International Conference of Science, Engineering & Environmental Technology (ICONSEET), 3(26): 190-193, 2018
ISSN 0794-9650
www.repcomseet.com

Lethality Investigation of Toxicity of Extracts from Natural Plants: A Case Study of Blighia Sapida Leaves

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Abstract – This study investigated the mortality effects of the extract from Blighia Sapida Leaves, with brine shrimp lethality test which is a simple, high throughout cytotoxicity test of bioactive chemicals. The brine shrimp lethality test for hatchability of Artemis Salina Nauplius was carried out using standard methods. The results revealed that the extract used produced 40% hatchability of the cysts after 72 hours at a concentration of 0.125mg/ml. The test result shows that the extract has weak cytotoxic compound and justifies the trado - medical application of the plant, which can serve as a base-line for pharmaceutical industrial development.

Keywords: Cyst, Cytotoxicity, Hatchability, Mortality, Plant Extract, Trado-medical.

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1. Introduction

Brine shrimp lethality test is a simple, high throughout cytotoxicity test of bioactive chemicals. It is based on the killing ability of test compounds on a simple zoological organism-brine shrimp (Owolabi et al, 2007). This test was proposed and further developed by several groups. The brine shrimp lethality bioassay is widely used in the evaluation of toxicity of heavy metals, pesticides, medicines, especially natural plant extracts, etc (lnlaghe et al, 2009).

It is a preliminary toxicity screening for further experiment on mammalian animal's models was designed. One important aspect of this test is that the solvent used in this test may give false positive signals due to the toxicity of the solvent itself. It has been well-known that some organic solvent and detergents have high cytotoxicity in vivo (Binary et al, 2007). A systematic study on how high concentration of the solvents affects the results from brine shrimp lethality bioassay and guidance for maximum working concentration of the solvent is needed.

Firstly, pure compounds and crude plant extracts was dissolved in a solvent suitable for the tested herbal sample. However, a mixture of several solvents may be a more suitable solvent system for some plant sample. By dissolving the plants extract in a convenient solvent, a stock solution is made, which can be used for serial dilutions to prepare different concentrations. This is useful in the means of toxicity testing, because it is of high importance to determine the concentration range in which there is a linear correlation between the concentration and the hatchability of the brine shrimp cysts. Most experiments that involve the brine shrimp hatchability test for toxicity assessment of herbal extract include a concentration range of 10, 100, and 1000ug/ml. The results for the toxicity of the tested herbal preparations gained by using crude plant extracts were more accurate than testing pure compounds isolated from the same plant. This difference in the toxicity results is probably due to the chemical complexity of the crude or partially

purified extract, which seemed to be essential for the bioavailability of the active constituents of the examined plants. On the other hand isolated pure compounds seemed to lose this specific bioactivity

The brine shrimp lethality test is considered a useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities in plant extracts. The brine shrimp hatchability test has been developed for toxicity testing of various concentrations of pure compounds and crude plant extracts.

The leaf of Blighia sapida also known as ackee apple leaf is an evergreen tree that grows to about 10meters tall, with short trunk and dense crown (Change et al, 2012). The leaves are par pinnately compound, 15-30 centimeters (5.9-11.8 in long with 6-10 elliptical to obovate-oblong leathery leaflets. Each leaflet is 8-12 centimeters (3.1-4.7) in long and 5-8 centimeters (2.0-3.1) in wide (Vinken Pierre et al, 1995). Although native to West Africa, the use of ackee in food especially prominent in Jamaica cuisine. Ackee is the national fruit of Jamaica and ackee salt fish is the natural dishes. (National Geographical Traveler. Retrieved 19 August 2016)

It uses is not limited to traditional application alone but also for scientific uses and its general purposes, which include; enhancement of immune system, maintain skin health, cuts and wound, cancer, vasodilator activity, etc (Madziga et al, 2010).While its traditional uses includes; coolant, expectorant, stimulant, treatments of diabetes, ulcers, conjunctivitis, etc (Sarker&Nahar, 2007). The oil of the ackee arils contains many important nutrient especially fatty acids. It is also used in the production of soap on some part of Africa (http:/en. Wikipedia. Org/wiki/sapindales) This study aimed at determining the therauphetic effects of Blighia sapida plant extract and the possibility of transforming ackee leaf into valuable medicinal use.

