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Cytotoxicity (Brine shrimp Lethality Bioassay) of Barringtoniaracemosa Leaves, Stem-Bark and Root Extract

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ABSTRACT

Objective: *The present study was conducted to test for in vivo Brine Shrimp Lethality Assay (BSLA) of Barringtonia racemose leaves, stem-bark and root extracts after dissolving in five solvents (n-hexane, dichloromethane (DCM) ethyl acetate chloroform and methanol) and complement with cytotoxicity results with known pharmacological activities of the plant.*

Methods: *Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration), 10 nauplii were added into three replicates of each concentration of the plant extracts, and after 24 h the surviving brine shrimp larvae were counted, and $LC_{50}$was assessed.*

Results: *The cytotoxicity for the leaves, stem-bark and root extracts of Barringtonia racemose showed a concentration dependent increment in mortality rate of the brine shrimp nauplii in relation to the solvent n-hexane dichloromethane, ethyl acetate, chloroform and methanol fractions of the leaf, stem and root and leaves extracts which were more potent against the brine shrimp with LC_{50} values of methanol with 42.968 for leaf, and stem with 69.186. The dichloromethane and ethyl acetate roots fractions of both the extracts exhibited low activity with LC_{50}values 27.397 and 27.400 ppm (µg/ml) respectively.*

Conclusion: *The result indicated bioactive components are present in this plant parts (leafs, stem-bark and roots) that could be accounted for its pharmacological effects.*

Key words: *Brine shrimp; Cytotoxicity; Barringtonia racemose; Leaves; Stem-Bark; Root.*

INTRODUCTION

Over the last decade, interest in drugs of plant origin has been growing steadily. The study of bioactive compounds from plant sources and extracts in the chemical laboratory is often hampered by the lack of a suitable, simple, and rapid screening procedure. There are, of course, many procedures for bioassay, but unless collaborative programs with biologists, biochemistry and pharmacologists are in place, the typical chemical laboratory is not suitable equipped to perform the usual bioassays with whole animals or isolated tissues and organs, as well aseptic techniques (Sam et al., 1993).

More to that, some of the negative effects obtained in the use of local plants as source of medicine are basically due to over-dosage and as well as adequate knowledge of other detrimental by-products contained in some plants (Lilybethand Olga 2013).

Thus, screening for biologically active plant constituents and the selection of the plant species to be studied is obviously always a crucial factor for the ultimate success of the investigation. Plants used in traditional medicine are more likely to yield pharmacologically active compounds. Therefore, in vivo lethality in a simple zoological organism, such as the brine shrimp lethality test (BST), developed for Meyer et al(1982),might be used as a simple tool to guide screening and fractionation of physiologically active plant extracts in such Laboratories, where one of the simplest biological responses to monitor is lethality, since there is only one criterion: either dead or alive. This general bioassay detects a broad range of biological activities and a diversity of chemical structures. One basic premise here is that toxicology is simply drug at a higher dose, thus if we find toxic compounds, a lower non-toxic, dose might elicit a useful, pharmaco-logical,

perturbation on a physiologic system (McLaughlin 1991).

However, it has been demonstrated that Brine shrimp toxicity correlates reasonably well with cytotoxic and other biological properties. Brine shrimp have been previously utilized in various bioassay systems. There have been many reports on the use of this animal for environmental studies, screening for natural toxins and as a general screening for bioactive substances in plant extracts such as *Barringtonia racemose.*

Barringtonia racemose(L). Which is also known as Putat, fish poison tree or powder puff tree is a type of highly valuable plant species due to its medicinal values. Geographically it is found to be widely distributed from eastern Africa and Madagascar to Micronesian and Polynesian Island as well as in mangrove site of Sarawak, this species was collected at Meranak at the Meranak river bank in kota-samarahan, Sarawak. The plant has been associated very well in various tribes around the world with diverse ethno-botanical uses (Osman *et al*., 2015). The mangrove tree is easily recognized by its large leaves, delicate white flowers and guava-like fruit that hang in long racemes. It has a straight, unbranched stem that leads to a rounded crown and is usually 4-8 m tall, but occasionally reaches 15 m. The bark is greyish brown to pink with white blotches and raised dots and lines. The branches are marked with leaf scars.

This species is classified as under-utilized crops (Osman *et al*., 2015). The leaves are alternate and carried in clusters at the ends of branches; the flowers are produced on hanging racemes up to 1m long. The buds are pinkish red and split open to bring forth masses of delicate stamens in white sprays (Chantaranothai, 1995). The seeds, bark, wood and roots contain the poison saponin and is used to stun fish. The bark, which also has a high tannin content, is frequently used in powdered form for this purpose. Extracts from the plant are effective insectides and are also used medicinally in South Africa (Kong *et al*., 2006).

