



BIOLOGICAL ACTIVITIES OF LEAF EXTRACTS OF *Morus* sp. S54 VARIETY

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ABSTRACT

The functional biological activities of leaf extracts of *Morus* sp. S54 variety were investigated in this study. The antifungal, antioxidant, total phenolic content and the teratogenic effect of the plant extracts were evaluated. Both ethanol and water extract showed antifungal activity against *Aspergillus niger* with diameter zones of inhibition of 19.24 mm and 17.60 mm, respectively. Extract of *Morus* leaf also exhibited DPPH radical scavenging activity with a mean of 12.93% and contained a total phenolic content of 67 mgAAE/g of sample. In teratogenicity assay, coagulation was the most toxic effect observed in higher concentrations of water extract. Tail malformation and growth retardation were the most common teratogenic effects observed in developing *Danio rerio* embryos.

Keywords: Antifungal, antioxidant, total phenolic content, teratogenic effect, *Morus* sp. S54 variety

INTRODUCTION

Plants are exemplary source of medicines and several drugs have been directly or indirectly derived from them which aids in the treatment of many serious diseases [1]. Mulberry leaves which contain many nutritional components are known as “the natural reservoir of botanical nutrients” and

are the best feed for silkworms [2, 3]. Thus, mulberry can be a good source of nutraceuticals. In addition to this, plants were also a good source of antifungal compounds which can be sources for novel drugs. Studies have shown that *Morus* leaf extract

possess antimicrobial activity [4], which can inhibit the growth of human pathogens.

Furthermore, another promising effect of plants is having antioxidant activity. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which causes cancers and heart diseases [5]. However, side effects of some synthetic antioxidants have been documented for the exhibit carcinogenic effects [6]. Due to these issues, natural antioxidants obtained from plants were of increasing interest [7]. Numerous reports have described antioxidant and radical scavenging compounds in herbs, fruits, vegetables and cereals extracts [8]. Studies have been reported that leaves of mulberry are potentially rich antioxidant sources [9, 10]. But despite the positive effects plants can contribute to human health, plant also pose deleterious effects depending on various concentrations. It is because plants chemical constituents can also act as teratogen, mutagen, and potentially toxic compounds [11], which we need to evaluate and examined.

With this research, it evaluated the functional biological activities of leaf extract of *Morus* sp. S54 variety specifically on the antifungal, antioxidant and teratogenic activities. The antifungal effect was tested against *Aspergillus niger* by measuring zone

of inhibition. The antioxidant property was evaluated based on the radical scavenging activity and the total phenolic content. Teratogenic effects were observed using zebrafish (*Danio rerio*) embryos.

MATERIALS AND METHOD

Source of Plant Sample

Matured green leaves of *Morus* sp. S54 variety were collected at Sericulture Research and Development, Don Mariano Marcos Memorial State University North Luzon Campus at Bacnotan, La Union. Collected plants were air dried and pulverized and were subjected to hot water and ethanol extraction procedure. Hot water extract was obtained by mixing 25 g of ground leaves of *Morus* with 500 ml of distilled water in flask and put in a water bath at 90°C for 1 hour. Thereafter, the suspensions were filtered using Whatman No. 1 filter paper. Meanwhile, in ethanol extraction, ground samples were added with 80% ethanol, and distilled water for 48 hours with 1g: 5 milliliters; sample: solvent ratio. Afterwards, it was filtered using Whatman No. 1 filter paper. Then filtrates were concentrated in rotary vacuum evaporator at 40°C, 120 rpm.

Antifungal Screening

Seven-day-old culture of *A. niger* was used in the preparation of fungal suspension with

cell density of 2.7×10^6 conidia/ml which was determined using a haemocytometer. Disc diffusion method was followed. One ml of the spore suspension was transferred into plate and poured with sterilized potato dextrose agar. After the agar solidified, paper discs impregnated with hot water and ethanol extracts, nystatin as control 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.

Antioxidant Screening

For the determination of the radical scavenging activity of leaf extract of *Morus*, the DPPH radical scavenging activity assay by Kolak *et al.* [12] was used. While on the total phenolic content the Folin – Ciocalteu method described by Hodzic *et al.* [13] was adopted. Each test was done in triplicate.

Teratogenic Activity Screening

Healthy and sexually matured zebrafish were acclimatized and were allowed to spawn. After spawning and fertilization, 12-hour embryos were exposed to different concentrations (3%, 1%, 0.5%, 0.1%, 0.05% and control). Extract was diluted to embryo water. Percentage mortality was recorded after 12, 24, 36, and 48 hours exposure. Morphological abnormalities were

assessed based on the parameters established by Nagel [14].

Statistical Analysis

Experimental units were laid out in a Completely Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) and treatments means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Antifungal Activity of *Morus* S54 Variety Leaves Extract

Hot water and ethanol extracts of *Morus* leaf were screened for their antifungal property against *A. niger* using disc diffusion method. Table 1 shows the mean diameter zone of inhibition of *Morus* leaf against *A. niger* after 48 hours of incubation. Both hot water and ethanol extracts of *Morus* showed antibacterial activity against *A. niger* with means of 17.60 mm and 19.24 mm, respectively. Niratker et al. [4] reported that the ethanolic extract of *Morus* spp. inhibited the bacteria and fungi. Therefore, the effect of the plant extracts may vary depending on the extraction solvent used.

