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Biological Activity of Bignay [*Antidesma bunius* (L.) Spreng] Crude Extract in *Artemia salina*

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In the present study, the leaves and fruits of bignay [*Antidesma bunius* (L.) Spreng] were extracted with methanol. The methanolic crude extracts were tested for its biological activity using the brine shrimp hatchability and lethality assay. Results showed biological activity of the extracts in both assays. Higher activity was observed in fruit extract and its LC_{50} was comparable to the positive control used. It is possible that bignay contains compounds with potential cytotoxic activity.

Key words: Bignay, *Artemia salina*, biological activity, cytotoxic

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INTRODUCTION

Bignay [*Antidesma bunius* (L.) Spreng] is an abundant and invasive shrub tree species found in the Philippines. It has wide-spreading branches forming a dense crown, evergreen, alternate leaves that are oblong and pointed and round or ovoid fruits, borne in grapelike, pendent clusters (often paired). Its leaves are usually used as flavoring when combined with other vegetables while its fruits are processed into jam and jelly ant its juices fermented into wine and brandy.

Recently, bignay was used to treat different illnesses ranging from colds to cancer[1]. Due to the absence of studies regarding the biological activity of bignay, this study was done to assess the biological activity of bignay's leaves and fruits 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[2].

MATERIALS AND METHODS

Extraction: Leaves and fruits of bignay were collected from Lipa, Batangas. The leaves were properly washed with tap water and sliced into small pieces. The sliced leaves were soaked with methanol for one week (250 g L^{-1}). The fruits were also washed, then crushed thoroughly in a blender and soaked in methanol for one week (300 g/600 mL).

Crude extracts were obtained by filtration. These were further concentrated by using a rotary evaporator. The collected dark green leaf extract and the yellowish fruit extract were then lyophilized to obtain powder form of the extracts.

Biological assay: The tests were conducted in 96 well microtiter plate with sterilized seawater (final volume 200 uL). A stock solution of 10,000 ppm was prepared for each extract with the use of artificial sea water. Different concentrations (10–1,000 ppm) of extracts were prepared from their stock solution. Artificial sea water was used as negative control while potassium dichromate was used as positive standard. One hundred microliter of each working solutions for each 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 Migliore *et al.*[3]. Dried cysts amounting to 100 mg were hatched in 100 mL seawater at 28°C, under conditions of continuous illumination and strong aeration. After 2 h, aliquots measuring 50 μL were placed in each well where the extracts had previously been deposited, and they were incubated at the same conditions of temperature and illumination under gentle shaking. After 12, 24 and 48 h of exposure, the free nauplii were counted under a stereoscopic microscope. 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. Approximately 12 h after hatching, the phototropic nauplii were collected with a micropipette from the lighted side and placed in a small vial. Ten brine shrimp were transferred to each well which contained the extracts previously deposited. Groups of 10 brine shrimps aged 12 h 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 ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not to starvation, the dead larvae in each treatment were compared to the dead larvae in the control. In any case, hatched brine shrimp nauplii can survive for up to 48 h without food[4] because they still feed on their yolk-sac[5]. 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 exhibit any internal or external movement during several seconds of observation.

RESULTS AND DISCUSSION

The biological activity of the extracts of bignay fruits and leaves to *A. salina* were determined using the hatchability and lethality assay. For the hatchability test, inhibition of hatching was observed in at least 10 ppm fruit extract for 12 h and 50 ppm for 24 h exposure (Fig. 1). This is higher than the leaf extract, which showed hatching inhibition at 50 ppm for 12 h and 100 ppm for 24 h of exposure (Fig. 2). At lower concentrations

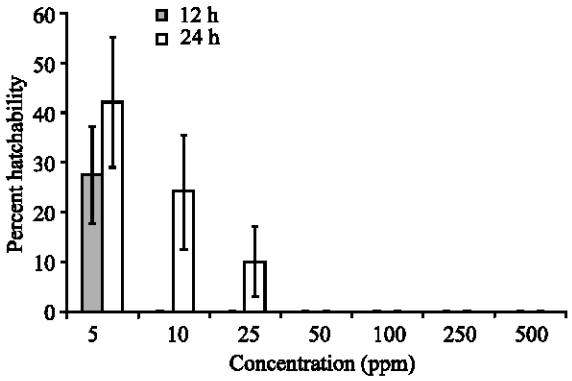

Fig. 1: *Artemia salina* (brine shrimp) hatchability test of fruit extract. Percentage hatchability of brine shrimp eggs was monitored after 12 and 24 h exposure on different concentrations of the fruit extracts

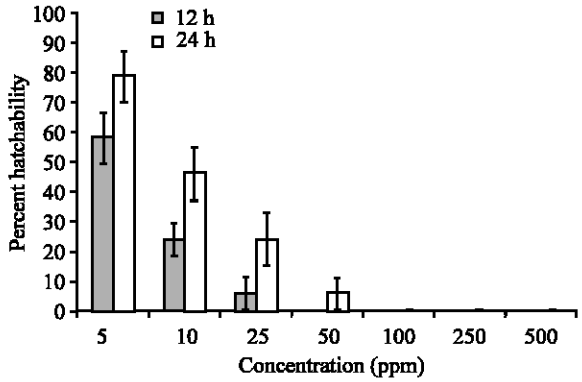

Fig. 2: *Artemia salina* (brine shrimp) hatchability test of leaf extract. Percentage hatchability of brine shrimp eggs was monitored after 12 and 24 h exposure on different concentrations of the leaf extracts