RESULT

Cytotocity

The lethality concentration of LC_{50} was assessed at 95% confidence using probit analysis. it has been observed and reported by Meyer et al., (1982) that LC_{50} value less 1000µg/mL is toxic while LC_{50} value of greater than 1000µg/mL

Table1. *Average death of Artemia salina at different concentration of Hexane crude extract of Barringtoniaracemosa Leaf, Stem-bark and Roots*

Methanol Crude Extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	4.33±0.58	5.00±0.58	5.70±0.58	6.33±0.58	7.00±1.00	10.0±0.00	37.285
Stem-bark	4.00±0.00	4.70±0.58	6.00±0.00	8.00±0.00	10.0±0	10.0±0.00	19.909
Roots	4.80±0.58	5.70±0.58	5.33±1.16	7.33±1.16	9.33±1.16	10.00±0.00	24.093
(-ve control)	0	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	10±0.00	7.455

The result is Mean+SD. N = 30, table 1. Above show the average death and LC_{50} of Artemia salina brine shrimp at different concentration of the hexane Leaf, stem-bark and roots extract of B. racemosa.

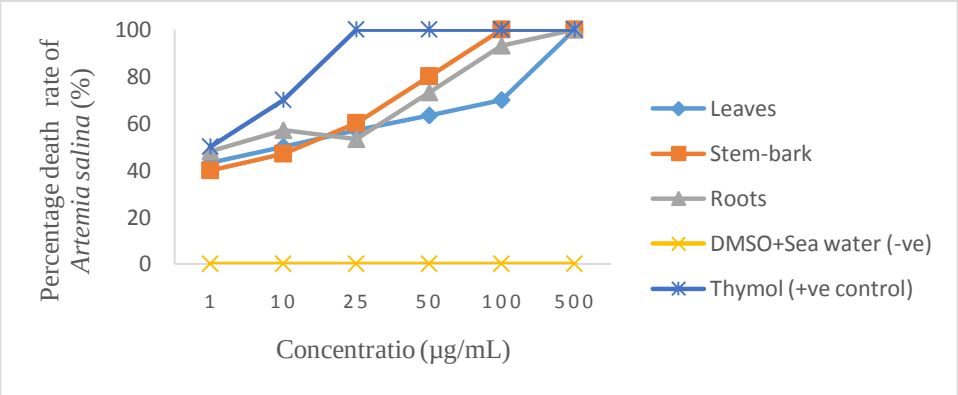

Figure1. *Average death of Artemia salina (%) as a function of various Hexane extract concentration on plant parts of Barringtonia racemose was monitored after 24hrs exposure of different concentration of the plant part*

Table2. *Average death of Artemia salina at different concentration of Dichloromethane crude extract of Barringtonia racemose Leaf, Stem-bark and Roots*

Dichloromethane Crude Extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	4.70±0.58	5.70±0.58	6.33±0.58	6.66±1.53	7.33±0.58	9.33±0.58	38.557
Stem-bark	3.70±0.58	4.33±0.58	5.33±0.58	5.33±0.58	8.70±0.58	9.00±0.00	69.186
Roots	4.00±0.00	4.70±0.58	5.70±0.58	8.00±0.00	8.67±0.58	10.0±0.00	27.397
(-ve control)	0	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	10±0.00	7.455

The result is Mean+SD. N = 30, table 1. Above show the average death and LC_{50} of Artemia salina brine shrimp at different concentration of the dichloromethane Leaf, stem-bark and roots extract of B. racemose.

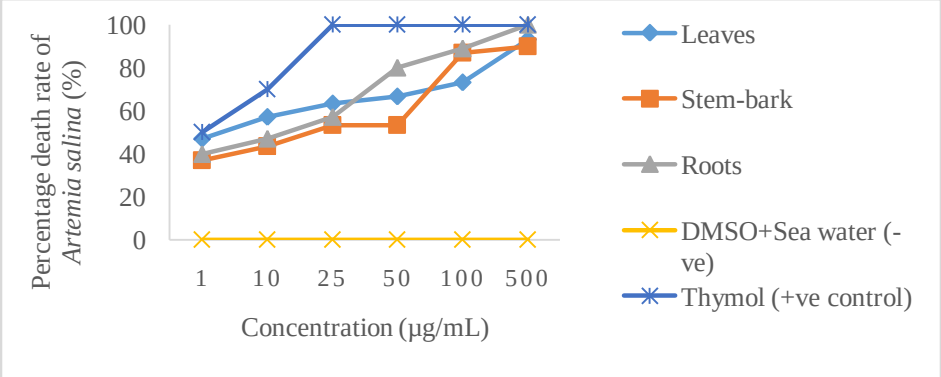

Figure2. *Average death of Artemia salina (%) as a function of various Dichloromethane extract concentration on plant parts of Barringtonia racemose was monitored after 24hrs exposure of different concentration of the plant part*

