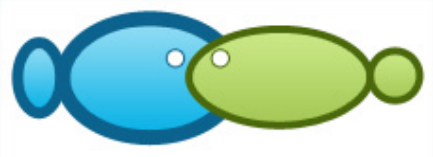

Acute toxicity of rabbitfishes *Siganus* spp. (Siganidae) crude venom extract on tilapia *Oreochromis mossambicus* (Cichlidae)

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Abstract. The overall objective of this work was to determine the acute toxicity of crude venom extract from rabbitfishes (*Siganus* spp.) on tilapia (*Oreochromis mossambicus*) fingerlings. A 24-hour static bioassay consisting of six treatments (one control and five concentrations) with three replicates was conducted under laboratory condition. The median lethal concentration (LC_{50}) was found to be 2.97 mLL^{-1} and the toxic reactions exhibited by fish include; discoloration, gulping air on water surface, erratic swimming, loss of reflex, slow opercular movement and settling at the bottom motionless. These manifestations by tilapia were severe during the first two hours which resulted to death of numerous individuals. However, after two hours, those individuals that have survived exhibited normal swimming behavior. Despite being categorized as relatively harmless, the rabbitfish crude venom has demonstrated fatal effect on tilapia fingerlings.
Key Words: secondary metabolites, fish bioassay, probit analysis.

Introduction. Fish stings and envenomation are common problems in tropical countries among fishers, beachgoers and seafood processors because they are exposed to many opportunities to encounter these problems as they undergo their activities. Over 200 species of fish are known to be venomous (Russell 1965; Deakins & Saunders 1967). However, phylogenetic analysis of fish presumed over 1,200 species found to be venomous (Smith & Wheeler 2006). Known venomous fish include; catfishes, scorpionfishes (stonefish, lionfish and waspfish), stingrays and rabbitfishes (Tam et al 2007). The stonefish (Family Scorpaenidae) are believed to be the most venomous fish in the world (Phoon & Alfred 1965; Sutherland 1983; Lyon 2004). Venomous fish possess pungent spines with venom glands in certain fins. When stung by the spines, the venom enters the wound, inducing intense pain and other local effects such as swelling and redness of the skin; in severe cases of poisoning, death may occur. Fish venoms are usually mixtures of heat-labile high molecular weight proteins with systemic toxic effect and low molecular weight amines which cause inflammatory reactions. The components which are responsible for pain and poisoning of the sting tend to be large, unstable proteins that are rapidly destroyed by heating. Fish venoms usually act directly on muscle tissue and have little, if any, effect on the nervous system or on the coagulation profile (Tam et al 2007; Chan et al 2010). However, Church & Hodgson (2002) argued that all piscine venoms produce profound cardiovascular changes, both in vitro and in vivo, including the release of nitric oxide from endothelial cells, smooth muscle contraction, and differing effects on atria. Atkinson et al (2006) and Tam et al (2007) recommended the primary treatment of venomous fish sting is to inactivate the heat-labile venom by immersing the injured parts in hot water (42^{o}C) for 30-90 minutes.

Like other venomous fish, rabbitfishes (Family Siganidae) use their venom for defensive purposes. Human envenomation occurs accidentally when swimmers or fishers mishandle or accidentally step on the spines of the dorsal fin. Clinical features of

rabbitfish sting are manifested by localized pain with no swelling or redness and foreign body and infection are the main concern (Church & Hodgson 2002).

The toxicity of most venomous fish remains a largely untapped source of novel compounds probably due to the labile nature of venoms as well as the difficulty in collecting sufficient samples. This study aims to determine the acute toxicity of crude venom extract of rabbitfishes (*Siganus* spp.) on tilapia (*Oreochromis mossambicus*) fingerlings.

