Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 (3):47-54
(http://scholarsresearchlibrary.com/archive.html)

Der Pharmacia Lettre
Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Radical Scavenging and Teratogenic Activities of Stem-Bark, Leaves, and Fruit Rind of Guyabano (*Annona muricata* Linn.)

Rich Milton R. Dulay[1]* and Jocelyn S. De Vera[2]

[1]*Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines*

[2]*College of Teacher Education, Pangasinan State University, Bolaney, Alaminos City, Pangasinan, Philippines*

***Corresponding Author:** [1]Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, Email: richmiltondulay@clsu.edu.ph*

ABSTRACT

This work reported the radical scavenging activity and the toxic and teratogenic effects of stem-bark, leaves, and fruit rind extracts of Annona muricata Linn. in Danio rerio embryos. Stem-bark extract recorded the highest radical scavenging activity of 87.92%, followed by fruit-rind (78.64%) and leaves (75.87%) extracts. In D. rerio assay, 100% mortality was noted in embryos at 1000 µg/ml and higher concentrations of stem-bark and 5000 µg/ml and higher concentrations of leaves and fruit rind extracts after 48 hours of exposure. Delayed development and malformations were determined as teratogenic parameters. Embryos at 500 µg/ml of stem-bark and 1000 µg/ml of both leaves and fruit rind and their higher concentrations recorded 100% delayed development. Similarly, these concentrations of the three extracts showed 100% malformations. However, higher concentrations did not show abnormalities due to arrested growth at earlier periods of observations. Extract treated zebrafish showed hook-like tail, bent tail, malformation of the eyes, less pigmentation, scoliosis, and yolk deformity. The toxic and teratogenic effects of A. muricata were dependent on the parts of the plant, concentration of the extracts and time of exposure.

***Keywords:** Annona Muricata, Antioxidant, Teratogen, Zebrafish, Free Radicals, Embryo-Toxic.*

INTRODUCTION

The Philippines is considered a developing country that still believes on the effectiveness of traditional medicine. Medicinal plants are the most valuable resource in traditional medicine, which are used to treat various illnesses such as dysentery, diarrhea, intestinal ailments, fever, cold, cough, sore throat, skin diseases, and others. The different parts of the plants are utilized in various preparations, and the most common preparation is boiling.

Guyabano (*Annona muricata* Linn.) is one of the effective medicinal plants. This fruit-bearing tree, which belongs to family Annonaceae, is reaching heights of 5 meters with alternate, shiny dark green, oval and leathery leaves and with pear-shaped and prickly green, sweet and sour taste fruit. This plant exhibits different pharmacological properties such as anti-hyperglycemic, anti-inflammatory, antinociceptive, anticancer, and antitumor [1-4].

Despite of the remarkable biological activities, the toxic and teratogenic effects and the radical scavenging activity of *A. muricata* have not yet been investigated. Herein, we evaluated the toxic and teratogenic effects of the water extracts of leaves, stem-bark, and fruit-rind of *A. muricata* in developing embryos of zebrafish. The radical scavenging activity of this medicinal plant was also determined.

MARTERIALS AND METHODS

Collection of plant samples

The stem-bark, leaves, and fruit rind of *P. americana* were collected from Bambanaba, Cuyapo, Nueva Ecija, Philippines. Each sample was separately placed in a plastic bag with proper label and brought in the laboratory. Samples were washed three times, air-dried in a shaded condition for 10 days, milled using a blender, and subjected to extraction.

Ethanol extraction

Each plant part (20 g) was soaked in 500 ml of 95% ethanol for 48 hours. These were filtered using Whatman No. 2 filter paper to separate the filtrate and plant material. Each filtrate was evaporated in a rotary evaporator to remove the ethanol. Extracts were labeled and prepared for radical scavenging and teratogenic assays.

DPPH radical scavenging activity assay

The stable 2,2'-diphenyl-1-1picrylhydrazyl (DPPH) radical was used to estimate the free radical scavenging activity of the extracts, following the standard method of Shimada et al. [5]. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated.

Spawning and fertilization

The procedures on spawning and fertilization were followed Nagel [6]. Mature female and male zebrafish at 1:2 ratio were acclimatized in a glass aquarium with water saturated with oxygen. They were fed two times a day with dry flakes and the quality of water was maintained. In spawning, fish were localized in a plastic mesh and the aquarium was covered with black plastic sheet for 12 hours. After spawning, eggs were exposed to lighted condition for another 12 hours. The eggs were fertilized after 30 minutes of exposure to light. Embryos were collected from the aquarium using a hose and examined for the uniformity of embryos using a microscope. Coagulated and unfertilized eggs were discarded and the normal embryos were used in the assay.

