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Research Article

TOXICITY AND ANTIOXIDANT PROPERTIES OF PACIFIC RAIN TREE (*Samanea saman* (Jacq.) Merr.) PODS

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ABSTRACT. Pacific rain tree (*Samanea saman*) of the Family Fabaceae was investigated for its pharmacological potentials. In this study, *S. saman* pods were tested for their cytotoxicity, embryotoxicity and antioxidant properties. The pods were collected and oven-dried prior to extraction with 95% ethanol for 48 hours and evaporation using rotary evaporator at 300 rpm. Using brine shrimp lethality assay, the extracts were found have cytotoxic effects against *Artemia salina* larvae with LC50 value of 4.65 ppm. Similarly, the ethanol extracts of rain tree pods exhibited embryotoxicity against zebra fish (*Danio rerio*). Zebra fish exposed to the rain tree pod extract for 48 hours were observed to have head and tail malformation, growth retardation, flexure, stunted tail, limited movement, coagulation, undetached tail, lack of somite and absence of heartbeat. The free radical scavenging activity of the samples were estimated using the stable 2, 2'diphenyl-1-1picrylhydrazyl (DPPH) radical assay. The extract recorded an EC50 of 70.92 ppm. Results of this study revealed that *S. saman* pods can be a potential source of antioxidants and other bioactive compounds with cytotoxic and teratogenic properties.

Keywords: *Rain tree, antioxidant, cytotoxicity, embryotoxicity, Samanea saman*

INTRODUCTION

Plants have been valued for their numerous beneficial uses for a long time. Aside from being consumed largely as food, plants have been recognized as natural sources of bioactive compounds with pharmacologic and nutritive potentials. In fact, it was estimated that about 13,000 plant species worldwide are known to have medicinal properties [1]. According to Lai et al. [2], natural products formed by living systems, notably from plant origin, have shown great advantage in treating human diseases. Thus, plants are frequently explored for their possible use in drug discovery.

In tropical countries, one of the widely distributed trees is *Samanea saman* (Jacq.) Merr. It is a large umbraculiform tree, growing over 20 meters high with a trunk diameter of about 1.5 meters. In the Pacific region, it serves as a shade along roads, in parks and pastures [3]. Traditionally, the plant has been used as a folk medicine for the remedy of

headache, cold, diarrhea, stomach-ache intestinal ailments, sore throat, and stomach cancer. The boiled bark is used as a bandage to cure constipation [4].

Several studies have reported on the different therapeutic activities of *S. saman*. It has shown various bioactive compounds with medicinal properties such as antioxidants, antibacterial, anti-diabetic, analgesic, anti-ulcer, insecticidal, antifungal, and cytotoxic activities [3,5]. In particular, biological evaluations of this plant have shown its prospective use in medicine. For instance, leaf extracts of *S. saman* have shown antibacterial, antifungal, insecticidal and cytotoxic activities [4]. Phytochemical screening of the leaves also shows the presence of bioactive compounds such as flavonoids, tannins, saponin, polyphenols, alkaloids, cardiac glycosides, terpenoids, steroids, and resin [6,7]. The bark, seed and root of the tree were also studied for their medicinal uses.

These properties, among others, suggests that the Pacific rain tree have derived medicines which could be important in the field herbal pharmaceutics. The medicinal plant had shown promise in drug discovery. Despite various studies supporting the potentially valuable phytochemistry and bioactivity of *S. saman*, there is limited information about its pods' bioactivity. With an objective of uncovering the pharmacologic potential of *S. saman* pods, this study focused on the assessment of its cytotoxicity, embryotoxicity and antioxidant properties.

MATERIALS AND METHODS

Specimen Collection

The pods were collected from *Samanea saman* trees located in Science City of Muñoz, Nueva Ecija, Philippines. Plant samples were authenticated on the basis of taxonomic characters through the assistance of an experienced botanist. The voucher specimen that contains the identification characteristics of the plant was deposited for future reference. Collected specimens were oven-dried at 70ºC for eight (8) hours. Seeds were removed and the pods were then powdered using mortar and pestle.

Preparation of Ethanol Extract

Ethanol extract of *S. saman* for the cytotoxicity assay was obtained following the methods of Jacob and David [8]. Fifty grams (50 g) of powdered *S. saman* pods was soaked in 95% ethanol (500 mL) for 48 hours at room temperature. The extract was then filtered and filtrate was subjected to a rotary evaporator with a speed of 300 rpm at 50ºC. Ethanol extracts were stored in a sterile glass vials and kept refrigerated for storage.