Material and Method

Extraction of Crude Venom. It was reported that rabbitfishes contain venom in dorsal, anal, pelvic fins spines as well as in basal tissues. Overall, a total of 410 g of dorsal, anal and pelvic fins spines with its basal tissues (Fig. 1a) were removed from 22 kgs fresh rabbitfishes (*S. canaliculatus* and *S. guttatus*) collected from fish market. Spines and basal tissues were minced, homogenized and macerated on ethanol for 72 hours (Fig. 1b). Initially, primary and secondary extracts were removed by filtration using cloth (Fig. 1c). Combined extracts were further filtered using whatman filter in vacuum pump. Finally, crude venom extract was concentrated using rotary vacuum evaporator (Fig. 1d). The total volume of crude venom extract derived was 60 mL (Fig. 1e). This was used in the range finding test and the final definitive test.

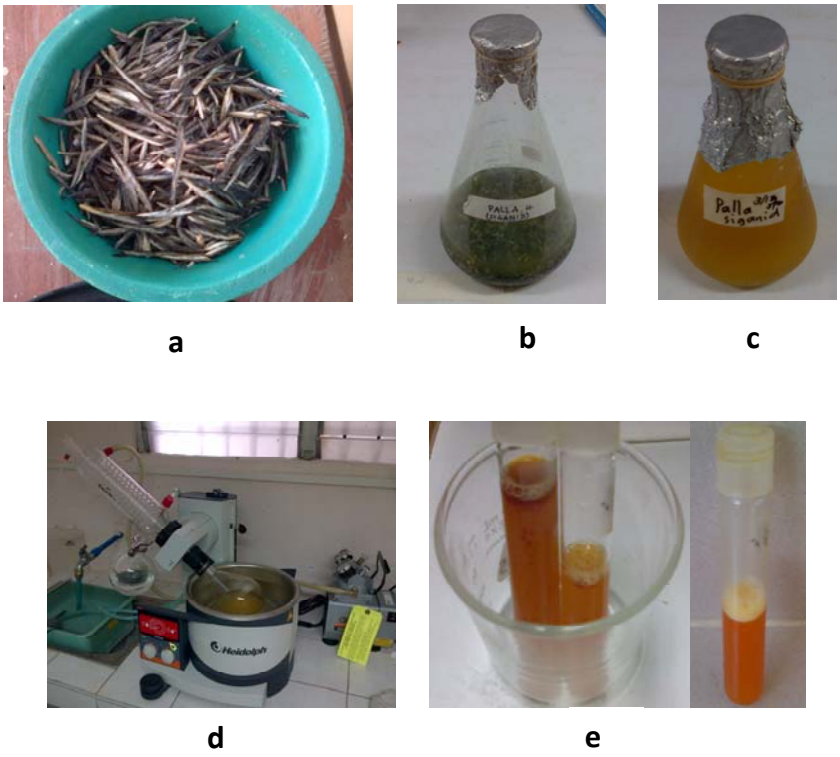

Figure 1. Extraction of crude venom from rabbitfish (a – fresh dorsal, anal and pectoral spines along with basal tissue, b – maceration in ethanol, c- filtered extract, d- filtered extract concentrated in rotary vacuum evaporator, e- concentrated crude venom extract).

Acclimation of Fish. Three hundred individuals of tilapia (*O. mossambicus*) fingerlings with an average length of 44.11±0.50 mm (mean TL,±s.e) and weight of 1.28±0.05 g (mean body weight, ±s.e.) were bought from tilapia hatchery. These specimens were acclimated in aerated freshwater tank for seven days and fed with fish pellets.

Range-finding test. During the range-finding test, feeding was halted and fish in healthy conditions which were observed as visibly free of any deformities, lesions, or

disease were selected for the experiment. A 24-hour range finding test was conducted on six treatments with five concentrations (0.01, 0.10, 1.00, 2.00, 3.00 mLL^{-1}) and a control (0 mLL^{-1}) with 10 individuals in each treatment without replication. Those fishes which did not show any tactile response were considered dead. Fifty percent mortality was recorded at 3.00 mLL^{-1} concentration after 24 hours period. Thus, the final concentrations were determined for the definitive test which followed the next day based on the above result.

