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BIO-TOXICITY STUDY OF SOME SELECTED PLANT BY *ARTEMIA SALINA* (LEACH) TEST

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Abstract

Plant derivatives in current research are often used as anti-microbial directly without a study of its toxic effects. Current study carried out to determine toxicity of five plant extracts (LC50) by Brine Shrimp Test (BST). Eight dilutions was prepared to test ranged (640, 320, 160, 80, 40, 20, 10 and 5 mg/L). The percentage of lethality of the brine shrimp larvae was calculated for each concentration after 48hr for 50% killed mortality of the larvae. The LC50 values of the plant extracts were: Castor was 5ig/ml (low); chili: 20 mg/ml (low); neem: 30 mg/ml (safe); lemon grass: 473 mg/ml (safe) and ginger: 495 mg/ml (safe). Future research need to focus on toxicity studies for plant extracts or botanicals natural products for more safety.

Key words : Bio-Toxicity, plant extracts, *Artemia salina,* Brine Shrimp Test *(BST).*

Introduction

The flora includes plants from families Meliaceae, Rutaceae, Solanaceae, Asteraceae, Malvaceae, Compositae, Leguminosae, Labiatae and Canellaceae that contain several secondary phytochemical substances, such as alkaloids, glycosides, tannins, flavonoids, essential oils, saponins and organic acid. Many of these phytochemicals demonstrate effects similar with pesticides or operate as anti-feedants, inhibit growth and repel diseases (Rajinder *et al*., 2011; Naghdi *et al*., 2011). Many studies recommended to using plant extracts as anti-microbial to replacement of antibiotics, pesticides, fungicides and weedicides as natural products. Those studies in mostly used crude or partition of crude in applied experiment bioassay without mentioning the negative effects of such us natural products. In both states of using low or high concentrations, may be will led to site effects in the case of use, whether for short or long time without realizing the harmful effects resulting from the presence of some active compounds poisonous directly or overlapping with other toxic compounds. The researchers ignorance of the risks that lead to the use of such materials requires extensive studies to reach of recommendations for toxicological tests by following approved standard methods. The above scenario showed an urgent need to study the toxicity of natural products of plant origin before recommending their final use.

Lower the amount, more toxic is the material. *In vitro* lethality to shrimp larvae can act as a fast and simple tool for checking bioactive compounds when isolating natural products. This analysis is fast, cheap, and has been established for fractions and observing of natural products that are physiologically active (Sheikh *et al*., 2004; Costa *et al*., 2010, Valadbeigi *et al*., 2016). Brine Shrimp Test (BTS) *Artemia salina* (Leach) using larvae is a very useful monitory instrument for cytotoxicity of natural product such as plant extracts. The biocompound from some plant extracts may contain active compounds that can be toxic to human health. Hence it was important to determine the LC50 values of the extracts that showed positive anti-fungal activity using the Brine Shrimp Test (BST) in the study. Current study come out to study of some plant extract as example to detect of toxicity by using BTS test and submit recommendations before direct using for natural products in anti-bioassay tests.

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Material and Methods

Preparation of Test Extracts

Based on earlier work the concentrations for the test were prepared using the method of (Nguta *et al*., 2012). 640 mg of crude plant extract of each plant under study was dissolved in organic solvent DMSO in 10 ml volumetric flask. The flask was stirred manually until the samples reached a fully dissolved and homogenous state. The solution (crude plant extract + DMSO) was then transferred to 1000ml conical flask and distilled water added to each flask to complete the volume of the solution to (640mg/L). This stock solution was used to prepare 7-

serial dilutions of comprising 320, 160, 80, 40, 20, 10 and 5 mg/L that used for test.

BST Evaluation for Bioassay

Artemia salina (Leach), was collected from a fish aquarium. 50mg of *Artemia salina* (Leach) eggs were added to a 100 ml conical flask containing 75ml sea water. The conical flask was kept in a clean and sterile chamber under room condition for 48 hrs for the eggs to hatch into shrimp larvae. Ten clean and sterile 50ml conical flasks were used for the BTS evaluation. 20 hatched larvae were added with scapula into each flask containing 45ml of sea water. 1ml of each of the 8 dilutions (640, 320, 160, 80, 40, 20, 10 and 5 mg/L) was then added with a pipette to each flask and the samples were observed for 48hr for 50% mortality of the larvae. The percentage of lethality of the brine shrimp larvae was calculated for each concentration (Pelka *et al*., 2000) formula as follows:

$$\% \text{ Mortality} = \left[\frac{(\text{Number of live larvae} - \text{Number of dead larvae})}{\text{Number of live larvae}} \right] \times 100 \quad \ldots. (1)$$

The experiment was done in 3 replicate using CRD and regression analysis for mortality (%) was performed using Microsoft Excel 2010 (Probity method analysis) following method Nguta *et al*., (2012).

