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Efficacy of ethanolic leaf extracts of *Carica papaya* and *Terminalia catappa* as molluscicides against the snail intermediate hosts of schistosomiasis

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The study evaluated the molluscicidal effects of ethanolic leaf extracts of two Nigerian indigenous medicinal plants; *Terminalia catappa* and *Carica papaya*. Different concentrations 2500, 630, 160, 40 and 10 ppm were prepared from the stock solution of the extracts. Adult *Biomphalaria pfeifferi* and *Bulinus globosus* were exposed to these different graded concentrations. Mortalities were observed at intervals of time. There were strong positive correlations between mortalities observed in snails and extracts' concentrations; $R_2 = 0.997$ and 0.952 in *T. catappa* exposed *B. pfeifferi* and *B. globosus* respectively and $R_2 = 0.925$ and 0.937 in *C. papaya* exposed *B. pfeifferi* and *B. globosus* respectively. The lethal concentration (LC50) values for *B. pfeifferi* and *B. globosus* were (864.1, 1095.7 ppm) and (2716.3, 619.1 ppm) for *T. catappa* and *C. papaya* ethanol extracts respectively. The corresponding LC90 values were (1222.8, 1874.9 ppm) and (4515.9, 1180.7 ppm) for *T. catappa* and *C. papaya* ethanol extracts respectively. The results from the lethal concentration values showed that *B. pfeifferi* is more susceptible to *T. catappa* while *B. globosus* is more susceptible to *C. papaya* ethanolic leaf extracts.

Key words: Molluscicidal effects, medicinal plants, lethal concentrations, susceptibility.

INTRODUCTION

The number of cases due to schistosomiasis has been estimated to be around 200 million worldwide with 650 million people being at risk of infection (Chitsulo et al., 2000). Suggestions have been made on the possible resistance of schistosoma parasite to praziquantel; the mainstay drug in use over the decades. The freshwater planorbid snails; *Bulinus globosus* and *Biomphalaria pfeifferi* are the intermediate hosts of *Schistosoma haematobium* and *S. Mansoni*, that cause urinary and intestinal schistosomiasis respectively in Nigeria.

Snail control through the use of synthetic molluscicides also forms an important part in the integrated control programme for schistosomiasis. In the recent years, much attention has been given to the study of plant molluscicides because they might provide a cheap,

biodegradable and effective control way in rural areas of developing countries, where schistosomiasis is endemic (Brackenbury and Appleton, 1998). However, the toxicity of these molluscicides to non-target organisms and ecosystem destruction render them less efficient.

Several plant species have been proved to have molluscicidal properties against different snail species. The crude water extracts of *Alternanthera sessilis* showed molluscicidal activity against *B. globosus* (Azare et al., 2007). The histopathological effects of *Tetrapleura tetraptera* extract on some fresh water snails have also been reported (Adewumi and Ogbe, 1986). Others include the work by Ebele (1998) on the molluscicidal potency of *Polygoenum laningerum* on the fresh water snail, *B. globosus*, *Euphorbia splendens* Var. *Hislopia* on *B. glabrata* (Schall et al., 2001) and molluscicidal, ovicidal and cercaricidal activities of the parts of *Dalbergia sissoo* (Adenusi and Odaibo, 2008).

Carica papaya (Caricaceae) is believed to probably

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originate from southern Mexico and Costa Rica and then introduced as a plantation crop in all tropical and sub-tropical regions (Krishna et al., 2008). It contains broad spectrum of phytochemicals including polysaccharides, minerals, vitamins, proteins, enzymes, alkaloids, glycosides, fats and oils, lectins, saponins, flavonoids, sterols, etc. (Jean, 1999). The antimicrobial, antifungal and antihelminthic activities of the seed and latex of the plant have been extensively reported (Catzada et al., 2007; Okeniyi et al., 2007). *Terminalia catappa* (Combretaceae) commonly called tropical almond is grown in the tropical regions of the world as an ornamental tree (Nwosu et al., 2008).

In traditional medicine, *T. catappa* leaf, bark and fruit are used in treating dysentery, rheumatism, cough and asthma. The leaves have demonstrated anti-sickling activity (Moody, 2003) and are used in getting rid of intestinal parasites, treatment of eye problems, wounds and liver problems (Corner, 1997). The chloroform and methanol extracts of the bark and root, displayed strong antimicrobial activities (Pawar and Pal, 2002). In another study, addition of ethanol extracts of *C. papaya* and *T. catappa* independently to soft cheese ('wara'), suppressed the growth of enterobacteriaceae, molds and yeasts (Adetunji, 2008).