Table3. *Average death of Artemia salina at different concentration of Ethyl acetate crude extract of Barringtonia racemose Leaf, Stem-bark and Roots*

Ethyl acetate Crude Extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	3.33±0.58	3.70±0.58	5.00±0.00	4.70±0.58	6.00±0.00	8.33±0.58	142.45
Stem-bark	2.70±0.58	5.33±0.58	6.00±0.00	8.33±0.58	9.33±1.15	10.0±0.00	25.03
Roots	4.00±0.00	4.70±0.58	5.70±0.58	8.00±0.00	8.67±0.58	10.0±0.00	27.40
(-ve control)	0	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	10±0.00	7.455

The result is Mean+SD. N = 30, table 1. Above show the average death and LC_{50} of Artemia salina brine shrimp at different concentration of the ethyl acetate Leaf, stem-bark and roots extract of B. racemose.

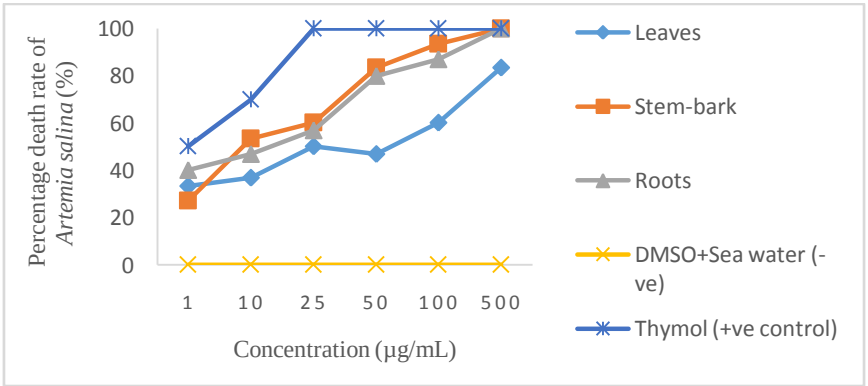

Figure3. *Average death of Artemia salina (%) as a function of various Ethyl acetate extract concentration on plant parts of Barringtonia racemose was monitored after 24hrs exposure of different concentration of the plant parts*

Table4. *Average death of Artemia salina at different concentration of Chloroform crude extract of Barringtonia racemose Leaf, Stem-bark and Roots*

Chloroform Crude Extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	3.00±1.00	4.70±0.58	6.00±1.00	7.33±0.58	6.67±0.58	7.70±0.58	76.638
Stem-bark	3.00±0.00	4.70±1.16	5.70±0.58	6.33±0.58	9.33±1.15	10.0±0.00	29.392
Roots	4.00±1.00	5.70±0.58	7.70±0.58	8.00±0.00	8.67±0.58	9.70±0.58	10.531
(-ve control)	0	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	10±0.00	7.455

The result is Mean+SD. N = 30, table 1. Above show the average death and LC_{50} of Artemia salina brine shrimp at different concentration of the chloroform Leaf, stem-bark and roots extract of B. racemose.

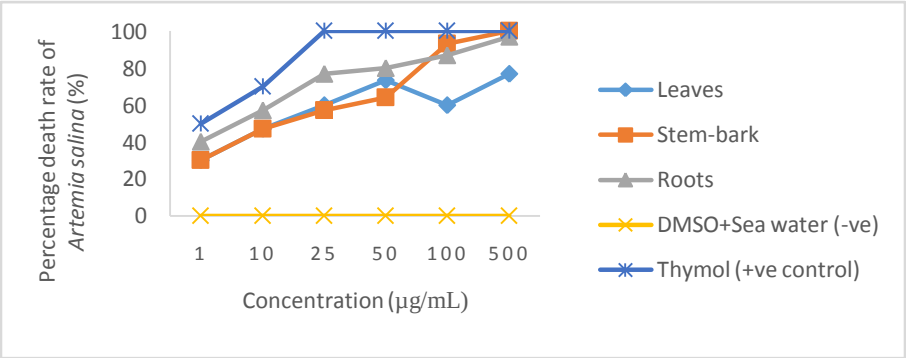

Figure4. *Average death of Artemia salina (%) as a function of various Chloroform extract concentration on plant parts of Barringtonia racemosewas monitored after 24hrs exposure of different concentration of the plant parts*