Toxicity and teratogenicity assay

The protocol on the toxicity and teratogenicity using zebrafish embryos was adopted from Dulay et al. [7]. Four embryos at segmentation phase were exposed to the different concentrations of each extract. Mortality was determined after 12, 24, 36, and

48 hours of extract exposure. The percentage hatchability, delayed development, and malformation were also recorded. The morphological abnormalities of the treated embryos were based on the parameters established by Nagel [6]. The validity of the results was also noted. Analysis of Variance (ANOVA) was used to analyze the data and Least Significant Difference (LSD) was used to compare the means at 5% level of significance.

RESULTS AND DISCUSSION

Radical scavenging activity of A. muricata extracts

Medicinal plants contain various phytochemicals. These phytochemicals exhibit promising biological activities such as antioxidant. Antioxidants are natural agents that have the ability to eliminate or reduce the free radicals responsible in causing various diseases. A stable 2,2diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is a simple method used to determine the antioxidant activity of sample. In the present study, the DPPH radical scavenging activities of the extracts of the three plant parts of *A. muricata* were determined. The results of the radical scavenging assay of the three extracts are presented in Table 1. Among the three plant parts, stem-bark extract recorded the highest radical scavenging activity of 87.92%, followed by fruit-rind extract with 78.64%. Although noted as having the lowest activity, the scavenging activity of leaves extract (75.87%) was statistically comparable with the fruit-rind extract. These scavenging activity values of the three plant parts of *A. muricata* are within the range of scavenging activity values of *Hibiscus cannabinus*, where the fruit, stem and leaves have 65%, 75%, and 84% activity, respectively [8]. The results of the present study strongly indicate that the antioxidant properties of the plant are dependent on their parts. Patel et al. [8] reported that the leaves of *Parthenium hystrophorous* and *Gmelina arborea* have higher scavenging activity than their stems, whereas *Calotropis procera* and *Kigellia pinnata* showed higher scavenging activity in their stems than their corresponding leaves. The strong radical scavenging activity of *A. muricata* could be attributed to its phytochemical components. This plant contains flavonoids and phenolic compounds [9], which have been reported to exhibit antioxidant properties [10, 11]. Due to this important antioxidant possessed by *A. muricata*, this plant could a valuable resource of natural compounds to combat free radicals that cause oxidative damage resulting to various chronic diseases including cancer, diabetes, arthritis, inflammation, atherosclerosis, as well as neurodegenerative diseases and ageing process.

Table:1-Radical scavenging activity of the three plant parts of *A. muricata*

***A. muricata* Extract**	**Radical Scavenging Activity (%)**
Stem-bark	87.92[b]
Leaves	75.87[c]
Fruit-rind	78.64[c]
Cathechin	95.12[a]

Means having the same letter of superscripts are not significantly different from each other using DMRT at 5% level of significance.

Toxic effects of A. muricata extracts

Zebrafish embryo is a reliable animal model in toxicity and teratogenicity assays of potentially toxic edible and medicinal plants. In the present study, segmentation phase embryos of zebrafish were exposed to the varying concentrations of the extracts of the three plants parts of *A. muricata*. Table 2 depicts the percentage mortality of extract treated embryos at different periods of observation. At 12 hours' post treatment application (hpta), 100% of mortality was recorded in embryos exposed to 10000 µg/ml concentration of the three extracts and at 5000 µg/ml concentration of stem-bark. Coagulation was the most distinct toxic effect of the extracts. A 100% mortality of embryos at 5000 µg/ml concentration of leaves and stem-bark extracts was observed after 36 hours of exposure. However, at 48 hpta, embryos at 500 µg/ml concentration of the three extracts showed mortality but significantly lower than that of the higher concentrations. Embryos at stem-bark, leaves, and fruit rind have 33.33%, 25.00%, and 16.67%, respectively. A 100% mortality was noted in embryos at 1000 µg/ml concentration of stem-bark and 5000 µg/ml concentration of leaves and fruit rind extracts. All extracts at 100 µg/ml and lower concentrations recorded no mortality. The results of the study clearly suggest that the toxic effects of *A. muricata* on developing embryos are dependent on the parts of plant, concentration of extract, and time of exposure. Comparing the three plant parts, stem bark had the most toxic effect. This significant toxic effect could be attributed to the bioactive chemical components of *A. muricata*, and one of the novel phytochemicals is annonaceous acetogenin. This potent anticancer compound is composed of a series of polyketide-derived fatty acid with tetrahydrofuran rings and methylated gamma-lactone bonded together [12].