Little is known about the molluscicidal properties of *C. papaya* and no work so far has been conducted on molluscicidal properties of *T. catappa*. This work therefore assessed the efficacy of the two plants as molluscicides, against two major snail intermediate hosts of schistosomiasis in Nigeria.

MATERIALS AND METHODS

Sampling of snails

One hundred and thirty two young adult snails were collected by searching from the water outlet region of Awba reservoir, 'a man-made lake' in the University of Ibadan, Ibadan, Nigeria. They were properly washed in the water and transferred into a sterile glass jar and were then taken to the laboratory.

Preparation of plant extracts

The leaves of *C. papaya* and *T. catappa* were oven dried at 138°C and crushed into powder with mortar and pestle. The extraction of the plant extracts using 75% ethanol was done using soxhlet extractor, in the Department of Chemistry, University of Ibadan, Ibadan, Nigeria. Boiling flasks of 250 ml capacity were washed with detergent and were properly rinsed with clean water. They were dried in the oven at 105-110°C for about 30 min. They were transferred into desiccators and were allowed to cool. Five grams of the crushed leaves were weighed into a labelled thimble.

The boiling flask was filled with 200 ml of 75% ethanol and the extraction thimble was plugged tightly with cotton wool. The soxhlet apparatus was then assembled to allow for reflux for about 6 h. After 6 h, the thimble containing the sample was removed with care and the ethanol on the top was drained into a container. The extracts were then concentrated using water bath, which removed the ethanol component, leaving behind greenish-brown and

brownish viscous oil of *C. papaya* and *T. catappa* respectively.

Extracts bioassay

The stock solution was prepared by dissolving 1g of semi-solid plant extracts in 10 ml of distilled water. Serial dilutions with sterile distilled water were made from the stock solution to obtain; 2500, 630, 160, 40 and 10 ppm respectively.

Six adult *B. globosus* (of shell length 6.0 to 8.0 mm) and 5 *B. pfeifferi* (shell diameter 7.0 to 15.0 mm) were introduced simultaneously into appropriately labelled 100 ml beakers containing the different extracts' concentrations of *C. papaya* and *T. catappa*. The control experiment without extract concentration was also set up. All experiments were conducted at room temperature (26 to 28°C). The snails were not fed during the course of the experiment (Sermsart et al., 2005).

Personal observations have shown that healthy snails can survive without food for at least five days in water with good conditions. Mortalities were observed after 24 h and the data obtained from the mortalities of the snail after the recovery periods were plotted on probit regression graph to obtain the LC50 and LC90 respectively. The escape behaviour of snails in response to extract's concentrations was also observed.

However, the data on the number of snails escaping in each extract's solution was not taken into consideration. The correlations coefficients (R^2) between mortalities recorded and plant extracts' concentrations were also determined, to study the nature of association between the two variables.

RESULTS

Snails were observed crawling out of extract solutions and aggregating at the water-air interface some hours later, after exposure. Mortalities of *B. globosus* and *B. pfeifferi* to ethanolic extracts of *C. papaya* and *T. catappa* leaves, showed a similar pattern. Generally, mortalities increased with extracts' concentrations (Figures 1 and 2). No mortality was recorded in *B. pfeifferi* exposed to extracts' concentrations; 160, 40 and 10 ppm of the two plants after a 24 h exposure. One hundred percent (100%) mortalities were observed in both *B. globosus* and *B. pfeifferi* exposed to extracts' solution 2500 ppm of both plants.

There were strong positive correlations between mortalities observed in snails and extracts' concentrations; $R^2 = 0.997$ and 0.952 in *T. catappa* exposed *B. pfeifferi* and *B. globosus* respectively and $R^2 = 0.925$ and 0.937 in *C. papaya* exposed *B. pfeifferi* and *B. globosus* respectively.

The lethal concentration (LC50) values for *B. pfeifferi* and *B. globosus* were 864.1 ppm, 1095.7 ppm and 2716.3 ppm, 619.1 ppm for *T. catappa* and *C. papaya* ethanolic extracts respectively. The corresponding LC90 values were 1222.8, 1874.9 and 4515.9, 1180.7 ppm for *T. catappa* and *C. papaya* ethanolic extracts respectively (Figure 3). The confidence limits (95%) and the regression equations for the toxicity of *C. papaya* and *T. catappa* extracts on *B. globosus* and *B. pfeifferi* as obtained from the probit analysis is summarized in Table 1.