Table5. *Average death of Artemia salina at different concentration of methanol crude extract of Barringtonia racemose Leaf, Stem-bark and Roots*

Methanol Crude Extract	Average death of Artemia salina Concentration (µg/mL)						LC50 (µg/mL)
	1	10	25	50	100	500	
Leaves	4.33±1.52	5.00±1.00	6.33±0.58	6.33±0.58	8.00±1.00	9.33±0.58	42.968
Stem-bark	3.70±0.58	4.00±1.00	6.00±1.00	7.33±0.58	9.00±1.00	9.70±0.58	37.889
Roots	4.33±1.15	4.77±0.58	8.70±2.31	9.33±1.15	10.0±0.00	10.0±0.00	18.446
(-ve control)	0	0	0	0	0	0	-
(+ve control	5±0.00	7±0.00	10±0.00	10±0.00	10±0.00	10±0.00	7.455

The result is Mean+SD. N = 30, table 1. Above show the average death and LC_{50} of Artemia salina brine shrimp at different concentration of the methanol Leaf, stem-bark and roots extract of B. racemose.

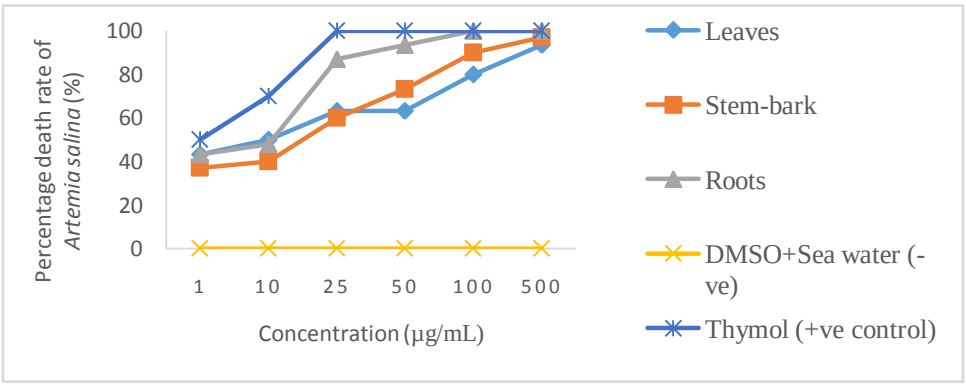

Figure5. *Average death of Artemia salina (%) as a function of various Methanol extract concentration on plant parts of Barringtonia racemosewas monitored after 24hrs exposure of different concentration of the plant parts*

DISCUSSION

The extracts of the three plant parts of *Barringtonia racemose* tested showed a significant lervical activity when compared with other plants studied. The lethality concentration of the leaves, stem-bark and roots were observed at 1ppm -500ppm as presented on the tables. Biological activity of the were determined using the lethality assay, Artemia salina is said to be very vulnerable to toxins at the early developmental stage. In the tables 1-5. The degree of lethality shown by the extractives was found to be directly proportional to the concentration of the extractives range from the lowest concentration to the highest. For the hexane extracts of the leaves, stem-bark and roots of the plant the LC_{50} was found to be 37.285 µg/mL, 19.909 and 24.093., dichloromethane 38.557, 69.186 and 27.397, ethyl acetate 142.45, 25.03 and 27.397. Chloroform and methanol was 76.638, 29.392, 10.531 and 42.968, 37.889, 18.446 µg/mL respectively after 24hrs of exposure. This is higher in comparism to the positive control (Thymol) LC_{50} (7.445) and very toxic when compared to the fact that less than 1000µg/mL is toxic as reported by Meyer *et al*. (1982). The figure above, 11-15 indicated dose dependent relationship where in the percentage death rate of Artemia salina (%) increases with increases in the concentration of the extract. The very high lethality rate detected of the extracts after 24hrs activity was noticed in roots of chloroform and methanol crude extracts with 10.531µg/mL and 18.446 µg/mL. However, Moshi *et al*. (2010), Elumba et al. (2013) and Magdalene et al. (2014) suggested that some of the plant extracts with LC_{50} below 100µg/mL which are categorized as toxic, does not always indicated its danger or out-right toxicity toward human, but may also suggest a potential antitumor or anticancer activities. Exposure or administering this type of plant may unlikely to have negative effects on human (Moshi *et al*., 2010).

