TOXICITY TEST OF FRESH AND COOKED *Turbo intercostalis* (TOHILON) IN BRINE SHRIMPS ASSAY

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ABSTRACT

This study was conducted to determine the toxicity effect of fresh and cooked meat in brine shrimps assay. Fresh meat and cooked meat extracts were used in determining the physical properties in terms of: color, odor, pH, and solubility. Chemical properties in terms of: alkaloids, flavonoids, saponins and tannins. Fresh meat extract has a yellowish green in color and unpleasant odor, pH of 6.58, miscible in water and ethanol but immiscible in hexane. Saponin is the only chemical property present in fresh meat. The artificial seawater is the only solution that is nontoxic. The rest of the solution with fresh meat extract are toxic. Cooked meat extract has a yellowish color and with pleasant odor, pH of 6.54, miscible in water and ethanol but immiscible in hexane. Saponin is the only chemical property present in cooked meat. Only in cooked meat Solution B, the number of death is lower than 50%. The same with cooked meat, 0.1 milliliter Solution B + artificial sea water the number of death is below the lethal concentration. In artificial water solution, no death occur. All the rest of the solutions are toxic.

INTRODUCTION

Toxicity is the capacity of a substance to poison. The toxicity of a substance is therefore not an inherent property but the detrimental manifestation of its biochemical effect in a living system. The turbinids are sometimes called turban snails for their turban-like shells. Most of these turban snails have thick and hard calcareous opercula which are flat on the inside, but convex outside. They are used in shell jewelry and bigger ones are used as paper weight. Most of these snails were collected for food. The Ribbed Turban snail commonly known as tohilon is commonly seen turban snail species on the rocky shores or reef flats of Singapore and Philippines. The shell is usually greener with lots of brown patches. The length of the shell varies between 25mm and 80mm.

METHODOLOGY

The study was conducted at University of Eastern Philippines, University Town, Catarman Northern Samar, Philippines. The researcher used the experimental method in determining its toxicity test of tohilon in terms of its fresh and cooked meat. In this experiment, three treatments were used: determination of physical properties of tohilon; determination of chemical properties of tohilon; and the Lethal Concentration, LC50.

Determination of Physical Properties

Color test: Ten (10) mL of the tohilon extract was placed in a test tube and the color was determined by five evaluators, the most perceived of their sense of sight is the resulting color of tohilon extract.

Odor test: Ten (10) mL of the tohilon extract was contained in a test tube to be determined by five evaluators and the most perceived odor of their sense of smell is the resulting odor of tohilon extract.

pH value: Forty (40) mL of the tohilon extract was measured in 80mL beaker and a digital pH meter was used to determine the concentration of the extract. The digital pH meter was dipped down in the extract and reading was recorded.

Solubility test: Two (2mL) of tohilon extract was placed in three test tube separately. Then the following three selected solvents, 3mL of hexane, 3mL of distilled water and 3 mL of ethanol was added to the tohilon extract. The solution was shaken vigorously and after 2 minutes, the result was recorded.

Determination of Chemical Properties

Preparation of Tohilon Extract for Fresh Meat

The fresh tohilon meat was removed on its shell and thoroughly washed with clean water. Then, the water was drained and the collected meat was weighed, at about 120g. Distilled water of 240 mL was added then it was blended. Clean chess cloth was used in filtering the sample.

Preparation of Tohilon Extract for Cooked Meat

Freshly collected tohilon is boiled in 500mL beaker at about 100-110˚C. Then, the meat was removed from the shell. The tohilon meat was cleaned with water and then water was drained. The tohilon were weighed for about 70g. Distilled water was

added (140mL) then it was blended. Clean cheesecloth filtered the sample.

For the test of the presence of alkaloids, ten (10)mL of tohilon extract was evaporated to a syrupy consistency over a steam bath. Five mL of 2M HCl was added. The solution was heated with constant stirring for about 5 minutes and cooled then filtered. The residue was washed with enough 2M HCl to bring the filtrate to a volume of 5mL, then the filtrate was separated into two parts. To the first part, 2-3 drops of Mayer's reagent added. The result was recorded. The Dragendorrf's reagent of 2 to 3 drops was added. The result was recorded.