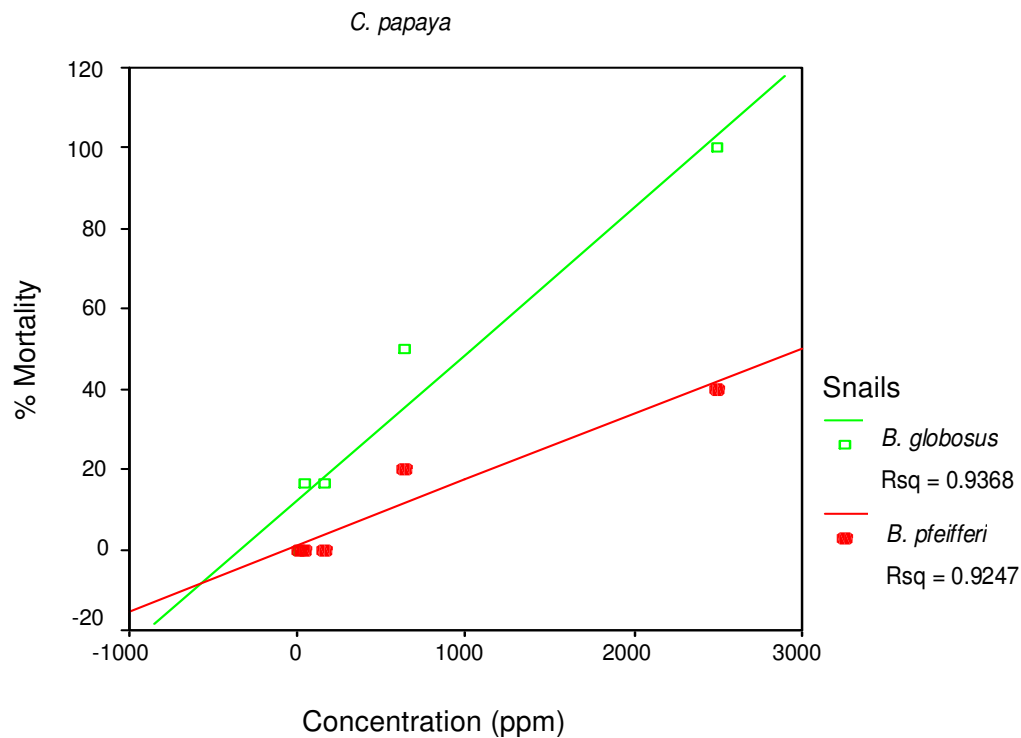


Figure 1. Snail mortality in response to concentration (ppm) of *C. papaya*.

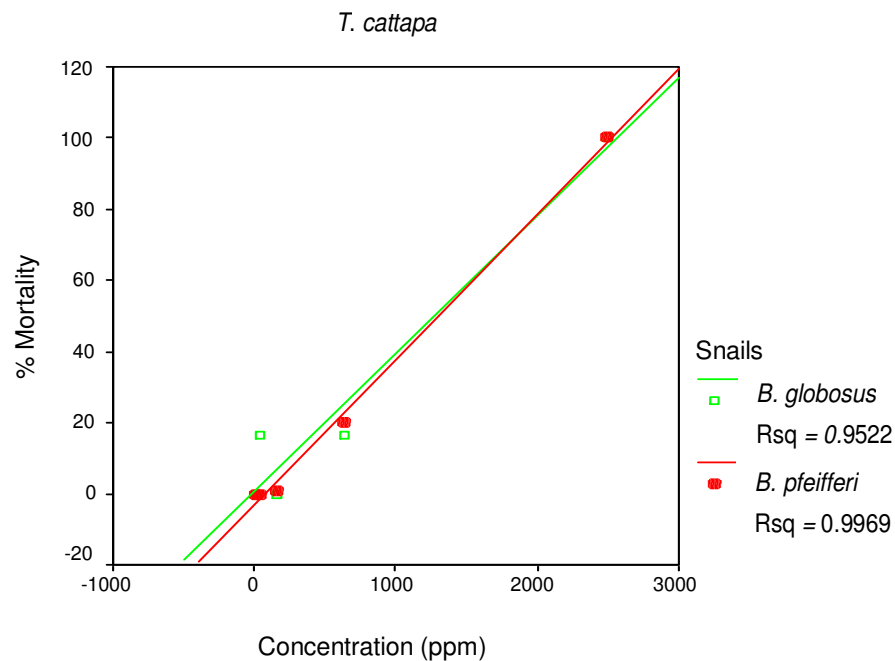


Figure 2. Snail mortality in response to concentration (ppm) of *T. catappa*.