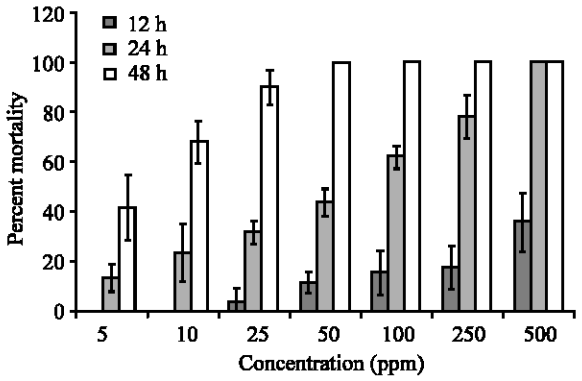

Fig. 3: *Artemia salina* (brine shrimp) lethality test of fruit extract. Percentage mortality of brine shrimp eggs was monitored after 12, 24 and 48 h exposure on different concentrations of the leaf extracts

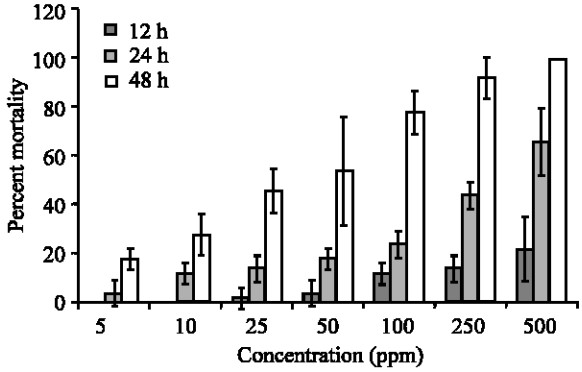

Fig. 4: *Artemia salina* (brine shrimp) lethality test of leaf extract. Percentage mortality of brine shrimp eggs was monitored after 12, 24 and 48 h exposure on different concentrations of the leaf extracts

(5-50 ppm), there is a dose dependent relationship wherein the percentage hatchability decreases as the concentration of the extract increases. The very low hatching rate detected after the 12 h treatment was probably due to an alteration in the development of *Artemia* embryos. It has been shown that *Artemia* is highly vulnerable to toxins at the early developmental stages[6,7]. It has also been observed that the hatched brine shrimp nauplii have deformities just like those in the positive control in comparison to the nauplii in the negative control.

In the lethality test, the same dose dependent relationship just like the hatchability was observed wherein the percentage mortality increases as the concentration of the extract increased. For the fruit extract, the LC_{50} was found to be 702.13, 130.67 and 5.7 ppm for 12, 24 and 48 h of exposure, respectively (Fig. 3). This is higher in comparison to the leaf extract which have LC_{50} of 1104, 339.4 and 69.8 ppm for 12, 24 and 48 h of exposure, respectively (Fig. 4). The positive control have an LC_{50} of 20.19 ppm. 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)[8] since at this stage, the life cycle of the nauplii have reached second and third instar and exhibit greatest sensitivity to test compounds[4].

For the past 30 years, the *Artemia* nauplii have been used detect general toxicity[9], in teratology screens[7,10] and in ecotoxicology[6]. 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[2,11]. With the preliminary results on the two assays, it is possible that the fruits and leaves of bignay contain substances that have cytotoxic activity.

CONCLUSIONS

The fruits and leaves of bignay were extracted with methanol. The collected crude extracts were lyophilized and then tested for its biological activity using the brine shrimp hatchability assay and lethality assay. Results showed dose dependent relationship on the activity and concentrations used. Higher activity was observed in fruit extract than in leaf extract. With these results, it is possible that the fruits and leaves of bignay contain substances with cytotoxic activity.

REFERENCES

1. Magsino, M., 2003. Don't laugh, bignay is really hard wood. Philippine Daily Inquirer. 10th April, 2003 pp: A1-A4.
2. 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 Medica, 45:31-34.
3. Migliore, L., C. Civitareale, G. Brambilla and G.D. Di Delupis, 1997. Toxicity of several important agricultural antibiotics to *Artemia*. Water Research, 31: 1801-1806.
4. Lewis, G.E., 1995. Testing the toxicity of extracts of Southern African plants using brine shrimp (*Artemia salina*). South African J. Sci., 91: 382-390.
5. Pelka, M., C. Danzl, W. Distler and A. Petschelt, 2000. Anew screening test toxicity testing of dental materials. J. Dentistry, 28: 341-345.
6. Sorgeloos, P., C. Remiche-Van Der Wielen and G. Persoone, 1978. The use of *Artemia nauplii* for toxicity tests. A critical analysis. Ecotoxicol. Environ. Safety, 2: 249-255.
7. Sleet, R.B. and K. Brendel, 1985. Homogeneous populations of *Artemia* nauplii and their potential use for in vitro testing in developmental toxicology. Teratogenesis, Carcinogenesis and Mutagenesis, 5: 41-54.
8. 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. Contamination Toxicol., 31: 391-398.
9. Persoone, G. and P.G. Wells, 1987. *Artemia* in Aquatic Toxicology: A Review. In: *Artemia* Research and its Applications. Morphology, Genetics, Strain Characterization Toxicology (Ed., Sorgeloos, P.) Belgium, Universita Press, pp: 259-275.
10. 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.
11. Solís, P.N., C.W. Wright, M.M. Anderson, M.P. Gupta and J.D. Phillipson, 1993. A microwell cytotoxicity assay using *Artemia salina*. Planta Medica, 59: 250-252.