Table:2-Mortality of *D. rerio* embryos after 12, 24, 36, and 48 hours of exposure to varying concentrations of the three plant part extracts of *A. muricata*

Plant Extract	Concentration (µg/ml)	Mortality (%)			
		12 hours	24 hours	36 hours	48 hours
Stem-bark	10000	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	5000	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	1000	0.00^{b}	25.00^{b}	50.00^{b}	100.00^{a}
	500	0.00^{b}	0.00^{b}	8.33^{bc}	33.33^{b}
	100	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}
	50	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}
	0	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{c}
Leaves	10000	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
	5000	16.67^{b}	66.67^{b}	100.00^{a}	100.00^{a}
	1000	0.00^{b}	8.33^{c}	33.33^{b}	66.67^{b}
	500	0.00^{b}	0.00^{c}	0.00^{c}	25.00^{c}

	100	0.00[b]	0.00[c]	0.00[c]	0.00[d]
	50	0.00[b]	0.00[c]	0.00[c]	0.00[d]
	0	0.00[b]	0.00[c]	0.00[c]	0.00[d]
Fruit-rind	10000	100.00[a]	100.00[a]	100.00[a]	100.00[a]
	5000	8.33[b]	41.67[b]	100.00[a]	100.00[a]
	1000	0.00[b]	0.00[c]	16.67[b]	41.67[b]
	500	0.00[b]	0.00[c]	8.33[bc]	16.67[bc]
	100	0.00[b]	0.00[c]	0.00[c]	0.00[c]
	50	0.00[b]	0.00[c]	0.00[c]	0.00[c]
	0	0.00[b]	0.00[c]	0.00[c]	0.00[c]

Treatment means of each plant extract having the same letter of superscript are not significantly different from each other at 5% level of significance using LSD.

Effect of A. muricata extracts on the hatching of zebrafish

Hatching is an indicator of successful and normal development of the embryo. The effect of the different concentrations of *A. muricata* extracts on the hatching process of zebrafish embryos was also determined in this study. The percentage hatchability of zebrafish after 48 hours of treatment exposure is shown in Table 3. It can be seen that embryos exposed to 50 µg/ml concentration of the three extracts and in control showed 100% hatchability. On the other hand, no hatched was observed at 500 µg/ml and higher concentrations of stem-bark extract and at 1000 µg/ml and higher concentrations of both leaves and fruit rind extracts. This only suggests that *A. muricata* extract could interfere and delay the hatching process of zebrafish. The delayed and impeded hatching could be due to the inhibited enzymes or substances responsible for this very important process.

Teratogenic activity of A. muricata extracts

Aside from the toxic effects, the teratogenic property of the three plant parts of *A. muricata* is also highlighted in this study. The delayed development and morphological abnormalities were considered in assessing the teratogenic effects of the extracts in zebrafish embryos. The percentage delayed development of embryos after 48 hpta is presented in Table 3. Apparently, the effect of the extracts on the delayed development of zebrafish was dependent on the parts of the plant and concentrations of the extracts. In stem bark extract, embryos showed 100% delayed development when exposed to 500 µg/ml and higher concentrations while those at 100 µg/ml concentration had 41.67%. Embryos at 1000 µg/ml and higher concentrations of leaves and fruit rind extracts also registered 100% delayed development. However, those at 500 and 100 µg/ml concentrations have 83.33% and 25.00% for leaves extract and 66.67% and 16.67% for fruit rind extract, respectively. No delayed development was noted to embryos treated with 50 µg/ml concentration of the three *A. muricata* extracts. In comparison, stem-bark had the most number of delayed embryos. This distinct delayed development apparently contributed to the morphological abnormalities of zebrafish.