For the test of the presence of flavonoids, the alkaline reagent was used. Five (5mL) of the tohilon extract was poured into a test tube and was treated with a few drops of sodium hydroxide solution. The result was observed upon addition of dilute acid. The process will be repeated thrice.

For the test of the presence of saponins, about 5mL of the tohilon extract was diluted in 20mL of distilled water and then agigated in a 100mL graduated cylinder for about 15 minutes. The process was repeated thrice.

For the test of the presence of tannins, the tohilon extract was centrifuged for 15 minutes allowing the solid particles of the extract to settle down at the bottom of the test tube. A clean supernatant liquid was decanted. About two (2mL) of tohilon extract was added to a few drops of one-percent lead acetate. A yellowish precipitate indicated the presence of tannins.

Confirmatory Test of Tannins

Five (5mL) of tohilon extract was diluted for about 2-3 drops of gelatin salt reagent. The formation of the jelly-precipitate indicates the presence of tannins.

Determination of Toxic Effect

For the tohilon test extract preparation, about 100mL of the tohilon extract was diluted in 5mL of methanol solution (Solution A). From Solution A, 0.5 mL was diluted to 10mL with methanol (Solution B). Then 0.1mL was pippeted of Solution B, 0.05mL of Solution A and 0.5mL of Solution A into separate vials 1, 2 and 3, then it was labelled 1, 2 and 3. Control vial was prepared containing 1mL of methanol.

Hatching the shrimp

A shallow rectangular dish (22cm x 32cm) was filled with the artificial seawater and a plastic divider was placed punched with several 2mm holes in the dish to divide into unequal compartments. Minute brown shrimp eggs were sprinkled into the larger compartment. Larger compartment was covered to keep away from light, then left the smaller compartment open. Smaller compartment was illuminated. After 48 hours, the hatched brownish orange nauplii was pipetted from the illuminated compartment of the dish. Note: The brine shrimp egg shells were left in the darkened side of the dish.

Counting the Nauplii

Nauplii was pipetted and counted macroscopically in the stem of the pipette, held against a well-lighted background.

Concentration of samples vials 1, 2 and 3

Each sample vials diluted to 5mL with artificial seawater makes a final concentration of 5mL respectively.

In a 9-inch pipette, ten nauplii were transferred into each sample vial labeled 1, 2 and 3 and control vial prepared. Artificial seawater was added to each vial and controls, to make a total volume of 5mL. A drop of yeast suspension (3mg/5mL of seawater) was added as food in each vial. The vials was kept under illumination.

Counting the Shrimps

A magnifying glass was used in counting the surviving shrimps. The survivors were counted after 6 hours, 12 hours, 18 hours and 24 hours. Number of deaths were accounted and percent death were determined for each dose level and for the control vials.

Determination of the Median Lethal Concentration

LC_{50}, was determined and 95% intervals from the 24 hour counts with the probit analysis method described by Finney. In the case where data are insufficient for this technique, transform the dose- response data into a straight line by means of a logit transformation (Hafner*et al* 1977). Derive the LC50 from the best-fit line obtained by linear regression analysis.

$$LC_{50} = \frac{no.of\ deaths}{30}\ x100$$

RESULTS AND DISCUSSION

Physical Properties

Table 1. Physical Properties of Fresh Meat and Cooked Meat

Properties	Fresh Meat	Cooked Meat	Interpretations
Color	Yellowish	Yellowish	
Odor	Unpleasant	pleasant	
pH	6.58	6.74	Weakly acidic
Solubility Ethanol Hexane Water	 Miscible Immiscible Miscible	 Miscible Immiscible Miscible	 Polar Non-polar Polar

The table showed that fresh meat has yellowish green in color and has unpleasant odor. pH of 6.58, a weakly acidic. Miscible in water and ethanol, it is polar since the sample and solvent mixed and immiscible in hexane, hence the sample and solvent was non-polar.