Results and Discussion

Bio-toxicity of Selected Plant Extracts (Brine Shrimp Test) LC50

The LC50 from table 1 values of the plant extracts were obtained from analyzing the percentage of the shrimp larvae killed as a result of the effect the concentrations of the plant extracts table 1. Best-fit line was obtained from the data by means of regression analysis (Abdul Rani *et al*., 2010; Veni and Pushpanathan, 2015). All of the extracts showed positive results indicating that the test samples are biologically active. The concentrations versus percent mortality (% Mortality) for 20 larvae for all test samples showed an approximate linear correlation. From the result, the median lethal concentration (LC50: the concentration at which 50% mortality of brine shrimp larvae occurred) was determined using the method of Nguta *et al.,* (2012).

From the results table 2, the most cytotoxic extracts were of Castor and chili, which exhibited slightly low levels of LC50. The extracts of Neem showed remarkable toxicity on the brine shrimp larvae at LC50 indicating safe toxicity category. The activity of Lemon grass and ginger demonstrated moderate and safe toxicity on brine shrimps. Results this study agree with the studies of Meyer *et al*., (1982); Commercial Vegeable Production Recommendation (2004) states that value greater than 20mg/L is non-toxic to humans, animals and can be used safely.

Table 1: Mortality percentage of larvae of shrimp (%) in Brine Sharimp Test bioassay of plant extracts at different concentrations (640 to 5 mg/L) after 48 hour.

Conc. (mg/L)	Castor *Mortality %	Chili	Neem	Lemon grass	Ginger
640	94	90	86	66	68
320	84	80	76	34	32
160	74	70	66	26	22
80	74	68	70	22	20
40	70	62	58	20	20
20	64	48	42	16	14
10	56	38	34	12	12
5	50	30	24	8	10

*Mortality percentage of lethality of brine shrimp larva (50 larva) was calculated at each concentration as follows: Mortality (%) = [(Number of live larvae – Number of dead larvae/ Number of live larvae × 100)].

The toxicity from Castor at different concentration (5, 10, 20, 40, 80, 160, 320, 640 mg/L) are presented in fig. 1 LC50 of crude extracts *Ricinus communis* L. (Castor). was 5mg/L. Toxicity studies revealed that the ethanol extracts exhibit low toxicity (LC50 of 5 mg/L) against *Artemia salina.* Percentage of the dead larvae was 50% at this toxicity level. The results on brine shrimps assay indicate that the extract has LC50 value greater than 2- 20mg/L, which signified that *Ricinus communis* L. (Castor) might be toxic to human However, there has been no report on the Bio- toxicity for this plant using Brine Shrimp Test.

After 48 hours, Brine shrimp lethality bioassay of crude extract Chili showed low cytotoxic activity against brine shrimp Larvae and LC50 Fig. 2 LC50 values of 5, 10, 20, 40, 80, 160, 320 and 640mg/L gave mortality percentages of 30, 38, 48, 62, 68, 70, 80 and 90% respectively. LC50 value at which 50% of *Artemia salina* (shrimp larvae) was found to be 20mg/L. This value indicates the might be toxic of *Capsicum frutescence*

Table 2: Brine Shrimp Test (BST) toxicity (mg/L) of crude plant extracts under study.

Plants	Values LC50 (mg/L)	*Toxicity category
Ricinus communis(Castor)	5	High
Capsicum frutescence L (Chili)	20	High
Azadirachta indica L. (Neem)	30	Low
Cymbopogon nardus L. (Lemon grass)	473	Safe
Zingiber officinale L. (Ginger)	495	Safe
DMSO (Di Methyl Sulfoxide)	>1000	Safe

*Toxicity level reference (Anon. Commercial Vegetable Production Recommendation, 2004).