The presence of morphological abnormality is the most important teratogenic effect in *D. rerio* teratogenicity assay. Therefore, in the present study the percentage malformation of *A. muricata* extract-treated embryos after 72 hpta was also determined and the results are depicted in Table 3. It can be seen that 100% malformation was registered in embryos at 500 µg/ml of stem-bark extract and at 1000 µg/ml of both leaves and fruit rind extracts. The different malformations of hatched zebrafish after 72 hours of exposure are shown in Figure 1. Apparently, extract treated zebrafish larvae showed hook-like tail, bent tail, malformation of the eyes, less pigmentation, scoliosis, and yolk deformity. However, higher concentrations did not show any malformations due to coagulation and dead embryos at earlier periods of observation. Normal embryos were observed at 50 µg/ml of all extracts and embryo water, which indicated the validity of the assay.

Table-3: Percentage hatchability and delayed growth after 48 hours and percentage malformation after 72 hours of *D. rerio* exposed to varying concentrations of the three plant part extracts of *A. muricata*

Plant Extract	Concentration (µg/ml)	Hatchability (%)	Delayed development (%)	Malformation (%)
Stem-bark	10000	0.00^{c}	100.00^{a}	Coagulated*
	5000	0.00^{c}	100.00^{a}	Coagulated*
	1000	0.00^{c}	100.00^{a}	Dead*
	500	0.00^{c}	100.00^{a}	100.00^{a}
	100	58.33^{b}	41.67^{b}	33.33^{b}
	50	100.00^{a}	0.00^{c}	0.00^{c}
	0	100.00^{a}	0.00^{c}	0.00^{c}
Leaves	10000	0.00^{c}	100.00^{a}	Coagulated*
	5000	0.00^{c}	100.00^{a}	Dead*
	1000	0.00^{c}	100.00^{a}	100.00^{a}
	500	16.67^{c}	83.33^{ab}	75.00^{b}
	100	75.00^{ab}	25.00^{c}	16.67^{c}
	50	100.00^{a}	0.00^{c}	0.00^{c}
	0	100.00^{a}	0.00^{c}	0.00^{c}
Fruit-rind	10000	0.00^{c}	100.00^{a}	Coagulated*
	5000	0.00^{c}	100.00^{a}	Dead*
	1000	0.00^{c}	100.00^{a}	100.00^{a}

	500	33.33[b]	66.67[b]	41.67[b]
	100	83.33[a]	16.67[c]	8.33[c]
	50	100.00[a]	0.00[c]	0.00[c]
	0	100.00[a]	0.00[c]	0.00[c]

Treatment means of each plant extract having the same letter of superscript are not significantly different from each other at 5% level of significance using LSD. *No malformation was recorded due to the coagulated and dead embryos at earlier period of observation.

Figure-1: Abnormal zebrafish larvae at 500 µg/ml of stem-bark extract (A) and at 1000 µg/ml of leaves extract (B) and fruit rind extract (C) of *A. muricata* and normal control embryo (D) at 72 hpta.

CONCLUSION

In conclusion, *A. muricata* exhibits antioxidant activity via DPPH radical scavenging activity. It also possesses toxic and teratogenic properties in developing *D. rerio* embryos. These activities are dependent on the parts of the *A. muricata*, concentration of the extracts and time of exposure. The significant effects to zebrafish embryos prove that this plant could be a valuable source of bioactive compounds with promising human healthful benefits.

REFERENCES

1. Adeyemi, AO., et al., *Afr J Trad, CAM*, **2009**. 6(1): p. 62-69.
2. De Sousa, OV., et al., *Int J Mol Sci*, **2010**. 11, p. 2067-2078.
3. Hamizah, S., et al., *Asian Pacific Journal Cancer Prevention*, **2012**. 13, p.2533-2539.
4. Torves, MP., et al., *Cancer Cell Letters*, **2012**. 323: p. 29-40.
5. Shimada, K., et al., *Journal of Agricultural and Food Chemistry*, **1992**, 40, 945.
6. Nagel R, *ALTEX*, **2002**. 19(1) p. 38-48.
7. Dulay, RMR., et al., *Int J Med Mushrooms*, **2012**. 14(5): p. 507-512.
8. Patel, VR., et al., *Advances in Biological Research*, **2010**. 4(1): p. 23-26.
9. Pandya, N., *Int J Res Pharm and Biomedical Sciences*, **2011**. 2(4): p. 1404-1414.
10. Shahidi, F. and Wanasundara, PD., *Cri Rev Food Sci Nutr*, **1992**. 32: p. 67-103.
11. Miller, AL., *Alt Med Rev*, **1996**. 1, p.103.
12. Mat Daud, N., *Integr Cancer Sci Therap*, **2016**. 3(4): p.543-551.