Cooked meat extract has yellowish in color and pleasant odor. pH of 6.74, a weakly acidic. Cooked meat was miscible on both water and ethanol, it is polar since the sample and solvent mixed. Cooked meat was immiscible in hexane for they are non-polar.

Chemical properties

Table 2.Chemical Properties of Fresh Meat and Cooked Meat

Tohilon Extract	Test	Result	Interpretation
Fresh Meat	Alkaloids Dragendorff's reagent	Clear yellow, no precipitate form	Negative
	Mayer's reagent	Cloudy yellow, no precipitate form	Negative
	Flavonoids	Jelly-like, white precipitate form	Negative
	Saponins	Foamy surface	Positive
	Tannins	No jelly-like formation	Negative
Cooked Meat	Alkaloids Dragendorffs reagent	Golden yellow, no precipitate form	Negative
	Mayer's reagent	Cloudy white, no precipitate form	Negative
	Flavonoids	Cloudy white	Negative
	Saponins	Foamy surface	Positive
	Tannins	Cloudy yellow, no jelly-like form	Negative

Table 2 showed that fresh meat and cooked meat are both negative in alkaloids test. In flavonoids test fresh meat form a jelly-like white precipitate is form then, cooked meat form a cloudy white. Result in flavonoids in fresh meat and cooked neat were both negative. In saponin test, both fresh meat and cooked meat are positive, foamy surface is form. Tannins test in fresh meat, no jelly like is form. Cooked meat, cloudy yellow no jelly-like is form. Results indicates that fresh meat and cooked meat is negative in tannins test.

Toxicity test in Fresh Meat and Cooked Meat

Table 3. Result in Percent (%) Death of Fresh Meat

Solutions	No. of Death after 24 hours	Percent(%)Death
0.05 mL soln.A + artificial seawater	29	97%
0.1 mL soln.B + artificial seawater	28	93%

0.5mL soln.A + artificial seawater	30	100%
5mL methanol	30	100%
Extract alone	30	100%
Artificial seawater	0	0%(no death occur)

Table 3 showed that solution A, 97% death was observed, solution A is toxic. Solution B, 93% death occur, result indicates that solution B is toxic. Solution C, D, and E there were 100% death observed. Result indicates that solution C,D and E were toxic. Solution F, no death was observed.

