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**BIOLOGICAL ACTIVITIES OF *Morus alba* L. LEAVES (ALFONSO VARIETY)**

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**ABSTRACT**

This paper demonstrated the antioxidant, antifungal, and toxic and teratogenic effects of the leaves extracts of *Morus alba* L. (Alfonso variety). Leaves extract exhibited scavenging activity against DPPH radicals with 52.59% and contain a total phenolic content of 36 mg AAE/1g sample. Ethanol extract showed 27.41 mm diameter zone of inhibition while water extract had 19.83 mm diameter zone of inhibition against *Rhizopus oryzae*. In teratogenicity assay, the varying concentrations of the water extract significantly affect the survival of zebrafish embryos in concentration and exposure time manner. Coagulation was the most distinct toxic effect while growth retardation and tail malformation were the obvious teratogenic effects of the extract to zebrafish.

**Keywords:** *Morus alba*, antifungal, antioxidant, toxicity, teratogenicity.

**INTRODUCTION**

*Morus alba* or mulberry is commonly utilized as food for silk worms and is known as natural reservoir of phytochemicals such as flavonoids, tannins, alkaloids, saponins, and terpenoids which can be associated to its medicinal properties [1]. In addition, this plant contains organic compounds such as

proteins, carbohydrates, fats as well as inorganic compounds which include fibers, mineral contents and some vitamins [2, 3, 4], indicating its promising potential in nutraceutical industry.

Plants are natural source of bioactive compounds responsible to their functional

activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer. Antioxidants are agents that scavenge free radicals that cause damage to important biomolecules if they are unstable and reactive. They play an important role in preventing several diseases and in neutralizing harmful free radicals and reactive oxygen species. Antimicrobial property of plant, on the other hand, is of great interest to so many researchers nowadays. These properties can be sources of effective novel drugs that can address the health problems caused by human pathogenic microbes. Despite of these valuable benefits of plants, their other chemical constituents can also act as teratogen, mutagen, and potentially toxic compounds [5], which we need to assess.

Herein, we evaluated the functional activities of *M. alba* leaves extracts specifically on the antioxidant, antifungal, and teratogenic activities. The antioxidant property was evaluated based on the radical scavenging activity and the total phenolic content. The antifungal effect was tested against *Rhizopus oryzae*. Zebrafish (*Danio rerio*) embryos were used as animal model in teratogenic activity.

## MATERIALS AND METHOD

### Source of Plant Sample

Matured green leaves of *M. alba* Alfonso variety were collected from Sericulture Research and Development Institute, Don Mariano Marcos Memorial State University, Bacnotan, La Union, Philippines. These were air-dried for 5 days, pulverized using a food processor, and extracted using ethanol and water as solvents. In ethanol extraction, 20 g of plant sample was soaked in 100 ml 80% ethanol for 48 hours. Extract was filtered using Whatman No. 1 and concentrated in rotary vacuum evaporator at 40°C, 120 rpm. In water extraction, 25 g of plant sample was soaked in 500 ml distilled water and were placed in a water bath at 90°C for 2 hours. This was filtered and subjected to treatment concentrations preparation.

### Antioxidant Screening

Ethyl acetate (10 ml) was added into the leaves sample to extract the antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol. The free radical scavenging activity of the samples was estimated using the stable 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical following the standard method of Shimada et al. [6] with modifications. 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 microtiter 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.

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton [7] with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed as mg of ascorbic acid equivalents (AAEs) per gram of sample. Triplicate test was done per sample.

### Antifungal Screening

Seven-day-old culture of *R. oryzae* was used in the preparation of fungal suspension and the cell density ( $2.7 \times 10^6$  conidia / ml) was determined using haemocytometer. Disc diffusion method was followed. One ml of the spore suspension was transferred into plate and eventually poured with sterilized potato dextrose agar at 40°C. Paper discs impregnated with ethanol and hot water extracts, nystatin as standard were placed equidistantly on the medium. Plates were

incubated at 30°C and the zones of inhibition were measured after 48 hours. Each test was done in triplicate.

### Toxic and Teratogenic Assay

Teratogenicity assay established by Dulay et al. [8] was adopted in this study. Acclimatized zebrafish with a ratio of 1 female : 2 male were confined in a plastic mesh and allowed to spawn and fertilize. After fertilization, embryos at segmentation phase (12 hour post fertilization) were collected, rinsed, and placed in a watch glass to check the uniformity of the phase of embryos. Unfertilized egg and coagulated embryos were discarded. Embryos were exposed to various concentrations (3%, 1%, 0.5%, 0.10%, 0.05% and control) of the extract. Mortality and morphological abnormalities were determined at certain periods of exposure. Assessment of the different morphological abnormalities was based on the parameters established by Nagel [9].

