepared by dissolution crude extracts (butanol and ethyl acetate extracts) and pure products (GO1 and 12-Glucosyl-GO1) both from *Glinus oppositifolius* in distilled water containing 1% dimethyl sulfoxide. To prepare the stock solution, 0.4 g of the extracts was dissolved in 1000 milliliters of dimethyl sulfoxide water solution to form a 400 ppm (0.4g/1000 ml) solution. This stock solution was then used to make a serial dilutions to obtain the 200, 100, 50 and 25 ppm.

Assay on molluscicidal activity: The evaluation of the molluscicidal activity of the extracts was determined in accordance with the world health



Fig. 1: *Bulinus Truncatus* were Feeding Fresh Lettuce Leaves, by Adama Dénou



Fig. 2: *Biomphalaria Pfeifferi* were Feeding Fresh Lettuce Leaves by Adama Dénou

organization technique. Five adult snails with shells and having the same size were exposed to 50mL of each test solution for 24 hours. The transparent tanks containing five snails were covered with compress gaze by using a sticker label in order to retain the snails in the tank. After exposure, the snails were rinsed and kept in the control medium (dimethyl sulfoxide water) for 1 hour then observed for

molluscicidal effect. A control group was kept in dimethyl sulfoxide water under the same experimental conditions. First five different concentrations were used for the crude extracts from *G. oppositifolius* : 400, 200, 100, 50 and 25 ppm. Secondly two different concentrations were used for the pure products from *G. oppositifolius*: 400 and 200 ppm. For each concentration, five snails were used with three repetitions. The animals were handled in accordance with the principles of animal welfare in scientific experiments. Mortality was recorded, and dead snails were removed immediately. Dead snails were identified by discoloration of the shell, the expulsion of the body of the shell, lack of movement and lack of reaction to irritation of the foot with a needle. Extracts with at least 50% mortality after this test were considered potent.

**Statistical analysis:**

The differences in mortality rate between study groups were assessed by using Excell sheet. Analysis of variance was used to compare the effect of the crude extract and pure product on snails by calculating lethal concentration fifty (LC50).

**III. RESULTS AND DISCUSSION**

The results of molluscicidal activity on snails are presented in tables I and II. Although BuOH extract has shown 100% of deaths on both snail species at 200 ppm, EtOAc extract was more active with 80% of deaths on *Bulinus truncatus* at 100 ppm but BuOH is more active on *Biomphalaria pfeifferi* with 80% of deaths at 100ppm. *B. truncatus* are more sensitive to crude extracts from *G. oppositifolius* than *B. pfeifferi*. At 24hours exposure BuOH extract shown LC50: 64.3 and 91.7 ppm respectively for *Bulinus truncatus* and *Biomphalaria pfeifferi* whereas EtOAc extract LC50 were 86.2 and 116.6 ppm for the former and the latest snails respectively.

We noted that the pure products had low activity on the two species of snails. However GO1 is more active than 12-Glucosyl- GO1 on *Bulinus truncatus* with 40% at 200 ppm.

Total extracts of *G. oppositifolius* were more active than pure products from the same plant. Experimentation has shown that *Bulinus truncatus* are more sensitive than *Biomphalaria pfeifferi*.

Tableau I: Molluscicidal activity of crude extracts (BuOH and EtOAc) of *Glinus oppositifolius* on snails after 24 hours exposure.

CRUDE EXTRACTS OF <i>G. OPPOSITIFOLIUS</i>	CONCENTRATIONS (PPM)	MORTALITY RATE (%) <i>BULINUS TRUNCATUS</i>	MORTALITY RATE (%) <i>BIOMPHALARIA PFEIFFERI</i>
BUOH	400	100	100
	200	100	100
	100	60	80
	50	40	0
	25	0	0
ETOAC	400	100	100
	200	100	100
	100	80	40

	50	20	0
	25	0	0
CONTROL	0	0	0

BuOH: butanol extract; EtOAc: ethyl acetate extract

Tableau II: Molluscicidal activity of pure products (GO1 and 12-glucosyl-GO1) from *Glinus oppositifolius* on snails after 24 hours exposure.

