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Bioactivity Study of *Barringtonia asiatica* (Linnaeus) Kurz. Seed Aqueous Extract in *Artemia salina*

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Abstract: The aim of this study is to look at the biological activity of the seeds of *Barringtonia asiatica*. The water crude extract of botong seeds was tested for its biological activity using the brine shrimp hatchability and lethality assay. Results showed high biological activity of the extract in both assays. The LC_{50} obtained was better than the positive control used. It is possible that botong seeds contain compounds with potential biological activity that can be used to treat cancer or tumor. Phytochemical tests showed the presence of terpenoids and saponins in the crude extract.

Key words: *Barringtonia asiatica*, seed, brine shrimp assay, bioactivity

INTRODUCTION

Barringtonia asiatica (Linnaeus) Kurz or fish killer tree grows extensively in coastal region of tropical Asia and the Pacific (Herlt *et al*., 2002). Locally known as botong in the Philippines, they are found along the seashore throughout the country and often cultivated for windbreaks and for shade purposes. The different parts of this species have many uses. The ground seeds are used as fish poisons and source of starch, while the oil from the seeds is used as illuminant (Huang *et al*., 2004).

The seed was found to contain about 2.5% of fixed oil, consisting of olein, palnitin and stearin, 0.54% gallic acid and 3.271% barringtonin, a glucoside (Chopra *et al*., 1958). Two major saponins have been isolated from a methanol extract of the seeds and their structures have been isolated by two dimensional NMR spectroscopy (Herlt *et al*., 2002). The seeds of botong were reported to have piscicidal activity and an oleanane glycoside, ranuncoside was elucidated as the principal compound responsible for the activity (Burton *et al*., 2003).

Limited studies have been done regarding the biological activity of *Barringtonia asiatica*. Antimicrobial activity against two gram-positive bacteria, two gram-negative bacteria and two fungi was found on a related species (*Barringtonia acutangula*) (Rahman *et al*., 2005). The known potent activity of botong and its availability makes it an ideal candidate as source of phytochemicals with possible medical and other beneficial uses. This study was conducted to find out if the phytochemicals in botong seeds possess biological activity against brine shrimp (*Artemia salina* L.). The brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity. Its cytotoxicity data showed strong correlation with costly cytotoxicity tests using human cancer cell lines (Meyer *et al*., 1982; Molina-Salinas and Said-Fernandez, 2006). There is no study yet on the biological activity of the botong seed extract on brine shrimp. The seed extract will be then tested for the possible phytochemicals that are present and responsible for the biological activity.

MATERIALS AND METHODS

Extraction: Mature fruits of *Barringtonia asiatica* were collected from Infanta, Quezon, Philippines on May 2005. The seeds were removed from the fibrous mesocarp and the brown seed coating. It was then grated, packed in aluminum foil and stored in the freezer ready for extraction.

The extraction of the plant material was done by soaking the grated sample in distilled water for 24 h. Crude extract was obtained by filtration and further concentrated by using freeze drier. The collected brownish extract was lyophilized to obtain powdered form of the extracts.

Biological assay: The tests were conducted in 96-well microtiter plate (final volume of 200 μL) using sterilized seawater. A stock solution of the crude extract (10,000 ppm) was prepared using artificial sea water. Different concentrations of extracts ranging from 1-500 ppm were prepared from the stock solution. Controls were also prepared and used with distilled sea water as negative control and potassium dichromate as positive

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standard. One hundred microliter of each working solutions of the extract was transferred in an individual well of a 96-well microtiter plate.

Brine shrimp hatchability test: The brine shrimp hatchability test is based on the procedure done by Migliore *et al.* (1997). Dried cysts of about 100 mg were allowed to hatch in 100 mL seawater at 28°C, under conditions of continuous illumination and strong aeration. After 2 h, 50 μL aliquots were placed in each well where the extracts had previously been deposited. They were incubated at the same conditions of temperature and illumination under gentle shaking. The free nauplii in each well were counted under a stereoscopic microscope after 12, 24 and 48 h of exposure. Five replicates were used for each treatment and control. The percentages of hatchability were calculated by comparing the number of free nauplii in each treatment with the number of free nauplii in the control. Later the percentage of hatch inhibition (HI%) was calculated as:

HI (%) = % hatchability in the control-% hatchability in each treatment

Brine shrimp lethality test: Dried cysts were incubated (100 mg cyst per 100 mL) in a hatcher at 28-30°C with strong aeration, under a continuous light regime. Phototropic nauplii were collected with a micropipette from the lighted side and placed in a small vial 12 h after hatching. Ten brine shrimps were placed in each well which contained the extracts previously deposited. These brine shrimps were exposed to various concentrations of the extract. The mortality was determined after 12 h (mainly nauplii in instar I/II), 24 h (nauplii in instar II/III) and 48 h (mainly nauplii in instar III/IV) of exposure. The larvae did not receive food. To make sure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation, the number of dead larvae in each treatment were compared to the number of dead larvae in the control. In any case, hatched brine shrimp nauplii can survive for up to 48 h without food (Lewis, 1995) because they still feed on their yolk-sac (Pelka *et al.*, 2000). However, in cases where control deaths were detected, the percentage of mortality (% M) was calculated as:

M (%) = Percentage of survival in the control-percentage of survival in the treatment

The number of survivors was counted under a microscope and percentage of mortality was calculated. Larvae were considered dead if they did not show signs of any internal or external movement during several seconds of observation under a microscope.

Phytochemical test: The presence of saponins and terpenoids in the extract was determined using the froth test, hemolytic assay and Liebermann-Burchard test for saponins and the Salkowski test for terpenoids. Froth test was done wherein 2 g of the powdered sample was boiled in 20 mL of distilled water in a water bath and filtered (Oboh *et al.*, 1999). The filtrate (5 mL) was mixed with 5 mL distilled water and shaken vigorously for a stable persistent froth. On the other hand, hemolytic assay was done by mixing the crude extract with red blood cell solution in microplate wells using the method by Mojica and Merca (2005). Lastly, for the Liebermann-Burchard test, 10 drops of the crude extract was placed in dry test tubes. It was added with 3 drops of acetic anhydride and one drop of concentrated sulfuric acid and observed for any color changes (Said *et al.*, 1990).

For the test for terpenoids, 5 mL of the crude extract was mixed with 2 mL of chloroform and concentrated sulfuric acid (3 mL) was carefully added to form a layer.

RESULTS AND DISCUSSION

Brine shrimp hatchability and lethality test assay were used to determine the biological activity of the *Barringtonia asiatica* (botong) seed's aqueous extract. For the hatchability test, inhibition of hatching was observed in at least 10 ppm extract for 12 h and 100 ppm for 24 h exposure (Fig. 1). At lower concentrations (1-50 ppm), there is a dose dependent relationship wherein the percentage hatchability decreases as the concentration of the extract increases. The low hatching rate observed after the 12 h treatment was probably due to an alteration in the development of *Artemia* embryos. Studies have shown that *Artemia* is highly vulnerable to toxins at the early developmental stages (Sorgeloos *et al.*, 1978; Sleet and Brendel, 1985). The hatched brine shrimp nauplii in treated with the crude extract were found to have deformities just like those observed in the positive control in comparison to the nauplii in the negative control.

In the lethality/mortality test, the same dose dependent relationship just like the hatchability test was observed wherein the percentage mortality increases as the concentration of the extract increased (Fig. 2). The LC_{50} was found to be 275.7, 32.34 and 2.25 ppm for 12, 24 and 48 h of exposure, respectively. This is higher in comparison to the positive control which have LC_{50} of 510.4, 139.4 and 20.25 ppm for 12, 24 and 48 h of exposure, respectively. Activity increased significantly up to 48 h exposure particularly for the fruit extract. Maximum sensibility is usually reached after 48 h of exposure (the oldest age class tested) (Sanchez-Fortun *et al.*, 1996)