Definitive Test. The definitive test was carried out in five concentrations (3.0, 3.5, 4.0, 4.5, 5.0 mLL^{-1}) and a control (0 mLL^{-1}) with three replications for tilapia (33-59 mm, TL) each in 1 liter plastic container with aerated freshwater (pH 7.8–8.0). The experimental set-up used Completely Randomized Design (CRD) and calculation of acute toxicity followed the procedures given by Finney (1978).

Gross mortality of fish in each concentration was recorded every hour for the first 12 hours and every two hours thereafter for 24 hours, with dead fish removed every two hours. Tilapia were not fed throughout the test. The LC_{50} values were determined from maximum likelihood estimates of linear functions relating log crude extract concentration to probit transformations of percent mortality (Finney 1978). The LC_{50} values were determined using mean assayed concentrations and cumulative mortality. Statistical comparisons between LC_{50} values were based on the standard error of the difference. When it became apparent that no statistically significant differences in LC_{50} values had occurred between bioassay replicates ($p>0.05$), the replicates were pooled, and a single LC_{50} was calculated for crude extract.

Results and Discussion. The 410 g spines and basal tissues of rabbitfishes had produced 60 mL of ethanolic crude venom extracts. This gives a 15% turnover of crude venom from fresh spines and basal tissues. This means that 1 kg of spines and basal tissue could produce 150 mL of crude venom extracts. The ratio of spines and basal tissue to body weight of fish was 1.5% which indicates that it needs a lot of fish to derive more venom extracts.

Probit analysis revealed a LC_{50} to be 2.97 mLL^{-1} (Fig. 2). This value was found to be higher than those reported in the literature. This higher recorded concentration required to kill the subject could be attributed to the kind of test species wherein in this case tilapia, which is known as hardy species. Accordingly, the aquatic toxicity rating scales for fish set by the United States Fish and Wildlife Service (USFWS) research and information bulletin categorized this value as relatively harmless (>1,000 mgL^{-1}). However, the rating scales presented by the USFWS correspond to pure venom or pure compound used in the toxicity test. In this study, the ethanolic venom extract was used, thus when crude extract used herein is further purified it might have obtained an even lesser concentration. Akinbulumo et al (2004) reported a lower LC_{50} (698 mgL^{-1}) of crude ethanolic extract of *Derris elliptica* roots tested to *O. niloticus.* This indicates that *Derris* extract is more lethal than venom of rabbitfish. In comparison with bioassay of *Moringa oleifera* aqueous seed extract on nile tilapia (Ayotunde et al 2011) against this study, *Moringa oleifera* aqueous seed extract is many times more lethal than rabbitfish venom. Ayotunde et al (2011) reported an LC_{50} of 252 mgL^{-1}. To test the sensitivity of synthetic chemicals on tilapia, Ayoola (2008) applied glyphosate herbicide, while Benli & Koksal (2005) used ammonia. Both results showed lower LC_{50} than the present study, suggesting synthetic chemicals are highly toxic on tilapia.

The behavior of fish observed in this study conformed to the observations by Akinbulumo et al (2004) on *O. mossambicus*. During the first two hours, fish displayed erratic swimming movements, jumping above the water surface, rapid opercular movements, loss of balance, incessant gulping of air on the surface, blackening of the whole body, unusual lethargy and fish settling at the bottom motionless with slow opercular movements. This erratic swimming behavior resembles the response of muscle tissue on the venom (Tam et al 2007), while the incessant gulping of air on the surface manifests the profound cardiovascular change and differing effects of venom on atria (Church & Hodgson 2002).

