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RELATIVE TOXICITY OF AQUEOUS EXTRACTS OF ROOT OF *PYCNANTHUS ANGOLENSIS* (WELW) AND *TALINUM TRIANGULARE* (JACQ) ON *BULINUS GLOBOSUS*

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ABSTRACT

Pycnanthus angolensis and *Talinum triangulare* are among the common valuable plants in West Africa due to their medicinal and nutritional value. This study was aimed at determining the relative toxicity of the ethanol roots extracts of *P. angolensis* and *T. triangulare* on *B. globosus*. Bioassay was evaluated at different concentrations of 160, 320, 480, 640 and 800 pm. Observations were made at 24, 48 and 72 hrs. Mortality of 100 % was recorded for 320 pm concentration of ethanol root extracts of *P. angolensis* at 72 hrs and 640 pm concentration of *T. triangulare* at 72 hrs respectively. Through out the duration of the experiment, *B. globosus* in the controls experiment were active. Based on the result of this present study, the roots of *P. angolensis* and *T. triangulare* are recommended as potential agents for the control of *B.globosus* which is one of the intermediate hosts of urinary schistosomiasis.

KEYWORDS: Relative toxicity, *Pycnanthus angolensis, Talinum triangulare Bulinus globosus.*

INTRODUCTION

In Nigeria and many West African countries, the issues of vector/ intermediate host related diseases are so alarming. This has resulted in man-power lost, coupled with the attendant economic implications. The resistant of these parasites responsible for these diseases has also aggravated the problems. For the effective control of these parasites like *Schistosoma*, multifarious approaches targeting the vector or intermediate hosts are urgently required (Okete *et al*., 2015).

At present Niclosamide (Baylucide, Bayer Germany) is the only commercially available synthetic molluscicide (WHO, 2005). However, this synthetic molluscicide tends to be biocidal at lower concentrations than those required to kill the snail hosts. This has necessitated the continued search for compounds which are safe, effective affordable and environment- friendly. It is inline with the above aforementioned background that the toxicity of the ethanol root extracts of *P. angolensis* and *Talinum triangulare* was evaluated on juvenile *Bulinus globosus*.

B. globosus is one of the indispensable intermediate hosts of *Schistosoma haematobium*- the causative agents of urinary schistosomisis. This snail host occurs throughout most parts of Africa and adjacent regions (Chitsulo *et al.*, 2000). Although the snails do not play an active role in transmission of the parasites from one host to another as do insect vectors; it is an indispensable intermediate host for the development of the parasites- *Schistosoma haematobium*.

Urinary schistosomiasis is a major disease of public health in humans, occurring in over 72 countries of the world (WHO, 2010). It is one of the neglected tropical diseases due mainly to the fact that its effects are not dramatic like malaria and HIV/AIDS, but are hidden diseases and somewhat subtle (Jordan and Webbe 1993). According to Gehad *et al*., (2009), the disease- schistosomiasis affects over 200 million people in different countries, including Nigeria. It is frequently considered the most important parasite disease after malaria among the infectious diseases of tropical and subtropical countries and is the third most prevalent parasitic diseases in the world in terms of overall morbidity burden, socio-economic and public health importance, and human impact (WHO, 2002).

Phyto-molluscicide is a simple inexpensive and appropriate technology for the control of snail vector, since the discovery of active ingredients such as saponnins in the berries of *Phytolacca, dodecandra*; naturally occurring molluscicides are receiving considerable attention (Brackenbury and Appleton, 1999). Studies have documented over 600 medicinal

plant species from over 100 families used in the treatment of various illness (Odugbemi, 2006). *Pynanthus angolensis* and *Talinum triangulare* are two of such medicinal important plants. *Pycanthus angolensis* belong to the family *myristicacea*e and is commonly called African nutmeg. In Nigerian languages; it is referred to as Akomu (Yoruba), Akujaadi (Hausa) and Egwunoma (Igbo), and Abakang (Ibibio), (Onwukaeme *et al*., 2007).

The laboratory evaluation of the ethanol leaf extracts of *Pycanthus angolensis* showed that it was molluscicidal at different concentrations *(*Okete *et al.*, 2015*).* According to a study by Agyare (2009), the plant was reported to be good for stomach ulcer treatment due to its anti-adhesive activity against *Helicobacter pylori* on human stomach cells. Investigation of the constituents of *Pycnanthus angolensis* roots has shown active constituents of cycloligene derivatives, namely *pycnanthuligene* A, and *pycnanthuligen*e D, both of which showed significant antimicrobial activities against a panel of drug resistant pathogens (Agyare, 2009).