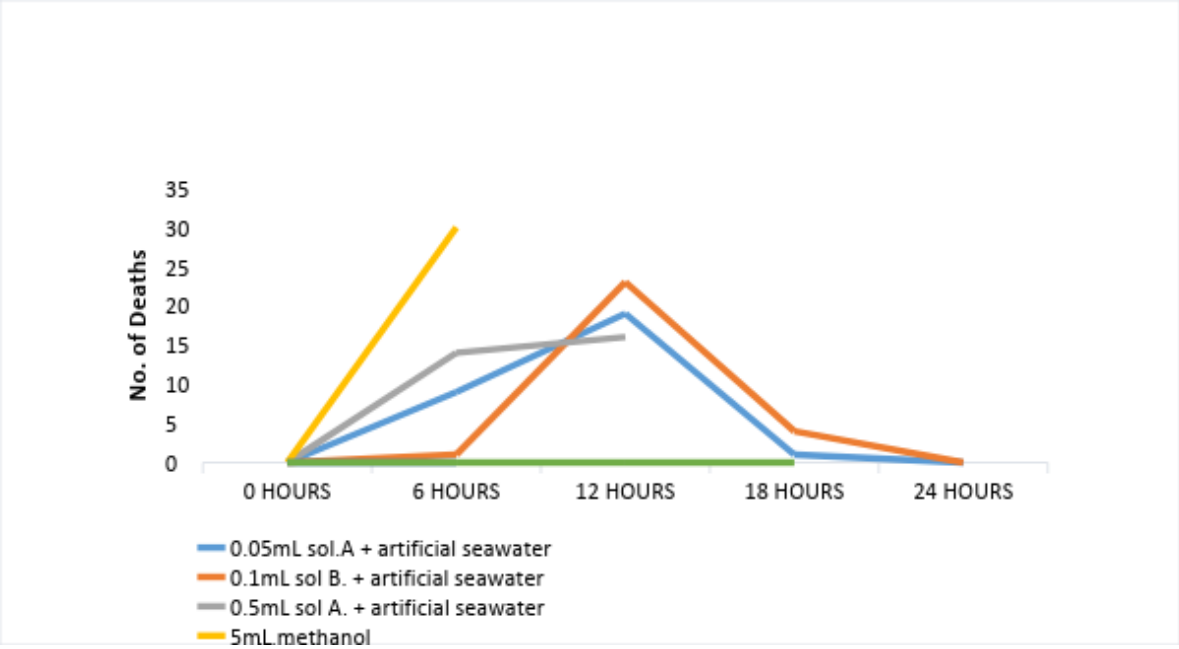

Figure 1. Dose response of Fresh meat extract

The graph showed that 0.1 mL solution B + artificial seawater, after 6 hours of the test only one died. After 12 hours, 23 brine shrimps died, then after 18 hours of the test there were 4 brine shrimps died, then after hours no death occur. Only 3 brine shrimp survived. For 0.5 mL solution A + artificial seawater after 6 hours 14 death occur. After 12 hours 16 death occur. All of the brine shrimps died. For 5 mL methanol alone, afterv 6 hours of the test all brine shrimps died. For extract alone, 30 brine shrimps died after 6 hours of the test. The extract is 100% toxic. For the artificial seawater, no death occur, the solution is not toxic because no death occur.

Table 4. Result in Percent (%) Death in Cooked Meat

Solutions	No. of deaths after 24 hours	Percent (%)Death
0.05 mL soln.A + artificial seawater	16	53%
0.1 soln.B + artificial seawater	14	47%

0.5mL soln.A + artificial seawater	30	100%
5mL methanol	30	100%
Extract alone	30	100%
Artificial seawater	0	0%

Table 4 showed that 0.05mL solution A + artificial seawater, 53% of the test shrimps die. The 0.05mL solution A + artificial seawater is toxic. In 0.1mL solution B + artificial seawater, 47% death occur. The 0.1mL solution B + artificial seawater is not toxic, the percentage death is less than the lethal concentration. Solutions 0.5mL solution A + artificial seawater, 5mL methanol and extract alone are all toxic. Brine shrimps in the solutions die. In artificial seawater alone, no death occur. Artificial seawater is not toxic.