### Statistical Analysis

All data were analyzed using Analysis of Variance and compared using Duncan Multiple Range Test at 5% level of significance.

## RESULTS AND DISCUSSION

### Antioxidant Activity of *M. alba* Leaves Extract

Antioxidants are important neutralizer and scavenger of free radicals that damage the system. In this study, the radical scavenging activity of *M. alba* leaves extract was determined and the total phenolic content was analyzed. The leaves extract of *M. alba* exhibited scavenging activity against DPPH radicals with 52.59% and contain a total phenolic content of 36 mg AAE/1g of sample. This activity of *M. alba* leaves could be attributed to the presence of the phytochemicals. Based on the study of Ramos et al. [1], phytochemicals that were present in *M. alba* Alfonso variety in traces and/or appreciable amount were flavonoids, tannins, alkaloids, saponins and terpenoids. In addition, Dai et al. [10] isolated three compounds from *M. macroura* bark, called guangsangon H, guangsangon I, and guangsangon J, which showed antioxidant activities. Furthermore, Khyade [11] reported that the leaves of mulberry, *Morus alba* (L) (Variety: Baramatiwali) extracted using 80% methanol was found to be high in antioxidant activity. The level activities of antioxidant in mulberry leaves give major advantage for medicinal purposes and each anti-oxidative property would play a role in preventing and treating cancer. Moreover, based on the results obtained of Imran et al. [12], mulberry

fruits were found to serve as a potential source of food diet and natural antioxidants.

#### **Antifungal Property of *M. alba* Leaves Extract**

The antifungal property of ethanol and water extracts of *M. alba* leaves was evaluated against *R. oryzae* using disc diffusion method. Table 1 presents the diameter zone of inhibition of *R. oryzae* treated with the two extracts of *M. alba* leaves after 48 hours. Apparently, both extracts showed bioactivity against the fungal pathogen. Ethanol extract recorded the highest inhibition of 27.41 mm. This was higher when compared to the diameter inhibition showed by the standard nystatin with 22.14 mm, although not statistically differed from each other. On the other hand, water extract had a diameter of 19.83 mm, which found statistically comparable with nystatin. This result strongly indicates the antifungal potential of *M. alba* leaves. Similar result was also reported by Niratker et al. [13], which the methanolic extracts of mulberry (*Morus indica*) has maximum antifungal activity. Moreover, Kumar and Chauhan [14] reported four new antifungal phytoalexins that were isolated from root bark of mulberry plant, and these were Moracin E, F, G and H, Kwanon D, E, F.

Table 1: Diameter zone of inhibition of <i>R. oryzae</i> treated with <i>Morus alba</i> L. leaves extracts after 48 hours	
Treatment	Diameter Zone of Inhibition (mm)
Ethanol Extract	27.41 <sup>a</sup>
Water Extract	19.83 <sup>b</sup>
Nystatin	22.14 <sup>ab</sup>
Means with the same letter of superscript are not significantly different from each other at 5% level of significance by DMRT.	

### Toxic Effect of *M. alba* Leaves Water Extract

In the present study, the toxic effect of *M. alba* leaves extract was determined using zebrafish embryo model. Embryos were exposed at varying concentrations of the extract and the mortality was periodically determined. The results of percentage mortality of extract treated embryos are shown in Table 2. It can be seen that the varying concentrations of the extract significantly affect the survival of zebrafish embryos, thus, indicating concentration dependent. A 100% mortality was noted to those embryos at 3% extract after 12 and 24 hours of exposure. No statistical difference was found on the percentage mortality between 3% and 1% after the first two periods of observation. After 36 and 48 hours of exposure, embryos at 0.10% and higher concentrations of extract recorded 100% mortality, indicating time of exposure dependent. Coagulation was the most distinct toxic effect of the extract. However, no mortality was observed to control embryos, which suggest the validity of the test. It was reported that ethanolic extract of *M. alba* leaves can be considered more toxic than an ethanolic extract from *Ocimum sanctum* leaves, which promoted biochemical, hematological, and histopathological alterations in mice [15]. Furthermore, David et al. [16] revealed that the most common toxic effect of water extracts of *Moringa oleifera* was coagulation.

### Teratogenic Effect of *M. alba* Leaves Water Extract

Aside from the toxic effect, the teratogenic effect of the *M. alba* leaves extract was also assessed. Growth retardation or delayed development is one of the most common teratogenic effects of the extract. Figure 1 shows the obvious delayed development of the embryos exposed to higher concentrations of the extract. Successful hatching of larva was observed to those at control and 0.05% extract. On the other hand, tail malformation was the most distinct abnormality found in extract-treated embryos. This abnormality was possibly due to the delayed embryonic development. Therefore, *M. alba* leaves extract exhibited both toxic and teratogenic effects to the developing embryos of zebrafish.

