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Molluscicidal Effect of Ethanolic Leaf Extract of *Ocimum gratissimum* Against Adult *Bulinus globosus* in Abua/Odual Local Government Area, Rivers State, Nigeria.

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Abstract
The molluscicidal effect of seven different concentrations of ethanolic extract of Ocimum gratissimum (Scent leaf) against ten adult Bulinus globosus was investigated under laboratory conditions. The snails, collected from the vegetation at the banks of the streams in Anyu, Obedum and Emirikpoko communities in Abua/Odual Local Government Area, Rivers State, using a scoop net and some snails also hand-picked from vegetation where possible, were introduced simultaneously into appropriately labelled 1000ml beakers containing the different extracts' concentrations of O. gratissimum. The liquid extract obtained from 1kg dried and powdered leaf of O. gratissimum, exhaustively macerated in five litres of 99% ethanol for 48 hours was subsequently concentrated to dryness in a vacuum at 70^0C using a rotary evaporator and stored in a glass container. The stock solution was dissolved in deionised water and various concentrations administered were prepared for use by serial dilution. Mortality was assessed after twenty-four hours of exposure of the snails to the extract. One hundred per cent (100%) mortality was recorded in snails exposed to extract concentration of 40 ppm and 100 ppm. The 24-hour LC_{50} for the leaf extract was 15.34 ppm. Result showed no effects on the control. The study revealed that there is a very highly significant correlation ($r = 0.987$, $P<0.1\%$) between the mortality of Bulinus spp. of snail and the concentrations of O. gratissimum.

Keywords*: ethanolic extract, Bulinus globosus, Ocimum gratissimum, Schistosoma haematobium*

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I. Introduction

Schistosomiasis, caused by trematodes in the genus *Schistosoma*, is a chronic and debilitating neglected tropical disease [1], estimated in 2015 to have currently infected 200 million individuals worldwide [2]. The disease is the second most significant parasitic disease [3], especially in developing nations in both tropics and subtropics [4]. Schistosomiasis exhibits a marked geographical specificity that is associated with the endemicity of the snail intermediate hosts. The causative agent of urinary schistosomiasis, *S. haematobium*, is transmitted by *Bulinus* species of snail [5, 6]. *S. haematobium* infection is widely distributed in Nigeria and is hyper endemic in many loci in the northern and southwestern states, with moderate to low endemicity in the south eastern states [7, 8]. Control of urinary schistosomiasis deposits a great socioeconomic toll on infected individual in endemic regions in Nigeria. Like all parasitic diseases, effective control strategy involves an integrated approach aimed at reducing transmission, morbidity and incidence especially in highly endemic areas [9]. This method of control has been used for schistosomiasis in some endemic areas with desirable results ([10, 11]. Schistosomiasis control involves the use of chemotherapy, control of the snail intermediate hosts through the application of molluscicides, environmental and biological methods; health education; clean water supply and basic sanitation [12]. Chemotherapy with anti-schistosomal Praziquantel (PZQ) has been widely and effectively used for the reduction of morbidity of schistosomiasis [13, 14]. Elimination of snail intermediate host of schistosomiasis has been widely advocated as an arm of the integrated control for schistosomiasis in endemic countries [15]. The World Health Organization has tested thousands of synthetic compounds for the control of the snail host. Although effective, the high cost of these synthetic molluscicides and their negative impacts on the environment have so far proved them to be entirely inappropriate [16, 17]. With the growing awareness of environmental pollution, efforts are being made to discover molluscicidal products of plant origin worldwide [18, 19, and 20].

Being products of biosynthesis, Organic extracts are biodegradable in nature [21]. The use of plants with molluscicidal properties is simple, inexpensive and appropriate for the local control of the snail vector in

the endemic areas of poor nations of the world [22]. The use of plant molluscicides is attractive due to the economic advantage of cultivating the plants locally instead of importing synthetic compounds [23]. Several groups of compounds present in various plants have been found to be toxic to target organisms at acceptable doses ranging from <1 to 100 ppm. Laboratory screening of Nigerian medicinal plants has shown that some of these contain chemicals which are among the most potent natural molluscicides available today [22]. In view of the fore going, an attempt has been made in this study to evaluate molluscicidal effect of leaf extracts of *O. gratissimum* (Scent Leaf), an aromatic medicinal plant belonging to *Lamiaceae* family [24], which might be useful in the control of vector snails in the study area.