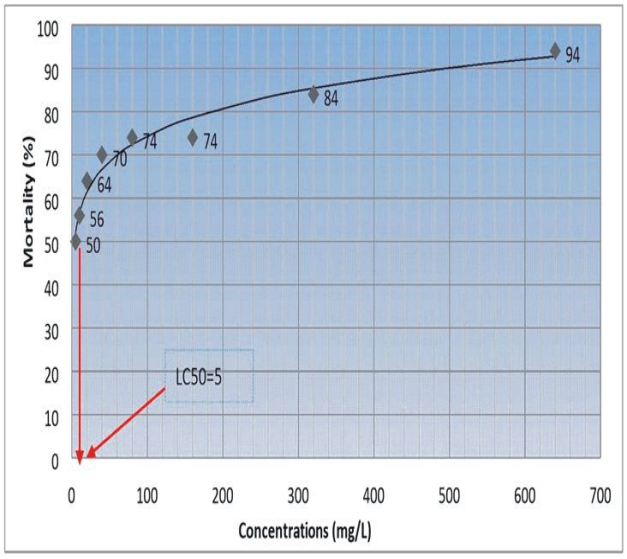

Fig. 1: Brine shrimp test (LC50) of the ethanol extract of *Ricinus communis* L. in brine shrimp lethality bioassay.

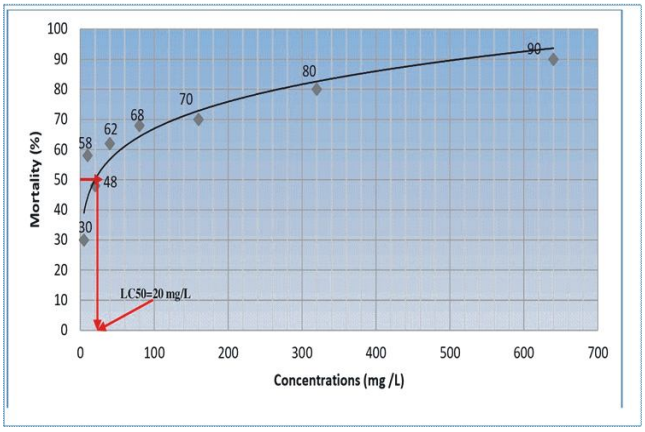

Fig. 2: Brine shrimp test (LC50) of the ethanol extract of *Cymbopogon nardus* L. in brine shrimp lethality bioassay.

L. (Chili) Table 2.

Toxicity result of Neem of fig. 3 showed that the concentration of 20 and 40mg/L gave mortality percentages of 42 and 58% respectively. The data analysis of the Larvae mortality of the brine shrimp gave a LC50 value of 30 mg/L (50% of the shrimp larvae were killed) after 48 hours. This indicates that Neem exhibits low toxicity against the brine shrimp. Toxicity from other concentrations (5, 10, 80, 160, 320 and 640 mg/L) recorded mortality percentages of 24, 58, 70 66, 76 and 86% consecutive.

The result showed moderate brine shrimp lethality of Lemon grass, where the LC50 value was found to be 473mg/L as shown in fig. 4. Maximum mortalities took place at a concentration of 620mg/L (66% mortality) while least mortalities were at 5mg/L (8% mortality). Other concentrations; 10, 20, 40, 80 and 160 mg/L showed low toxicity and gave percentage mortalities of 12, 16, 20, 22,

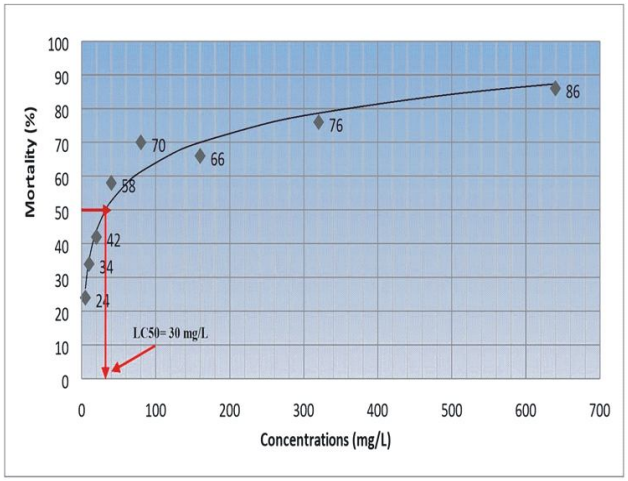

Fig. 3: Brine shrimp test (LC50) of the ethanolic extract of *Azadirachta indica* L. in brine shrimp lethality bioassay.