Brine Shrimp (Artemia salina) Lethality Assay

The cytotoxicity of *S. saman* was initially evaluated through the brine shrimp (*A. salina*) lethality assay. A modified hatchery for *A. salina* eggs was made and filled with artificial sea water which was prepared by dissolving 34 g of commercialized salt in 1 L of sterile distilled water. A pinch of *A. salina* eggs was then added to the artificial sea water and allowed to hatch for 48 hours. Proper aeration and adequate illumination were ensured.

Different extract concentrations (10, 100, 1000 and 10000 ppm) with a final volume of 5mL were prepared and poured into clean 40-mm Petri plates. These concentrations were chosen according to previous studies indicating that the plant has low cytotoxicity

in lower concentrations [1]. Plain artificial sea water without the extract was used as the negative control. Vincristine sulphate was used as the positive control by dissolving in DMSO following recommended concentrations [1]. Eggs that hatched, or the nauplii, were then transferred into the Petri plates. Ten (10) brine shrimp nauplii were used per concentration and the evaluation was done in three (3) replicates. The plates were left uncovered under a lamp and were added with a drop of yeast suspension (3 mg in 5 mL of saline solution) to serve as food for the nauplii.

After 24 hours, the number of dead nauplii per treatment was counted and the percentage mortality was determined. The lethality concentration (LC_{50}) was also calculated using Probit analysis. According to Meyer et al. [9], an LC50 value above 100 µg/mL are non-toxic, while LC_{50} value below 100 µg/mL are toxic.

Spawning of Mature Zebrafish

After acclimatization, adult female and male zebra fishes (1:2 ratio) were contained in a glass aquarium filled with untreated, clean tap water with continuous aeration. To promote spawning, the glass aquarium was covered with a black plastic sheet for 12 hours. Once spawning was finished, the eggs were exposed to a lighted condition using a fluorescent bulb for 12 hours. The fertilized embryos were siphoned out of the aquarium 12 hours post fertilization.

Preparation of Embryo Water

To serve as embryo water, a working solution using a combination of 1 mL Hank's solution 1, 0.1 mL Hank's solution 2, 1 mL of Hanks solution 4, 1 mL of Hank's solution 5, 0.35 grams $NaHCO_3$ and 96.5 mL double distilled water. Hank's solution 1 was prepared by combining 8 g of NaCl, 0.4 g of KCl and 100 mL of double distilled water. To prepare Hank's solution 2, 0.67 g Na_2HPO_4, 7 H_2O, 0.60 g KH_2PO_4 was combined with 100 mL double distilled water. Hank's solution 4 is a combination of 1.92 g $CaCl_2$, 2 H_2O and 100 mL double distilled water while Hank's solution 5 was prepared using 2.46 g $MgSO_4$, 7 H_2O and 100 mL double distilled water. The pH of the working solution was checked and adjusted to 7.2 by gradually adding 1 M NaOH.

Embryotoxicity of Rain tree Pod Extract

S. saman pods ethanol extracts were diluted with embryo water. Two concentrations were prepared separately, 0.05 ppm and 0.5 ppm. Pure embryo water (0 ppm) was used as negative control. The fertilized embryos were observed first under a light microscope before exposure to the different treatments.

After complete examination of normal embryos, 4 mL of each treatment concentration was doled out into each well of a 12-well enzyme-linked immunosorbent assay (ELISA) plate. Three fertilized embryos were placed into each well using a medicine dropper. Plates were then incubated at 26 ± 1°C. Tests were performed in triplicates.

Microscopic Observations and Analyses

The embryotoxic activity of *S. saman* pod extract was microscopically examined after 12, 24, and 48 hours of exposure. The morphological endpoint of zebra fish was determined and evaluated according to Schute et al. [10].

The extract is considered lethal if the observed effects towards the embryo include coagulation, undetached tail, lack of somites, and absence of heartbeat. It is teratogenic if observations include malformations of head, tail and heart, scoliosis, yolk deformity, and growth retardation. A normal observation is indicated by the absence of any deformities or abnormalities while coagulation and the absence of visual heartbeat over an extended period indicates that the embryos are dead.