DISCUSSION

The continuous crawling out from the test extract solutions and aggregation at the water-air interface, in

snails exposed to sub-lethal concentrations of test extracts was taken as an irritative, avoidance behaviour similar to that described by Evans et al. (1986) and Brackenbury (1999) in the pulmonate snail, *Bulinus*. The

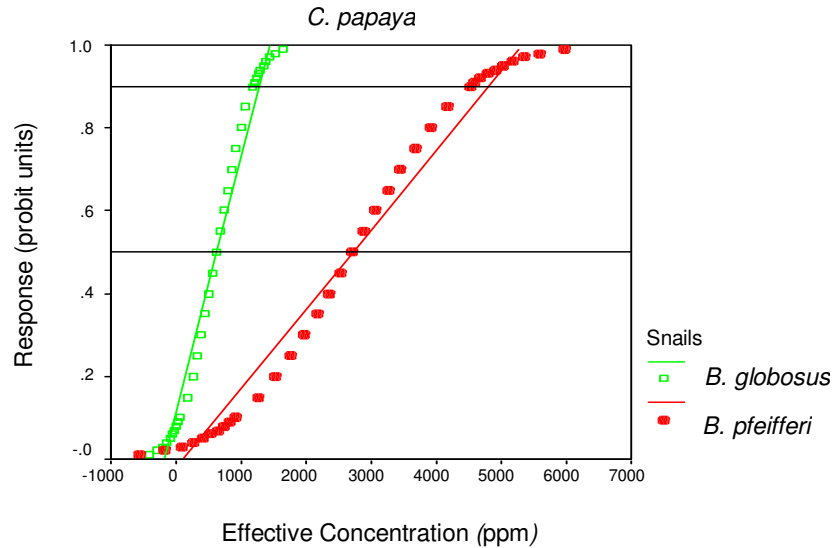


Figure 3. Probit regression graph for the determination of lethal doses of *C. carica* extracts on snails.

Table 1. Probit analysis of lethal concentration determination of plant extracts against snail intermediate host of schistosomiasis.

Extracts/snails	Regression equations	X ² (P>0.05)	LC50(ppm)	LC90(ppm)	Lower limit	Upper limit
TC/Bpf	Y = 0.004-3.087X	0.556	864.1	1222.8	-3.520	-2.654
TC/Bg	Y = 0.002-1.802X	53.21	1095.7	1874.9	-1.916	-1.688
CP/Bpf	Y = 0.001-1.934X	36.82	2716.3	4515.9	-2.066	-1.802
CP/Bg	Y = 0.002-1.413X	15.76	619.1	1180.7	-1.524	-1.301

Note: TC = *T. catappa*; CP = *C. papaya*; Bpf = *B. pfeifferi*; Bg = *B. globosus*.

abilities the snails have to crawl out of the plants' extracts, with concentrations not lethal to them and then aggregate at the water-air interface, where their soft tissues were not in close and continuous contact with the extracts observed in this study, are in consonance with the report of Adenusi and Odaibo (2008). This type of extract-leaving behaviour has been found to increase the survival of *Biomphalaria straminea* exposed to sub-lethal doses of niclosamide (Sarquis et al., 1997) and *B. glabrata* exposed to *Phytolacca dodecandra* (Jurberg et al., 1988).

The seemingly quick activities of extracts of *T. catappa* and *C. papaya* at 2500 ppm may be probably due to their acute toxic effects at this concentration. This is desirable, as it reduces the possibility of escaping behaviour (Sarquis et al., 1997). Similarly, immobility and lack of tactile response in snails exposed to copper sulphate and *T. tetraptera* have been reported (VanAardt and Coertze, 1981; Bode et al., 1996).

The results from the lethal concentration values showed that *B. pfeifferi* is more susceptible to *T. catappa* while *B. globosus* is more susceptible to *C. papaya* ethanolic extracts of leaves. Therefore, *T. catappa* seems

to be a better plant molluscicide for *B. pfeifferi* and *C. papaya* for *B. globosus*. Although several studies have assessed the molluscicidal activities of many plants against *B. pfeifferi* and *B. globosus*, no study on the toxicity of these folkloric medicinal plants has been conducted in Nigeria on the snail intermediate hosts of schistosomiasis. Since their antihelminthic activities have been established, this new approach widens the spectrum of their medicinal values.

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