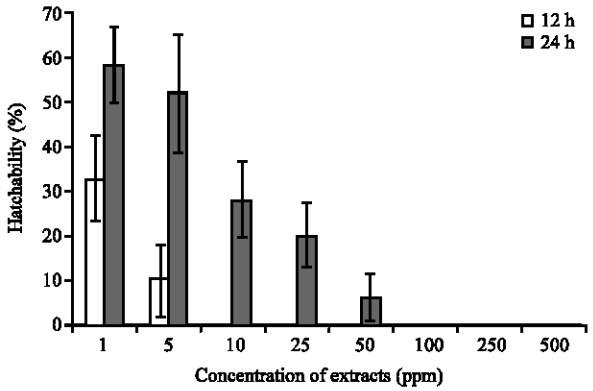

Fig. 1: *Artemia salina* (brine shrimp) hatchability test of *Barringtonia asiatica* crude water extract. Percentage hatchability of brine shrimp eggs was monitored after 12 and 24 h exposure on different concentrations of the extracts

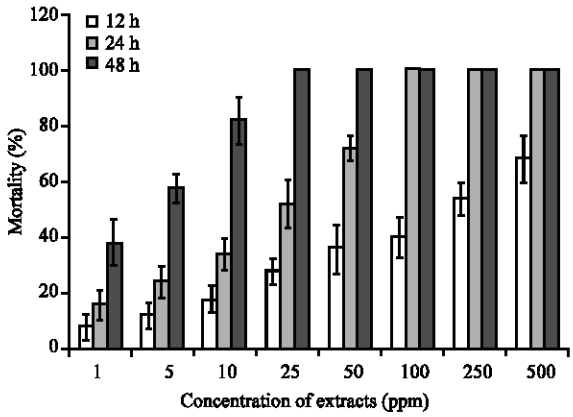

Fig. 2: *Artemia salina* (brine shrimp) lethality test of *Barringtonia asiatica* crude water extract. Percentage mortality of brine shrimp eggs was monitored after 12, 24 and 48 h exposure on different concentrations of the extracts

since at this stage, the life cycle of the nauplii have reached second and third instar and exhibit greatest sensitivity to test compounds (Lewis, 1995).

For the past 30 years, the *Artemia* nauplii have been used detect general toxicity (Persoone and Wells, 1987), in teratology screens (Acey and Tomlison, 1988; Sleet and Brendel, 1985) and in ecotoxicology (Sorgeloos *et al*., 1978). From a pharmacological point of view, a good relationship has been found with the brine shrimp lethality test to detect antitumoral compounds in terrestrial plant extracts (Meyer *et al*., 1982; Solis *et al*., 1993). The extract could contain antitumor agents since the LC obtained is lower than the standard set by the United States National Cancer Institute. For a bioactive compound to be an effective antitumor agent it must have an LC_{50} equal or less than 30 ppm. This is also more potent than the previous study done on bignay [*Antidesma bunius* (L.) Spreng] which have an LC_{50} of 702.13 ppm for 12, 24 and 48 h of exposure, respectively (Micor *et al*., 2005). With these preliminary results, it is possible that the *Barringtonia asiatica* contain substances that might have cytotoxic activity.

For the phytochemical tests conducted, the crude extracts gave positive results for the presence of saponins. Froth test showed persistent honeycomb frothings on the extract and Leibermann-Buchard test showed the formation of a greenish color in the solution. Positive results were also obtained in the hemolytic test thus confirming the presence of saponins. On the other hand, a reddish brown coloration on the interphase was obtained in the Salkowski test confirming the presence of trepenoids. Literature had reported the presence of these phytochemicals (Herlt *et al*., 2002; Burton *et al*., 2003; Yang *et al*., 2006) in the seeds of *Barringtonia asiatica* and its related genus. It is possible that these compounds acting individually or synergistically were responsible for the observed activity of the extract.

CONCLUSION

Aqueous crude extract of *Barringtonia asiatica* seeds were tested for biological activity using the brine shrimp hatchability assay and lethality assay. Results showed dose dependent relationship on the activity and concentrations used. It is possible that the extracts contained substances with cytotoxic activity. Phytochemical tests using the crude extracts showed the presence of saponins and terpenoids.

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REFERENCES

Acey, R.A. and D.W. Tomlison, 1988. *Artemia salina* as a model system for assessing the effects of xenobiotics on embryonic development. FASEB J., 2: 8463.

Burton, R.A., S.G. Wood and N.L. Owen, 2003. Elucidaion of a new oleanane glycoside from *Barringtonia asiatica*. Arkivoc, 13: 137-146.