Extraction of Antioxidant Compounds

S. saman pods were homogenized using a food processor then ethyl acetate (10 mL) was added into each cultured broth to extract antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol for antioxidant analysis**.**

DPPH Scavenging Assay

The free radical scavenging activity of the samples was estimated using stable 2,2'diphenyl-1-1picrylhydrazyl (DPPH) following the method of Dulay et al. [11] with slight modification. Different concentration of extracts (1.5 mL) was mixed with 2.5 mL DPPH solution (6 mg DPPH + 100 mL methanol) to obtain 1, 10, 100, 300, and 1000 ppm preparations.

The negative control was prepared by adding methanol instead of extract while ascorbic acid was used as positive control. The mixture was vigorously shaken and was left in the dark for 30 minutes before its absorbance reading at 517 nm in a visible spectrophotometer (Cole Parmer Spectrophotometer 12000) was recorded. The EC_{50} was estimated using nonlinear regression curve fitting function, sigmoidal dose response of GraphPad Prism 6.0.

RESULTS AND DISCUSSION

Cytotoxicity of Samanea saman Pod Extract against Brine Shrimp Nauplii

The brine shrimp (*Artemia salina*) lethality assay evaluated the cytotoxicity of *S. saman* pod ethanol extracts. This is considered as a rapid, inexpensive and simple bioassay for testing plant extract lethality, which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. It also has been proved to be a convenient system for monitoring biological activities of natural products [12].

Table 1 shows that the mortality rate of brine shrimps after 24 hours of exposure to the ethanol extract of *S. saman* pods at 10 ppm concentration already reached 90%. The result also shows a dose dependent increase in the mortality rate of brine shrimp, with LC50 of 6.76 ppm.

Table 1*. Cytotoxicity of S. saman pod extract against A. salina nauplii.*

Treatment Concentration	Mortality	Regression Equation	LC_{50}
0 ppm	0%		
10 ppm	90%		
100 ppm	94%	y=0.863x + 4.284	6.76 ppm
1000 ppm	98%		
10000 ppm	100%		

The cytotoxic property of *S. saman* can be attributed to the presence of bioactive compounds. Phytochemical screening of *S. saman* pods revealed that it has a high content of tannin and saponin [13,14]. These secondary metabolites were reported to decrease cell viability and are thus cytotoxic [15]. According to Jacob and David [8], the presence of bioactive components in *S. saman* indicates that it can have potential uses as antiproliferative, antitumor, pesticidal, chemotherapeutic and other bioactivities.

Embryotoxicity of Samanea saman Pod Extract against Danio rerio Embryo

Zebrafishes (*Danio rerio*) were utilized as model organisms for testing the embryotoxicity or teratogenicity of *S. saman* pods [3]. As shown in Figure 1,a 0.5 ppm extract concentration exhibited a high mortality rate (88%) and low hatchability (12%) among the test organisms.

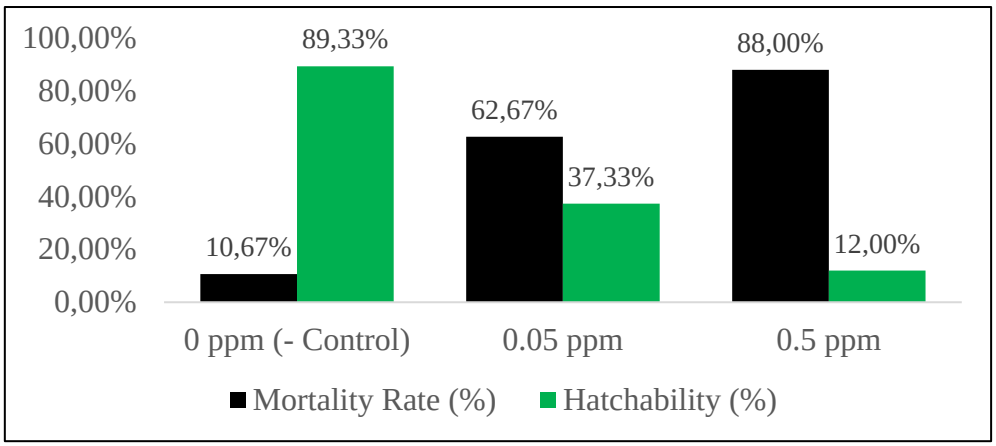


Figure 1. *Mortality rate and hatchability of zebra fish (Danio rerio) after 24 hours of exposure to S. saman ethanolic extracts.*

Morphological abnormalities in zebrafish embryos after exposure to 0.05 ppm ethanolic extracts are presented in Figure 2 and Table 2. Several developmental defects such as growth retardation, stunted tail, limited movement, coagulation and undetached tail were already observed in the zebrafish embryos that were exposed for 12 hours. When further exposed for 24 hours, more lethal effects have been observed. After 48 hours, all zebrafish embryos exposed to the ethanol extracts of rain tree pods were dead with malformations.