Antioxidant Activity

Antioxidant activity of leaf extract of *Morus* was determined using DPPH radical scavenging assay and the total phenolic

content was estimated using Folin-Ciocalteu method. Extract showed percentage radical scavenging activity of 12.93% whereas the total phenolic content of the plant extract was 67 mg AAE/ g sample. Khyade [15] revealed that mulberry might be a potential source of excellent natural antioxidants and anticancer. Also, the white, red, and black mulberry fruits were reported to contain high phenolic content [16]. The result is also supported by Vilorio *et al.* [10] in which the leaves *Morus alba* L. (Alfonso variety) exhibited scavenging activity against DPPH radicals with 52.59% and contain a total phenolic content of 36 mg AAE/g sample. However, Oyedemi *et al.* [17] reported that the weak and moderate activity in DPPH scavenging assay could also explain with the low flavonoid content, which have been reported to possess high antioxidant activity.

Embryotoxicity and Teratogenicity

The toxic effects of *Morus* leaf extract were determined using zebrafish embryo as an animal model. Embryos were exposed on different concentrations at varying time of exposure. Table 2 presents the percentage mortality of embryos exposed to the different concentrations of the extract. Apparently, mortality of embryos was observed at 1% and 3% of the extract after 12 hours of exposure with both 33.33% mortality. After

24 hours, the mortality of embryos exposed to these concentrations was significantly increased whereas 0.5% and 0.1% of extract also recorded 33.33% and 8.33%, respectively. A 100% mortality was noted after 36 hours at 1% and higher concentration and after 48 hour at 0.5% and higher concentrations. The 0.1% of extract maintained the 8.33% mortality up to the last observation period. The result of the study is congruent with the findings of Vilorio *et al.* [10], in which the varying concentrations of the *Morus alba* L. (Alfonso variety) water extract significantly affect the survival of zebrafish embryos at different time of exposure. In addition, Santos *et al.* [18] reported that after 36 and 48 hours of exposure, 100% mortality was observed in embryos at 3% of *Momordica charantia* leaf extract. This toxic effect of *M. alba* could be explained by the active components. Fujaya *et al.* [19] reported that mulberry extract contains phytoecdysteroid. The toxicity of phytoecdysteroids manifested itself by a decrease in larval weight, induction of cannibalism and an increase of mortality, together with disruption of development of moth [20].

Hatching is an indicative of successful developmental process of the embryos usually takes place between 48 to 72 hours of

post treatment application (hpta) depending on the thickness of the chorion. The percentage hatchability of embryos treated with different concentration of leaves extract after 48 to 72 hours was also recorded. Results showed that 0.1% and higher concentrations of the extract did not show hatched embryos after 72 hours of exposure due to the delayed growth and early arrested development of embryos.

Growth retardation is important parameter in determining the teratogenic effect. In this

study, delayed growth was the most common teratogenic effect of leaf extract of *Morus* that later causes mortality and morphological abnormalities in embryos. Tail malformation (bent tail) was observed morphological abnormality as teratogenic effect of *Morus* leaf extract. Previously, head and tail malformation were the most distinct morphological deformities in teratogenicity assessment conducted by Dulay and De Castro [21].

Table 1: Diameter zone of inhibition of leaf extract of *Morus* against *A. niger* after 48 hours of incubation

Treatments	Diameter zone of inhibition (mm)
Hot water extract	17.60 ^a
Ethanol extract	19.24 ^a
Nystatin	17.62 ^a
Negative control	6.00 ^b

Treatment means with the same letter of superscript are not significantly different at 0.05 level of significance using DMRT.

Table 2: Percentage mortality of zebrafish embryo exposed to the different concentrations of leaf extract of *Morus* at different time of exposure

Extract (%)	Mortality (%)			
	12 hours	24 hours	36 hours	48 hours
3.0	33.33 ^a	66.67 ^a	100.00 ^a	100.00 ^a
1.0	33.33 ^a	58.33 ^a	100.00 ^a	100.00 ^a
0.5	0.00 ^b	33.33 ^b	50.00 ^b	100.00 ^a
0.1	0.00 ^b	8.33 ^c	8.33 ^c	8.33 ^b
0.05	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^b
0.00	0.00 ^b	0.00 ^c	0.00 ^c	0.00 ^b

Treatment means with the same letter of superscript are not significantly different at 0.05 level of significance using DMRT.



Figure 1: Normal hatched zebrafish at embryo water (A), and bent-tail hatched zebrafish at 0.05% concentration of extract(B)

CONCLUSION

Morus leaf extract showed very promising biological activities such as antifungal and antioxidant. However, this plant also exhibited embryotoxic and teratogenic effects in developing embryos of zebrafish.

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