Talinum triangulare belonging to the family Portulacacea is an herbaceous, perennial, caules cent and glabrous plant widely grown in tropical regions as a leaf vegetable (Ezekwe *et al.*, 2001). It serves as indispensable constituents of the human diet supplying the body with mineral vitamins and certain hormone precursors, in addition to protein and energy (Philipson and Wright, 1991). Nutritionally, *T. triangulare* leaves has been shown to posses the essential nutrients like B. Carotene, minerals (such as Calcium, Potassium and magnesium), Pectin protein and vitamins (Aletor and Adeogun, 1995). Traditionally it is used as softener of other vegetables, and medically, *T. triangulare* have been implicated in the management of cardiovascular disease like stroke, obesity (Adewumi and Sofowora, 1980) and control of freshwater snails (Edu *et al.*, 2015).

MATERIALS AND METHODS

Methodology

Descriptions of experimental sites

The experiment was conducted at the University of Calabar, Calabar in Calabar Municipal Local Government Area of Cross River State, Nigeria in August 2013-November 2013. Cross River State covers a land area of 20,156km^2 and is located at 5^0 45^1 N, 80 3' E. It lies within the tropical climate and has three major vegetation zones namely: Mangrove, Rainforest, and Derived savannah zones. The Mangrove zones consist of creeks and swamps with annual rainfall of about 2000 mm.The rainforest zone with a mixture of tall and small trees and shrubs has a moderate total annual rain fall of 1500-2000 mm while derived savannah in the Northern zone of the state has thorny bushes, scattered trees and low grasses with rainfall of 500-1000mm per annum. Humidity is about 65-90%, ambient temperature of 22.2^0-23.8^{0}C minimum and 27^0-40^0 C maximum (Mofinews, 2006).

Collection of snails (*B. globosus*)

Snails were collected from Ubam River which serves as a boundary between Adim and Abini community, all in Biase Local Government Area of Cross River State Nigeria. The information gathered revealed that the river was not treated with molluscicide prior to snail collection with molluscicides (Personal communication with the users of Ubam River). Trapping method as outlined by Azim and Ayad (1984) and employed by Hairston (1990) was used for snail collection. Freshly cut palm leaves and cassava leaves were placed along the bank of the snail- endemic river usually in the evening. After 2 days, the leaves were recovered and inspected for the presence of snails. Collection of snails was done in the morning when water temperature was about 25^o C between 8 am and 12 noon. The collected snails were put in a sterile polythene bag along with water from the habitats and transported to Parasitology Research Laboratory, Department of Zoology and Environmental Biology, University of Calabar, Calabar.

Snail identification and culture in the laboratory

Snails were identified using keys outlined by Brown (1984) and Christensen and Frandsen (1985). Sand and earthenware pot were placed in 20 liters aquarium in an attempt to obtain a low temperature in the aquarium. Ten liters of the river water and the grass from the site of collection were then placed in the aquarium. A thermometer was placed in the aquarium to take the temperature throughout the period of the study. The snails were acclimatized for a period of 7 days between 25^o C and 26^o C

Plant collection and authentication

T. triangulare was bought from farmland within University of Calabar, Calabar-Nigeria. The plant specimens were put in sterile dark polythene bags and transported to the Herbarium Unit of Botany Department, University of Calabar, Calabar where they were identified by experts based on taxonomic keys available in the unit.

Phytochemical screening

Qualitative and quantitative phyto-constituents of the roots of *P.angolensis* and *T.triangulare* were obtained from The results of previous reports of Okete *et al* (2015); Edu *et al* (2015).

Preparation and preservation of plant powders

The selected plant parts were oven dried at a temperature of 65^0 C for three days. The plant parts were thereafter separately pulverized using electric blender (Ken Wood). Each plant material/part well-labeled in the container was then stored in a refrigerator in the laboratory.

Preparation of *T. triangulare* extracts

Aqueous extraction of plant substances was performed using the method of Harbone (2006) with a little modification. For each plant part, a stock solution was prepared by soaking 80 g of pulverized plant parts in 500

ml (160,000 ppm) de chlorinated water for 72 hours. The mixture was shaken vigorously for 2 hrs intervals to ensure proper soaking of the product. After which decanting of the extract was done and the liquid extract was filtered using Whatman No.1 filter paper. The aqueous extracts were put in the reagent bottles and stored at low temperature in the refrigerator. From known milliliters of each of the extracts, different concentration were later prepared (serial dilutions) using a known volumes of water taken from the snails habitat (Okete *et al.,* 2015)