Pure products from <i>G. oppositifolius</i>	Concentrations (ppm)	Mortality rate (%) <i>Bulinus truncatus</i>	Mortality rate (%) <i>Biomphalaria pfeifferi</i>
GO1	400	60	20
	200	40	0
12-Glucosyl-GO1	400	40	20
	200	20	0
Control	0	0	0

GO1: saponin from *G. oppositifolius*, 12-Glucosyl-GO1: 12-Glucosyl of saponin from *G. oppositifolius*.

The results of this study have demonstrated the potency of crude extracts and pure products of *Glinus oppositifolius* on *Bulinus truncatus* and *Biomphalaria pfeifferi*. In Mali very few activities have done on molluscicidal plants. But Diallo et al. reported that the aqueous extract of *Glinus oppositifolius* have shown 100% mortality of *B. Pfeifferi* only at 200 mg/L [17]. In Ethiopia the aqueous extract of *Glinus lotoides* fruits had molluscicidal activity against *B. pfeifferi* snails and cercariacidal activity against *Schistosoma mansoni* [18]. Schistosomiasis is a disease of the rural population and requires the participation of the local community in control programs. According to WHO, crude organic extract should present LC90 below 20ppm to be considered a good molluscicidal candidate for direct application in infected water [19]. However, Mendes et al. suggested that extracts between 20 and 100ppm would contain some amount of very active components [20].

#### IV. CONCLUSION

This study revealed that all substances tested have a dose-dependent activity on *Bulinus truncatus* and *Biomphalaria pfeifferi*. From these results add to the previous we conclude that *Glinus oppositifolius* is a promising plant in the fight against the schistosomiasis.

#### ACKNOWLEDGMENT

The first author, Adama Dénou, thanks a lot Siantiki Konaté for snails collecting.

#### REFERENCES

[1] WHO. Schistosomiasis. Fact sheet. 2016.  
[2] Naples J, Shiff C, Halden R. Reduction of infectivity of schistosome cercariae by application of cercariacidal oil to water. *Am J Trop Med Hyg*, 2005, 73:956–961.  
[3] Mengistu M, Shimelis T, Torben W, Terefe A, Kassa T, Hailu A. Human intestinal schistosomiasis in communities living near three