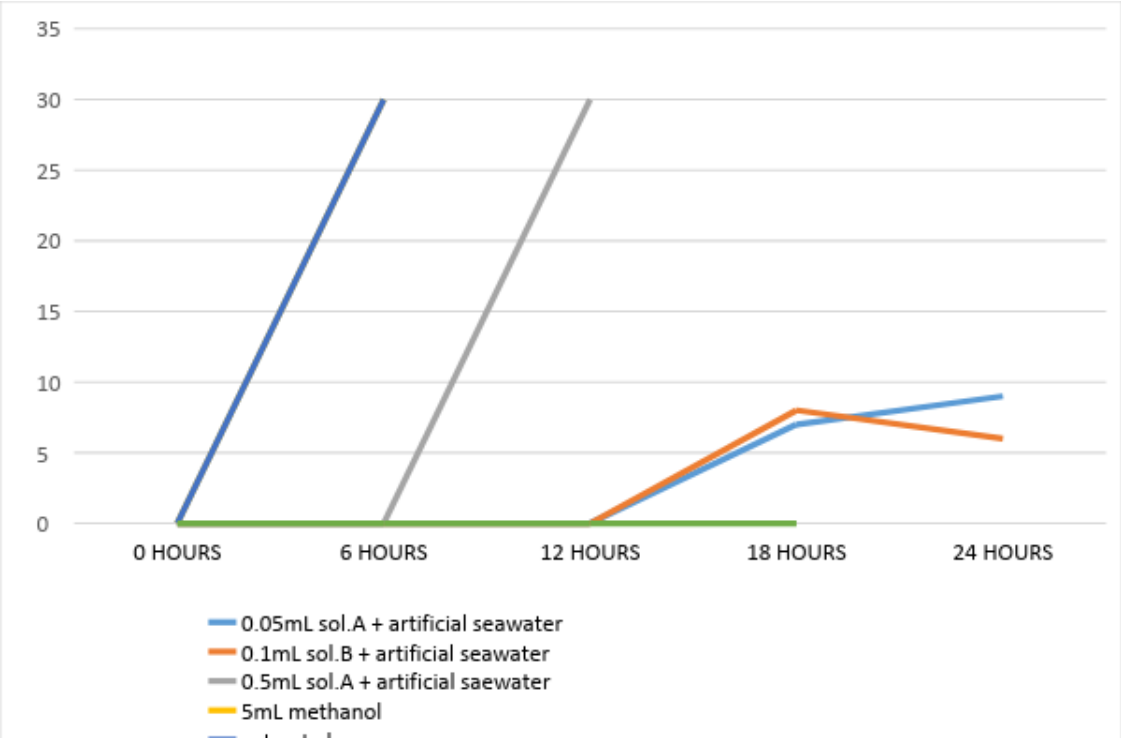

Figure 2. Dose response of cooked meat extract

The graph showed 0.5 mL solution A + artificial seawater, after 6 and 12 hours of the test there were no death observed. After 18 hours, 7 death occur and after 24 hours 9 death was observed. For 0.1 mL solution B + artificial

seawater, no death was observed after 6 hours and 12 hours of the test. Then, after 18 hours of the tezt * deaths were observed. After 24 hours, 6 brine shrimp died. For 0.5 mL solution A + artificial seawater after 12 hours of the test all brine shrimp died. Foir 5 mL methanol and extract alone after 6 hours of the test all of the shrimps died. In artificial seawater alone, after 24 hours of the test no death was observed.

Table 5. Comparison of the LC_{50} of Fresh meat and Cooked meat

Solution	Percent (%) Death	
	Fresh Meat	Cooked Meat
0.05 mL soln.A + artificial seawater	97%	53 %
0.1 soln.B + artificial seawater	93%	93%
0.5mL soln.A + artificial seawater	100%	100%
5mL methanol	100%	100%
Extract alone	100%	100%
Artificial seawater	0%	0%

Table 5 showed that fresh meat, artificial seawater no death was observed, it not toxic. Solutions 0.05mL solution A + artificial seawater, 0.1mL solution B + artificial seawater, 5mL methanol and extract alone, are all toxic. Percent death is higher than 50% lethal concentration.
In cooked meat extract 0.1mL solution B + artificial seawater and artificial seawater alone, the lethal concentration is less than 50%, solution B is not toxic. Solutions 0.05mL solution A + artificial seawater, 0.5mL solution A + artificial seawater and 5mL methanol are all toxic, percent death is above 50% lethal concentration.

CONCLUSION

Based on the findings of the study, the researcher formulated the following conclusions:

Fresh meat has a yellowish green in color and unpleasant odor. pH of 6.58, a weakly acidic. Miscible in both water and ethanol and immiscible in hexane. Cooked meat has yellowish in color and pleasant smell. pH of 6.74, weakly acidic. Miscible in water and ethanol but immiscible in hexane. Saponins is the only present chemical properties in both fresh meat and cooked meat. The Lethal Concentration of fresh meat is much higher than cooked meat.

REFERENCES

Books

Guevara, Beatrice Q.A Guide to Plant Screening: Phytochemical and Biological Research Center for Natural Sciences .Philippines.Publishing House, Beato Angelico Bldng. Espania, Manila.

Lewis Richard and Sax Irving N. Hawley' Condensed Chemical Dictionary, Revised Eleventh Edition. New York: Van NostrandKeinhold Company Inc., 1987.

Mathews, Christopher et al. Biochemistry 3rd Edition, Pearson Education Ltd, Singapore, 2002.