Extracts bioassay

The bioassay was performed as outlined by WHO (2005) and employed by Adenusi and Odaibo (2008, 2009), Singh and Yadav *et al.* (2010), and Philomena *et al.,* (2013). The different volume of 0.0 (control), 0.5, 1.0, 1.5, 2.0 and 2.5 ml from the stock solution of the extracts were added to equal volume (500 ml) of dechlorinated water (collected from the snails habitat) in bioassay boxes of 6. 5 cm depth x 14 cm length 8.5 cm breadth. Ten (10) snails ***(****B. globosus*) of weight range 6-12 g were immersed in different containers with varying serial dilution (Patole and Mahajan, 2010). There were triplicates for each concentration of each extract; three groups of ten snails each were kept in 500 ml of water collected from the snails' habitat as control groups. The concentration of each solution was calculated in ppm; 160, 320, 480, 620 and 800 ppm respectively. After 24h of exposure to the plant extracts, the snails were first examined using irritability function and hydro sensitivity. Those snails that were motionless or do not react to needle probe by closing their opercula were transferred to fresh declorinated water for another 24 hrs after which mortality was ascertained. According to El-Sheerbini *et al.* (2009), any plant extract that causes no mortality at 1000 ppm should be considered inactive and further investigation .The total examination period was 72 hrs.

Statistical analysis

Probit analysis of the raw data was carried out using Statistical Package for Social Science (SPSS) Software (Version 17.0) designed by Finney and Steven (1984) and employed by Philomena *et al.* (2013) to obtain the lethal concentration values. Analysis of variance (ANOVA) was used to test the significance differences in mean percentage mortality with different plant extract concentrations

RESULTS

The *B.globosus* became hyper active at first exposure to the different extract concentrations and then gradually became inactive. Exposure of the *B. globosus* to different concentrations of the ethanol root extracts of both plants resulted in mortality, though of varying degrees. The mortality effect in all the ethanol root extracts of all the plants used were concentration and time dependent.

The efficacy of the ethanol root extracts of *P.angolensis* and *T.triangulare* are shown in Table 1. The 78 h Lethal Concentration (LC_{50}) value of the ethanol root extracts of *P.angolensis* was 102.33 ppm while that of the ethanol root extract of *T.triangulare* was 199.53 ppm. The results revealed that the ethanol root extract of *P.angolensis* was more potent to juvenile *B.globosus* than the ethanol root extract of *T. triangulare.*

Table 1: Relative Toxicity of The Ethanol Root Extracts of *P.angolensis* and *T.triangulare* on Juvenile *Bulinus globosus.*

Extraxt Con. (ppm)	No.of *B.globosus* Exposed	Mortality value (%)					
		(Pa) 24 h	(Tt) 24 h	(Pa) 48 h	(Tt) 48 h	(Pa) 72 h	(Tt) 72 h
120	10	2(20)	1(10)	5(50)	3(30)	8(80)	6(60)
320	10	5(50)	3(30)	8(80)	6 (60)	10 (100)	8(80)
480	10	6(60)	5(50)	8(80)	8(80)	10 (100)	8(80)
640	10	7(70)	6(60)	10(100)	8(80)	10(100)	10(100)
800	10	8(80)	8(80)	10 (100)	10(100)	10(100)	10(100)
Control	10	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

Legeend
24, 48, 72= Exposure period in hours
Pa= *P.angolensis*
Tt= *T. triangulare*

DISCUSSION

Phyto-molluscicides occur in the leaves and roots of plants (Okete *et al.,* 2015). From the results of this present study, all the ethanol plants extracts exerted varying degrees of percentage mortality on the juvenile *B.globoscus* snails used in this experiment when compared to the controls. Although the reasons for the molluscicidal potency and mode of action of plant substances have not been fully studied, plant substances have been reported to cause mortality of several Genera of snails including *B.pfeifferi* snails (Otarigho and Olajumoke, 2012). Several authors including Kloos (1982) and Olofintoye, 2010) have attributed the molluscicidal potency of plant substances to some active phytochemical constituents including saponins. However, these authors did not explain the biocidal

activity of these phytochemicals. Cunha and Roque (2005) attributed the biocidal properties of saponins to haemolytic properties that disorganize the membrane. It is therefore thought that the saponins present in these plant substances may have exerted a haemolytic effects and subsequently leading to the death of these *B. globuscus* snails in this experiment.

The different phytochemicals may have also exerted their effects singly or in synergy. It is also probably that the phytochemicals in the extracts affected/altered the water chemistry/ physiology of the *B. globosus* and could thus result in the mortality.

The differential potency of the extracts against the test organisms is inline with the report of other researchers. For instance Olofintoye (2010), in his study on the comparative evaluation of molluscicidal effects of *Securidaca longepedunculata* and *Tephrosis bracteolata* observed the extract of S. *longepeunculata* was more potent than that of *T. bracteolata*, to adult B. globosus. The potency of plant extracts on test organisms / target species depends on several factors, one of which is the plant parts/species from which the extract is derived (Sukumar *et al.*, 1991). In the control of urinary schistosomiasis, phyto- molluscicide is a suitable option since it is an approach which is environment-friendly, cheap, easy to perform and its readily availability at the local levels.

The results obtained from this present research revealed the potency and possible application of the roots of *P. anglensis* and *T. triangulare* for the control of *B.globosus* which is one of the vectors of urinary schistosomiasis.

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