2. Material and Methodology

2.1 Sample Collection

The medicinal plant used for the case study was the leaves of ackee apple (Blighia sapida) and it was obtained by basket survey.

2.2 Sample Preparation

The plant material were collected and air dried at room temperature to remove the water on the leaf surfaces for 4 weeks, which was later kept in an oven in the chemistry laboratory for 8hours, electric blender was then used to crushed the leaf into powdery form.

3. Brine Shrimp Hatchability Test

Ten brine shrimp (Artemis Salina) cysts or eggs were obtained from the Federal Research Institute of Nigeria (FRIN) and counted on clean, sterile Petri dishes each. Sea water was incubated at 28°c-30°c for one hour. Extract was dissolved in the solvent used for the extraction and concentration ranging from 2.0,-0.125mg/mL that is (2.0, 1.0, 0.5, 0.25, 0.125mg/mL) was prepared with sea water by serial dilution. Varying concentration of the extract was added to the cysts on the Petri dishes. Each of the concentrations was replicated three times. The negative control was prepared by adding sea water only to the cysts whole positive control contain tetracycline (30mg/mL) (Gardi, 2012).

The Petri dishes containing varying concentration of extract were then incubated at 28°c for 72 hours while reading were then taken every 24hours to count the numbers of hatched nauplii. The test was performed according to Gardi, (2012). The negative control enables elimination of other factors that contribute to the total number of hatched naupilii. The solvent used to dissolve the crude plant extracts is

a relevant negative control for this purpose. The toxicity of tested plant samples was determined by comparing LC50 values with highly toxic substances suitable to be used as positive controls for the test, such as vincristine sulphate, potassium dichromate, cyclophosphamide, pure DMOS, Caffeine, etc.

4. Results and Discussion

4.1 Results

Table 1 showed artemis salina naupilius results at various concentration and replicates.

Table 1: Concentration of the Leathality Test (Mg/Ml)

Time	**2.0**	**1.0**	**0.5**	**0.25**	**0.125**	**+ c**	**- c**
(hrs)	a b c	a b C	a b C	a B C	a b C	A B C	a B C
24	3 4 3	3 4 3	3 4 3	5 5 5	5 4 5	0 0 0	7 7 7
48	6 6 6	6 7 7	7 7 7	8 8 8	8 9 9	0 0 0	7 8 9
72	8 8 8	8 8 8	8 8 8	9 9 9	9 9 9	0 0 0	9 8 9

a = 1st replicate

b = 2nd replicate

c = 3rd replicate

4.2 Discussion

Table 1 reveals the result of lethality test of Blighia sapida shoot extract which was designed to access its cytotoxicity properties. The extract used produced 40% hatchability of the cysts after 72 hours at a concentration of 0.125mg/ml which was in concordance with the work of Gardi, 2012. This result showed that the extract has weak cytotoxic compounds similar to the work of Madziga et al, 2010 and justifies its traditional uses for treatment of cough and any other related diseases, for examples; any skin diseases, stomach upsets, typhoid fever, jaundice e.t.c in low concentration. The toxicity test results showed that the plant was not toxic which reduce the danger of been poisonous to human health, hence the trado-medicinal application of plant sample.

5. Conclusion and Recommendation

5.1 Conclusion

From the work done so far, findings showed that the extract was not toxic which reduce the danger of been poisonous to human health, hence the trado-medicinal application of the plant sample. Therefore, it can be concluded that the result of brine shrimp hatchability could serve as a base line for clinical trial.

Invariably, identify the importance of plants in reducig the morbidity and mortality in developing countries. Most of the species are highly medicinal because they are used both to sustain health and cure illness (Osunwole 1999). Even locally in the study area, the ailment had been controlled in the use of Blighia Sapida, Psidium Guajava and Ocimum Gratissium. The compilation as Gbile (1988) will help to

preempt communication as problem that exists between ethnic groups, plant research scientist, herbalist, herb sellers and the entire consumers.

5.2 Recommendation

There is need for validation and standardization of Traditional medicinal practices so that this sector can be accorded it rightful place in health sector. Blighia Sapida (Ackee apple leaves) also known as isin in Yoruba, is thereby recommended for various treatments of ailment both scientifically and traditionally in the health system. Government should also provide a global forum for conservation, traders, manufacturers of herbal medicine and professionals in the field of traditional and alternative therapies.

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