Chopra, R.N., I.C. Chopra, K.L. Handa and L.D. Kapur, 1958. Chopra's Indigenous Drugs of India. Calcutta: U.N. Dhur and Sons Private Ltd.

Herlt, J.H., L.N. Mander, E. Pongoh, R.J. Rumampuk and P. Tarigan, 2002. Two major saponins from seeds of *Barringtonia asiatica*: Putative antifeedants toward *Epilachna* sp. larvae. J. Nat. Prod., 65: 115-120.

Huang, J., X. Lai, M. Zhong and S. Zhang, 2004. Survey of the chemical constituents and pharmacological activities of mangrove medicinal plant Barringtonia. Chinese Acad. Sci., 16: 167-171.

Lewis, G.E., 1995. Testing the toxicity of extracts of Southern African plants using brine shrimp (*Artemia salina*). S. Afr. J. Sci., 91: 382-390.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med., 45: 31-34.

Micor, J.R.L., C.C. Deocaris and E.R.E. Mojica, 2005. Biological activity of bignay [*Antidesma bunius* (L.) Spreng] crude extract in *Artemia salina*. J. Med. Sci., 5: 195-198.

Migliore, L., C. Civitareale, G. Brambilla and G.D. Di Delupis, 1997. Toxicity of several important agricultural antibiotics to *Artemia*. Water Res., 31: 1801-1806.

Mojica, E.R.E. and F.E. Merca, 2005. Biological properties of lectin from *Holothuria scabra* Jaeger. J. Biol. Sci., 5: 472-477.

Molina-Salinas, G.M. and S. Said-Fernandez, Salvador, 2006. A modified microplate cytotoxicity assay with brine shrimp larvae (*Artemia salina*). Pharmacologyonline, 3: 633-638.

Oboh, G., M.T. Olaleye and F.A. Akinyosoye, 1999. Hemolytic effect of saponin extracts from soybean seeds (*Glycine max*) on some mammalian erythrocytes. Rivista Italiana delle Sostanze Grasse, 76: 479-481.

Pelka, M., C. Danzl, W. Distler and A. Petschelt, 2000. A new screening test toxicity testing of dental materials. J. Dentistry, 28: 341-345.

Persoone, G. and P.G. Wells, 1987. *Artemia* in Aquatic Toxicology: A review. In: *Artemia* Research and its Applications. Morphology, Genetics, Strain characterization Toxicology. Sorgeloos, P. (Ed.), Belgium, Universita Press, pp: 259-275.

Rahman, M.M., D. Polfreman, J. MacGeachan and A. Gray, 2005. Antimicrobial activities of *Barringtonia acutangula*. Phytother. Res., 19: 543-545.

Said, I.M., L.B. Din, M.W. Samsudin, Z. Zakaria, N.I. Yusoff, U. Suki, A. Manaf, A.Z. Ibrahim and A. Latiff, 1990. A phytochemical survey of Uku Kincin, Pahang, Malaysia. Mal. Nat. J., 43: 260-266.

Sánchez-Fortún, S., S. Sanz and M.V. Barahona, 1996. Acute toxicity of several organophosphorous insecticides and protection by cholinergic antagonist and 2-PAM on *Artemia salina* larvae. Arch. Environ. Contam. Toxicol., 31: 391-398.

Sleet, R.B. and K. Brendel, 1985. Homogeneous populations of *Artemia* nauplii and their potential use for *in vitro* testing in developmental toxicology. Teratog. Carcinog. Mutagen., 5: 41-54.

Solis, P.N., C.W. Wright, M.M. Anderson, M.P. Gupta and J.D. Phillipson, 1993. A microwell cytotoxicity assay using *Artemia salina*. Planta Med, 59: 250-252.

Sorgeloos, P., C. Remiche-Van Der Wielen and G. Persoone, 1978. The use of *Artemia nauplii* for toxicity test. A critical analysis. Ecotoxicol. Environ. Saf., 2: 249-255.

Yang, Y., Z. Deng, P. Proksch and W. Lin, 2006. Two new 18-en-oleane derivatives from marine mangrove plant, *Barringtonia racemosa*. Pharmazie, 61: 365-366.