II. Material and Methods

Study Area

The study was conducted in Anyu, Obedum and Emirikpoko Communities of Abua/Odual Local Government Area in Rivers State, Nigeria. The population of Abua/Odual Local Government Area was projected at 282,410 at the 2006 population census. Anyu community is located on latitude 4° 52' N and longitude 6°25' E. Emirikpoko lies between Latitude 4° 52' 58" N and Longitude 6° 27' 05" E, while Obedum is located on Latitude 4° 54' 07" N and Longitude 6° 26' 17" E. The three communities enjoy the tropical rainforest vegetation; have a broad coastal plain topography with so many ponds and streams. The inhabitants are predominantly peasant farmers and artisanal fishermen who live in clustered homesteads of mainly mud houses, reinforced with bamboo sticks.

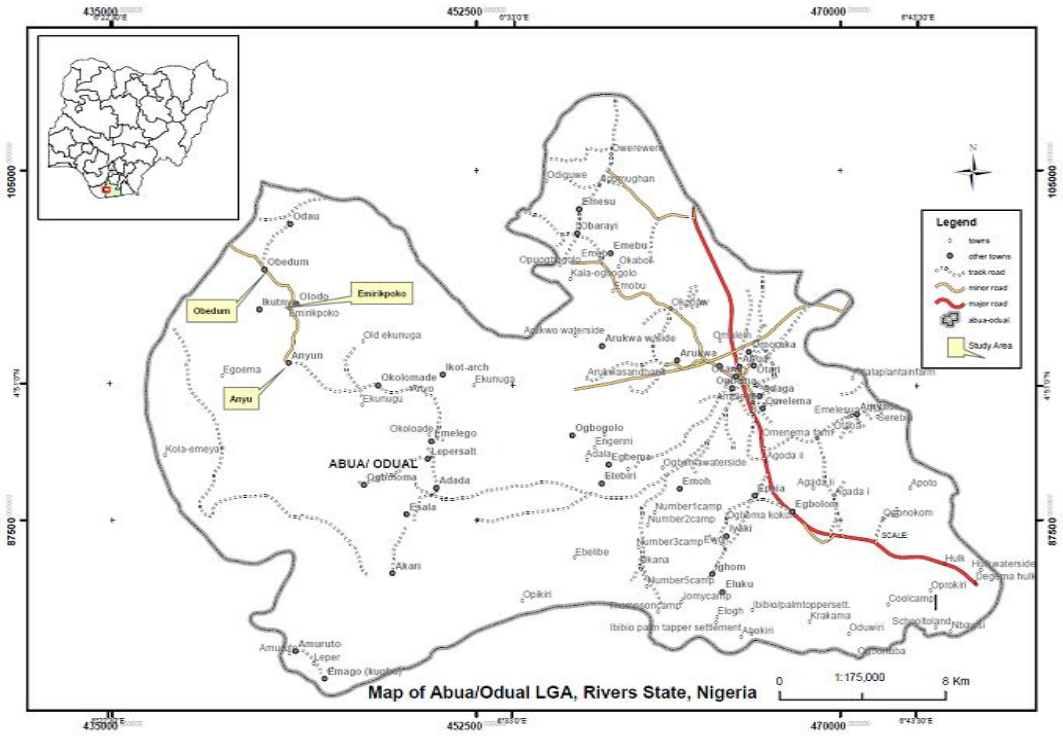

Figure 1., Map of Abua/Odual Local Government Area showing study area.