Masterton, W. et al. Chemical principles, 6th Edition, New York: Saunders College Publishing, 1985.

Masterton, William and Hurley Cecile N. Chemistry: Principles and Reactions, 3rd Edition. New York: Saunders College Puliblishing, 1997.

Ragann S. Manual of Analysis of Fruits and vegetables Products.New Delhi: Tata McGraw-Hill PublishinG Ltd.,

Rajvaiya, N., Markandey, D.K. Environmental Analysis and Instrumentation. New Delhi: A.P.H Publishing Corp. 2005.

Skoog, Douglas et al. Fundamentals of Analytical Chemistry, 8th Edition.Thompson Books/Cole, 2004.

Wolsey, W.C. et al. Chemical Principles in the Laboratory. 11th Edition, Minnesota: Thompson Books/Cole, 2002.

Journals

"Marine Environmental Research.Cadmium Toxicity", Volume 50,Issues 1.2000

M. Shuhaimi-Othman, R. Nur-Amalina, and Y. Nadzifah.2012. Toxicity of Metals to a Freshwater Snail, Melanoidestuberculata.Received 18 October 2011; Accepted 5 January 2012

Melanie A. Trenfield,Joost W. van Dam,Andrew J. Harford,DaviParry,ClaireStreten,KarenGibb,Rick A. van Dam.2016. "A chronic toxicity test for the tropical Marine snail*Nassariusdorsatus* to assess the toxicity of copper, aluminium, gallium, and molybdenum".Copyright © 1999 -2017 John Wiley & Sons, Inc.

TallaricoLde F[1], Borrely SI[2], Hamada N[2], Grazeffe VS[3], Ohlweiler FP[4], Okazaki K[2], Granatelli AT[3], Pereira IW[5], Pereira CA[6], Nakano E.2014. "Developmental toxicity, acute toxicity and mutagenicity testing in freshwater snails Biomphalariaglabrata (Mollusca: Gastropoda) exposed to chromium and water samples.Copyright © 2014 Elsevier Inc.

Taowu Ma., Shuangjiao Gong, Fe Zhou, Cheng Zhu, Kaidong Deng, QinghuaLuo, Zijian Wang. 2010. "Laboratory culture of the freshwater benthic gastropods Bellamyaaeruginosa (Reeve) and its utility as a test species for sediment toxicity.Published by Elsevier B.V.

S.L.Pandeswarae,P.R.Yallapragada.2010.Tolerance"accumulation and depuration in an intertidal gastropod, *Turbo intercostalis*, exposed to cadmium. Copyright © 2000 Elsevier Science Ltd.

Andy Davis, Jessica Holan, Catherine King.2015.Sensitivity of different life stages of common subantarctic

marine invertebrates to copper.Publisher Australian Antarctic Data Centre.

McNaught, Alan D; Wilkinson, Andrew; IUPAC (1997), "IUPAC Compendium of Chemical Terminology", IUPAC Compendium Chemical Terminology (2 ed.), Oxford: Blackwell Scientific, doi:10.1351/goldbook.F02424, ISBN 0- 9678550-9-8

Internet Sources

The American Heritage® Science DictionaryCopyright © 2002. Published by Houghton Mifflin.

ADAM, A. (1848) Narrative of the Voyage of H.M.S.Samarang — Vol. 2, p. 356-357.

Kasper, D.L., et al., eds. Harrison's Principles of Internal Medicine, 19th Ed. United States: McGraw-Hill Education, 2015.

FLECKER, H. (1936) Cone Shell Poisoning, with the Report of a Fatal Case — Medical Journal of Australia, 4th April, 9th May, and 20th June.

Christophersen, C. In: Alkaloids: Chemistry and Pharmacology; (edited by A. Brossi), Academic Press, Orlando, 1985, 24, p. 2

Collins English Dictionary - Complete & Unabridged 2012 Digital Edition © William Collins Sons & Co. Ltd. 1979, 1986 © HarperCollins Publishers 1998.

http://commons.wikimedia.org/wiki/Image:Tannic_acid_(looizuur).png