Fish was considered dead when it does not show any tactile response when touched. Highest mortality occurred during the first hour in all concentrations and none from the control, while death occurrence ended after 10 hours of test. This indicates that effects of venom on fish is immediate. Fish that survived showed normal behavior, displaying movements similar to those which are not subjected to venom test despite of some accumulation of fecal matter in the water media. In the control treatment, all fish survived with no obvious changes in fish behavior until the end of the experiment.

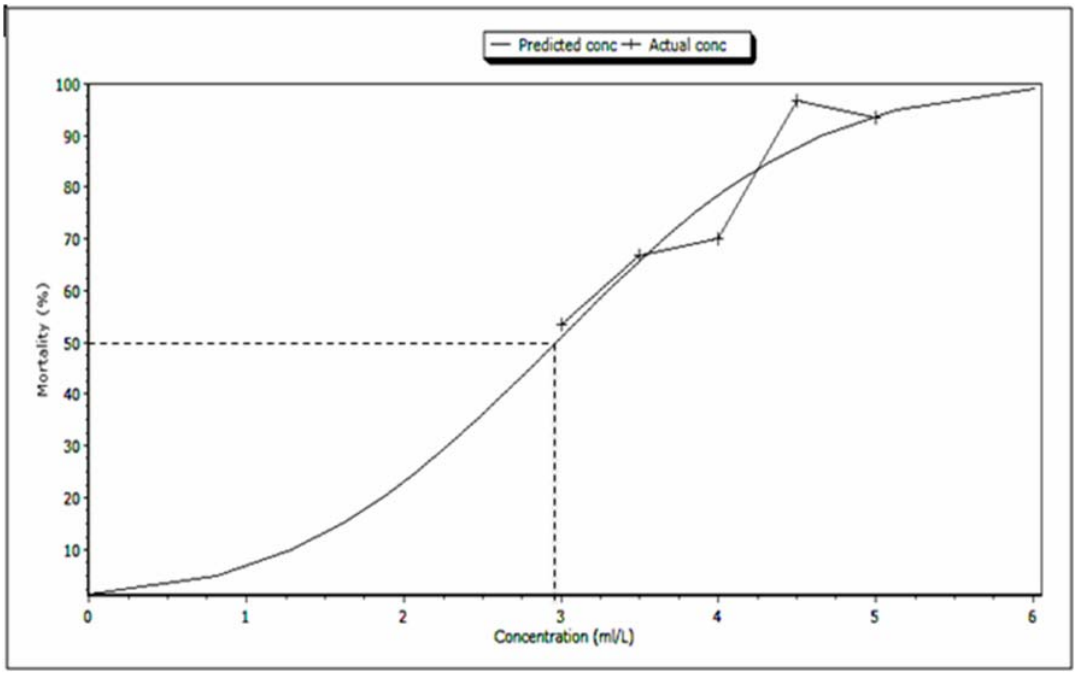

Figure 2. Average lethal concentration (LC_{50}= 2.97 mLL^{-1}) of crude venom extract from *Siganus* spp. on *O. mossambicus* fingerlings.

Conclusion. This study confirms that mortality was induced to fish during the experiment by crude venom despite its category as relatively harmless.

Acknowledgements. The author is grateful to the DOST-PCAARRD for the scholarship grant. To Dr. Anthony S. Ilano for the concept and idea regarding this study. To Dr. Filipina B. Sotto, head of USC marine biology station at Maribago, Lapu-lapu City, and staff Rey and Ben for their assistance in the use of laboratory facilities. CTU-Carmen Campus staff for providing tilapia fingerlings is highly appreciated. And finally to Ms. Senona A. Cesar, Mr. Francis Albert T. Argente and Ms. Charina I. Narido for their technical support.

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Received: 11 October 2016. Accepted: 10 November 2016. Published online: 23 November 2016.
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How to cite this article:
Palla H. P., 2016 Acute toxicity of rabbitfishes *Siganus spp*. (Siganidae) crude venom extract on tilapia *Oreochromis mossambicus* (Cichlidae). AACL Bioflux 9(6):1191-1195.