Collection of Snails and Plant Materials

Snails of the genus *Bulinus* were collected from the vegetation at the banks of the streams in the Anyu, Obedum and Emirikpoko communities in Abua/Odual Local Government Area, Rivers State, Nigeria, following standard malacological methods [25]. A scoop net was used to collect the snails and some snails were also hand-picked where possible. Snails collected were put in plastic containers, labelled and transported to the laboratory for analysis. Fresh leaves of *O. gratissimum* (Scent leaf) were procured at popular Oil mill market, Eleme junction in Obio/Akpor Local Government Area, Rivers State, Nigeria. They were identified in the Department of Plant Science and Biotechnology, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Preparation of Extract and Evaluation of Molluscicidal Activity

The plant material was air dried, then grinded in a blender to fine powder. 1kg of the powdered leaf of *Ocimum gratissimum* was exhaustively macerated in five litres of 99% ethanol for 48 hours. The liquid extract

obtained was subsequently concentrated to dryness in a vacuum at 70^0C using a rotary evaporator and stored in a glass container. This was dissolved in deionised water to make the stock solution from which the various concentration administered were prepared for use by serial dilution [26].

Ten adult *B. globosus* (of shell length 5.0 to 9.0 mm) were introduced simultaneously into appropriately labelled 1000 ml beakers containing the different extracts' concentrations of *O. gratissimum.* Each beaker was covered with nylon netting to prevent the snails from escaping. The control experiment without the extract was also set up. The snails were not fed during the experiment according the recommendations of [27]. Exposure and recovery periods were 48 hours in all the tests and all tests were carried out in duplicate. Mortalities were observed after exposure period of 24 hours [28]. Extrusion of the whole head-foot region permanently outside the shell or lack of any movement of the body when touched with a blunt stir rod was considered to determine mortality of the snail.

Data Analysis

Data obtained was analysed using EPA software, version 1.5. Probit analysis was used to determine the lethal concentration of the ethanolic leaf extract of the plant [29].

III. Results

Mortality of *B. globosus* in different concentrations of ethanolic leaf extract

Table 1 shows the effects of ethanolic leaf extract of *O. gratissimum on* adult *B. globosus* at different concentrations. Different percentage mortalities were recorded in different concentration levels of extract. Sixty percent (60%) mortalities were recorded in snails exposed to extract concentration of 15 ppm. One hundred percent (100%) mortalities were observed in snails exposed to extract concentration of 40 ppm and 100 ppm, while no mortalities were recorded in extract concentration of 5ppm and in the control. Generally, mortalities increased with extracts' concentrations (Fig.1).

Table 1., Mortality (%) of *B. globosus* in different concentrations of *O. gratissimum* ethanolic leaf extract.

Concentration (ppm)	(%) Mortality	log conc	Probit %
5	0 (0%)	0.699	0
10	1 (10%)	1	3.7184
15	6 (60%)	1.1761	5.2533
20	9 (90%)	1.301	6.2816
25	9 (90%)	1.3979	6.2816
40	10 (100%)	1.6021	8.72
100	10 (100%)	2	8.72
Control (H_20)	0 (0%)	-	-

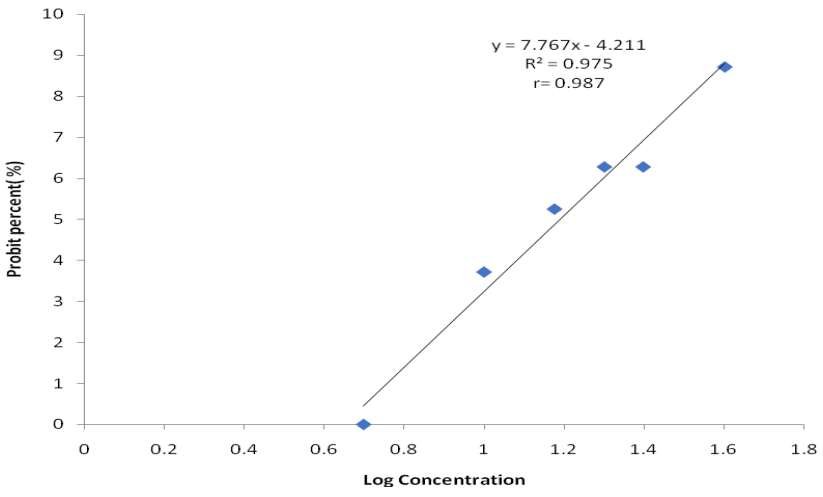

Figure 1. Probit plot of mortality of *B. globosus* exposed to different concentrations of ethanolic extract of *O. gratissimum.*

The lethal concentration of the plant extract

The lethal concentration of the plant extract resulting in 50% mortality of the snail (LC_{50}) was determined from the 24-hour counts mortality. The 24-hour LC_{50} for the leaf extract was 15.34 ppm. The LC_{90} value ranged from 18.67 to 40.68 ppm (Table 2). The study revealed that there was a very high correlation (r= 0.987, P<0.1%) between the mortality of *Bulinus* snail and the concentrations of *O. gratissimum.* There was a significant different between the extract concentration and snail mortalities in the study.