rivers of Jimma town, southwestern Ethiopia. *Ethiop J Health Sci.*, 2011, 21:111–118.  
[4] Ezeh C, Yin M, Li H, Zhang T, Xu B, Sacko M, Feng Z, Hu W. High Genetic Variability of *Schistosoma haematobium* in Mali and Nigeria. *Korean J Parasitol*, 2015, 53(1): 129-134.  
[5] Gryseels B, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet.*; 2006, 368:1106–18.  
[6] CDC, 2011. The burden of schistosomiasis. Available: [http://www.cdc.gov/globalhealth/ntd/diseases/schisto\\_burden.html](http://www.cdc.gov/globalhealth/ntd/diseases/schisto_burden.html).  
[7] WHO. Prevention and control of schistosomiasis and soil transmitted helminthiasis. Geneva: WHO Technical Report, 2002, series No912.  
[8] Domo DA, Kela SL. The efficacy of *Maytenus senegalensis* (L) extracts on experimentally infected rats with *S. mansoni*. *Int J Biomed Hlth Sci*, 2009, 5:157–63.  
[9] Ndamukong KJN, Ayuk MA, Dinga JS, et al. Prevalence and intensity of urinary schistosomiasis in primary school children of the Kotto Barombi Health area, Cameroon. *East Afr. Med. J.*, 2001, 78: 287-289  
[10] Willmott S. Report of an independent evaluation mission on the national Bilharzia programme in Egypt, 1985. *Trans R. Soc. Trop. Med Hyg.*; 1987, 81: 1-57.  
[11] Lemma A. Laboratory and field evaluation of the molluscicidal properties *Phytolacca dodecandra* (Endod). *Bull Wld Hlth Org*, 1970, 42:597–612.  
[12] Azare AB, Okwute SK, Kela SL. Molluscicidal activity of crude water leaf extracts of *Alternanthera sessilis* on *Bulinus* (phy) *globosus*. *Afr J Biotechnol*, 200, 6:441–444.  
[13] Molla E. Molluscicidal and cercariacidal activity of *Balanites aegyptica*. MSc Thesis. Addis Ababa University, 2011.  
[14] Rug M, Ruppel A., Toxic activities of the plant *Jatropha curcas* against intermediate snail hosts and larvae of schistosomes. *Trop Med Int Hlth*, 2000, 5:423–430.  
[15] Debes SC. En medisinplante fra Mali, *Glinus oppositifolius*. Master's Thesis. School of Pharmacy, University of Oslo, Norway, 1998.  
[16] Diallo D. Ethnopharmacological survey of medicinal plants in Mali and phytochemical study of four of them: *Glinus oppositifolius* (Aizoaceae), *Diospyros abyssinica* (Ebenaceae), *Entada africana* (Mimosaceae), *Trichilia emetica* (Meliaceae). Thèse de Doctorat. Faculté des Sciences, Université de Lausanne, Switzerland, 2000.  
[17] Diallo D, Marston A, Terreaux C, Toure Y, Paulsen Hostettmann K. Screening of Malian medicinal plants for antifungal, larvicidal, molluscicidal, antioxidant and radical scavenging activities. *Phytotherapy Research*, 20015,401–406.  
[18] Kiros G, Erko B, Giday M, Mekonnen M. Laboratory assessment of molluscicidal and cercariacidal effects of *Glinus lotoides* fruits. *BMC Research Notes*, 2014, 7:220  
[19] WHO. The control of schistosomiasis. Second Report of the WHO Expert Committee. WHO Technical Report 1993, Series No 830, Geneva.  
[20] Mendes NM, Rogerior OQ, Telma SMG, et al.. Screening of Asteraceae (Compositae) plant extracts for molluscicidal activity. *Memorias Instituto Oswaldo Cruz, Roi de Janeiro.*; 1999, 94: 411-412.

**Adama Dénou**, Pharmacist since 2008 (Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali), this author became Assistant of Pharmacognosy in 2009, 2010-2012: MSc science field: biology, science branch: pharmacognosy (Faculty of Sciences, University of Lome, Togo).

List of publications: Google.Scholar



**Adiaratou Togola**, Pharmacist since 2002 (Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali). Researcher at Department of Traditional Medicine. Education: 2004-2008 PhD, science field: pharmacy, science branch: pharmacognosy ( Faculty of Mathematics and Natural Sciences , School of Pharmacy, University of Oslo, Norway).

List of publications: Google.Scholar

**Kari Tvete Inngjerdigen**, Faculty of Mathematics and Natural Sciences, School of Pharmacy, University of Oslo, Norway. Education: Associate Professor of pharmacognosy.  
List of publications: Google.Scholar.

**Drissa Diallo**, Pharmacist since 1982 (National School of Medicine and Pharmacy, Mali). Education: Professor of pharmacognosy since 2004 (Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako, Mali), 2001-2016: Chief of the Department of Traditional Medicine. April 2006 - May 2010: Vice Dean of Faculty of Medicine, Pharmacy and Odonto-Stomatology, University of Bamako, Mali.  
List of publications: Google.Scholar.

**Berit Smestad Paulsen**, Faculty of Mathematics and Natural Sciences, School of Pharmacy, University of Oslo, Norway. Education: Professor of pharmacognosy.  
List of publications: Google.Scholar.