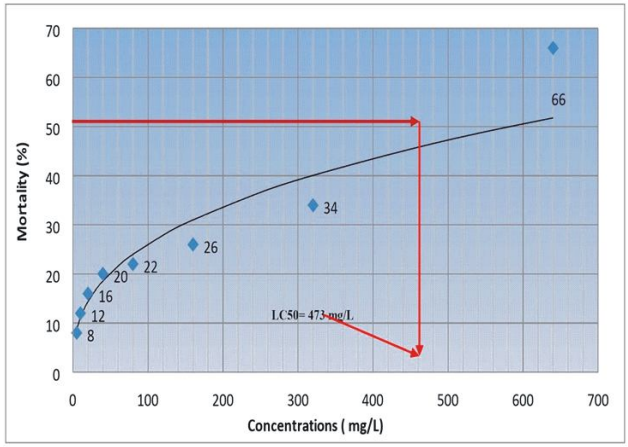

Fig. 4: Brine shrimp test (LC50) of the ethanolic extract of *Cymbopogon nardus* L. in brine shrimp Lethality bioassay.

26 and 26% sequentially. LC50 value of lemon grass was found to be 30mg/L and under toxicity category (safe) table 2.

The LC50 values of the brine shrimp obtained for extract of *Zingiber officinale* L. are shown in fig. 5. Active concentration of 495mg/L recorded 50% death of brine shrimp. Data analysis showed mortality percentages of 8, 12, 16, 20, 22, 26 and 34% for concentrations at 5, 10, 20, 40, 80, 160, and 320mg/L sequentially, and gave toxicity of less than 50% (mortality percentage) while the concentration at 620mg/L gave a mortality of 68%, indicating high toxicity. LC50 value of *Cymbopogon nardus* (495mg/L) was found to be 30mg/L and under toxicity category (safe) table 2.

According to Meyer *et al.,* (1982), extracts derived from natural products which have LC50 $\leq$ 1.0 mg/L are known to possess toxic effects. Therefore, the results of brine shrimps assay indicating that the extract has LC50 value greater than 20mg/L might not be toxic to humans

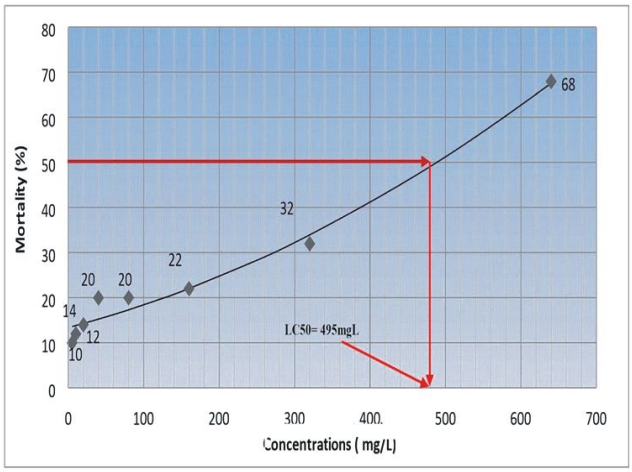

Fig. 5: Brine shrimp test (LC50) of the ethanolic extract of *Zingiber officinale* L. in brineshrimp lethality bioassay.

(Commercial Vegetable Production Recommendation, 2004). In this study, LC50 value of the crude extract from Castor, Chili, Neem, Lemon grass and Ginger were 5, 20, 30, 375 and 495 mg/L respectively.

The results of this study recorded low and save toxicity for all plant under study compared with study of (Alluri *et al*., 2005) that found extracts of *Aristolochia indica, Boswellia, Ginkgo biloba*, *Garcinia cambogia and Semecarpus anacardium have* showed high cytotoxicity with LC50. Result present study supported studies conducted by Khaled (2006) and Suad (2012) that ethanolic extracts of *Azadrichta indica* (neem) recorded levels of LC50 reached 20- 21mg/l and study Nowsad *et al.,* (2010) that found LC50 values recorded low toxicity of chili extract. Reports by (Yoga *et al*., 2007 ; Adoum, 2009; Najmeh *et a*l., 2011) reported analyzed the toxicity of *Psophocarpus tetragonolobus* L., *Canarium schweinfurthii* L., *Dissotis brazzae* L., *Isoglosa lacteal* L., *Nicotiana tabacum* L. and *Eucalyptus globules* L. recorded low toxicity ranged between 15.35-374 (mg/L) and can use it as natural source for medical purposes.

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