Solis *et al*. (1998) and Meyer *et al*. (1982) reported that brine shrimp lethality test is to detect antitumor compounds in terrestrial plant extracts. Thus, with the preliminary results on the cytotoxicity assays, it is possible that the plant parts of *Barringtonia racemose* contain substance that has cytotoxic activity.

CONCLUSION

The in vivo lethality in a simple zoological organism, such as the brine shrimp lethality test (BST), developed for Meyer *et al* brings about the evolution of the toxic action of plant extracts, this is indispensable to consider a treatment safe. Thus, it enables the definition of the intrinsic toxicity of the plant, and the effects of acute overdose (Padjama et al., 2002), a cheap and general bioassay that appears capable of detecting a spectrum of bioactivity present in crude extract is the brine shrimp lethality test. The lethality of the test sample in a simple zoological organism like the brine shrimp (*Artemia salina*) has been utilised by many researchers and has proven to be a useful tool in screening various chemical compounds found in various bioactivities. In this study, it was observed the solvent extracts exhibited brine shrimp cytotoxic activity with significant LC_{50} value. There was an observed concentration-dependent increment in the mortality rate of the brine shrimp; this is considered an indication of proof the cytotoxic effect of the plant extracts reported to have antimicrobial and antifungal activity.

The leaves, stem-bark and root extracts of *Barringtonia racemos e* is therefore considered containing active and potent components. However, *brineshrim*p lethality assay is inadequate in determining the mechanism of action of the bioactive substances in the plant, but it is useful in providing a preliminary screen that can be supported by a more specific bioassay, once the active compound has been isolated. hence, some useful drugs of therapeutic importance can bedeveloping out of the research work.

STATISTICAL ANALYSIS

Statistical analysis for biological activities was performed using SPSS programme. The LC_{50} values for toxicity assay were calculated using the Probit Analysis option in the SPSS.

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CONFLICT OF INTERESTS

The authors declared there is no conflict of interest.

REFERENCE

[1] Ana Beatriz PM, Moacir GP, Ines Maria CB. An Application of the Brine Shrimp Bioassay for

General Screening of Brazilian Medicinal Plants. Acta Farm. Bonaerense. 2002; 21 (3): 175-178.

[2] Chantaranothai P. Barringtonia (Lecythidaceae) in Thailand. Kew Bulletin. 1995; 50(4): 677–694.

[3] Elumba ZS, Teves FG, Madamba MRSB. DNA-binding and cytotoxicity of supercritical- CO_2 extracts of *Ganoderma lucidum* collected from the wild plant of Bukidnon province, Philippines. International Research Journal of Biological Sciences. 2013; 2(3): 62-68.

[4] Kong KW, Mat-Junit S, Aminudin N, Ismail A, Abdul-Aziz A. Antioxidant activities and polyphenolics from the shoots of Barring to niaracemosa (L.) Spreng in a polar to polar medium system. *Food Chem*. 2012; 134: 324-332.

[5] Lilybeth FO, Olga MN. Brine shrimp lethality assay of the ethanolic extract of three selected species of medicinal plants from Iligan City, Philippines. Int Res J Biosci. 2013; 2:74-77.

[6] Magdalene M, Del S, Clifford PB, Charity MLD. Cytotoxic effect of Betel vine. *piperbettlelinn*. Leaf extracts using Artemia salina leach (brine shrimp lethality assay). Journal of Miti disciplinary Studies. 2014; 3(1):100-111.

[7] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for active plants constituents. Plant Med. 1982; 45(19):31-34.

[8] McLaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Plant Med. 1991; 45:31-34.

[9] Moshi MH, Inonocent E, Magadula JJ, Otieno DF, Weisheit A, Mbabazi PK, Nondo, RSO. Brine shrimp toxicity of some plants used as traditional medicine in kagera region north western Tanzania. *Tanzania Journal of Health Research*. 2010; 12(1):1-6.

[10] Osman NI, Norriza HJS, Sidik AA. Pharmacological activities of *Barringtoniaracemosa* L. (Putat). A tropical medicinal plant species. *Journal of Pharmaceutical Sciences and Research*. 2015; 7(4):185-188.

[11] Padjama R, Arun PC, Prashanth D, Deepak M, Amit A, Anajna M. Brine shrimp lethality bioassay of Indian medicinal plants. Fitoterapia. 2002; 73:508-510.

[12] Sam TW. Toxicity testing using the brine shrimp: *Artemia salina*. Bio Act Nat. 1993; 18:441–456.

[13] Solis PN, Wright CW, Anderson MM, Guota MP, Phillipson JD. A micro well cytotoxicity assay using Artemiasalina. Planta Medica. 1993; 59:250-252.