Table 2., Estimated LC/EC Values and Confidence Limits

Exposure point	95% Confidence Limits		
	Conc.	Lower	Upper
LC/EC 1.00	7.698	1.035	11.233
LC/EC 5.00	9.421	2.008	12.754
LC/EC 10.00	10.492	2.853	13.679
LC/EC 15.00	11.283	3.609	14.363
LC/EC 50.00	15.342	9.372	18.383
LC/EC 85.00	20.859	17.381	32.943
LC/EC 90.00	22.432	18.699	40.683
LC/EC 95.00	24.983	20.451	56.670
LC/EC 9900	30.575	23.588	108.215

IV. Discussion

The result from the present study indicated that ethanolic leaf extracts of *O. gratissimum* have variable lethal effects on adult *B. globosus* snail depending on the length of exposure and the concentration of the extract. In this study the LC50 was relatively high. This indicates that the bio-molluscicidal activity will require a high extract to solvent ratio for its effectiveness. As expected the upper limit lethal effect of the extract revealed that at higher concentration, the *O. gratissimum* extract had total molluscicidal effect on the adult of *B. globosus.* This is consistent with the observation of [30] that the mortality of snails increased with increasing concentration and exposure period in their study of the molluscicidal activity of some Indian medicinal plants at different concentrations. The findings of [31] reported that many plant molluscicides showed high potency against adult snails but failed to show molluscicidal activity against juveniles or in some cases, has no ovicidal activity unless the concentration is increased. This is also a major setback of organic extracts in the control of parasites or vectors. The toxic activity of ethanolic extract may be due to various compounds; including phenolics, triterpenes, saponins and alkaloids which are known to possess molluscicidal activity and are present in various plants [32]. These compounds may jointly or independently contribute to produce molluscicidal actions against *B. globosus* snails.

As earlier stated, the lethal concentration of LC_{50} value (15.34 ppm) observed in this plant was higher than the finding of [33] who recorded LC_{50} of 0.19 ppm for *B. globosus* using ethanolic leaf extract of *T. bracteolate*. This indicates a low probability of effectively controlling the snails with moderate concentrations of the extract which will definitely hamper application of the extracts in naturally occurring waters. The World Health Organization reports that plant extracts showing LC_{50} less than 40 ppm (0.04%, 0.4µg/ml) has some potential to be applied as molluscicidal or larvicidal compound [34]. As feasible as the statement by [34], it remains a challenge to procure the quantity of the *O. gratissimum* to sustain a pest control programme.

V. Conclusion and Recommendations

The use of *O. gratissimum* leaf extract as molluscicides in the control of schistosome vector *B. globosus* may play a vital role in eradication of the disease since the plant is commonly available all year round in the study area. However, the extract can be effective only on small scales purposes. Urinary schistosomiasis can in principle be eliminated by an integrated approach, which takes into account the local conditions of the general problem of urinary schistosomiasis in Abua/Odual Local Government Area and in Nigeria. There is still an urgent need for further phytochemical investigation of this and other plants to sustain any progress that may have been recorded. Studies on natural molluscicides in laboratory and clinical applied field research should be well funded and strongly supported by the Government and the private sector in a current period of economic depression. Studies are recommended to observe the stability of *O. gratissimum* leaf extract under various physico-chemical conditions and to determine the active ingredient, with the aim of undertaking further field trials. Further laboratory tests are required to search for the presence of active component in the different parts, that is, stem, flowers, fruits and roots. Finally, the application of molluscicides must be carefully planned to take advantage of focal and seasonal patterns of transmission.

Conflict of Interest

The authors have declared that no conflicts of interests exist.

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