Extract (%)	Mortality (%)			
	12 hours	24 hours	36 hours	48 hours
3.00	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
1.00	83.33 <sup>a</sup>	91.67 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
0.50	41.67 <sup>b</sup>	91.67 <sup>a</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
0.10	41.67 <sup>b</sup>	66.67 <sup>b</sup>	100.00 <sup>a</sup>	100.00 <sup>a</sup>
0.05	8.33 <sup>c</sup>	41.67 <sup>b</sup>	66.67 <sup>b</sup>	83.33 <sup>b</sup>
0.00	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>

Means with the same letter of superscript are not significantly different from each other at 5% level of significance by DMRT.

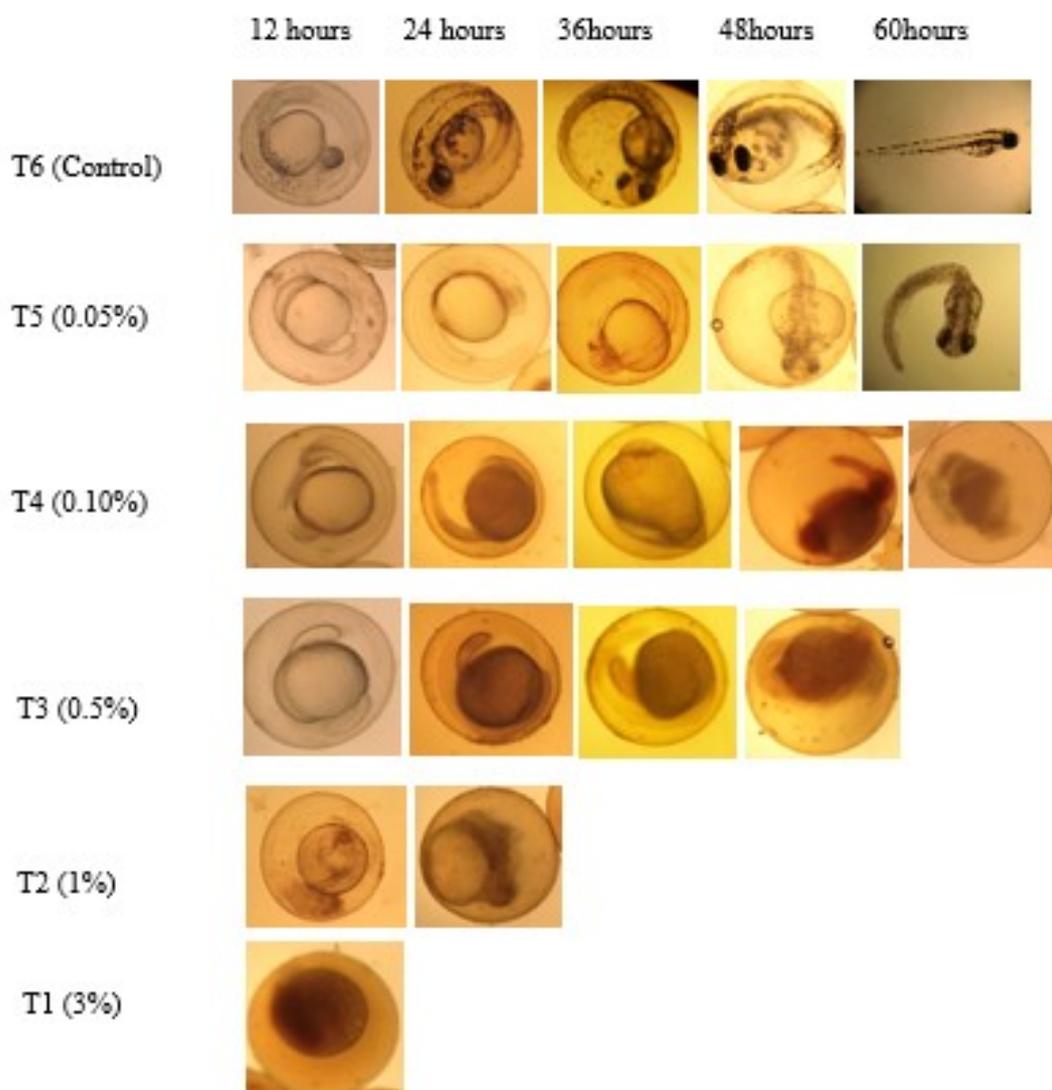


Figure 1. Morphological development of zebrafish embryos exposed to different concentrations of *M. alba* leaves extract after 12, 24, 36, 48, and 60 hours of exposure

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**CONCLUSION**

Altogether, *M. alba* (Alfonso variety) hold promising potential as source of novel compounds for pharmacological use. Leaves extract exhibited DPPH radical scavenging activity, contain phenolic compounds, and demonstrated antifungal effect against *R. oryzae*. However, despite of these advantages, this plant can also be a source of toxic compounds.

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