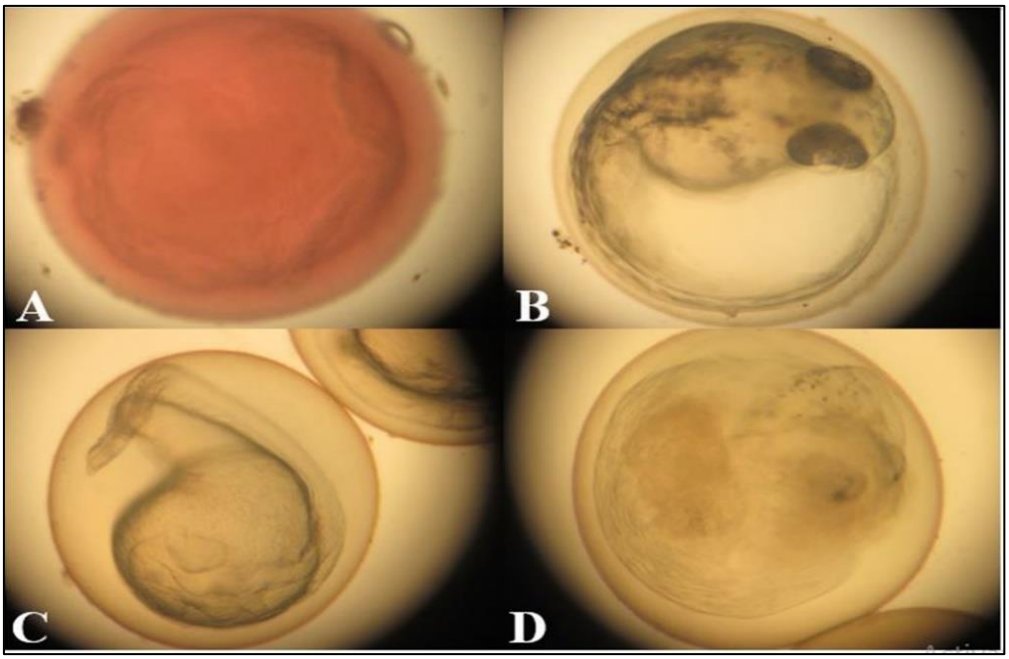


***Figure 2**. Morphological abnormalities in Danio rerio embryo (A) coagulated embryo, (B) unformed tail, (C) unformed head, (D) dead embryo.*

Embryonic development is a delicate and highly organized process. Hence, assessing the effects of herbal products on the development of embryos is important. Aside from their cytotoxic effects, secondary metabolites such as alkaloids, which were also detected in *S. saman*, may also have embryotoxic effects [16]. As shown by Green et al. [17], exposure of a developing embryo or fetus to alkaloids from plants, plant products, or plant extracts may cause developmental defects in humans and animals.

***Table 2.** Morphological endpoints of zebrafish (Danio rerio) embryos after exposure to 0.05 ppm S. saman ethanolic extract.*

Toxicological and Morphological Endpoints	**Time of treatment exposure**		
	12 hours	**24 hours**	**48 hours**
Head malformation		X	X
Tail malformation		X	X
Growth retardation	X	X	X
Flexure		X	X
Stunted tail	X	X	X
Limited movement	X	X	X
Coagulation**	X	X	X
Tail not detached**	X	X	X
No somite**		X	X
No heartbeat**			X

Note: observations with ** are lethal.

DPPH Radical Scavenging Activity of Samanea saman Pod Extracts

Figure 3 shows the DPPH radical scavenging activity of *S. saman* pod ethanol extract at different concentrations. Although lower than the antioxidant activity of ascorbic acid at 4.00 ppm, which is a purified compound, an EC_{50} of 70.92 ppm indicates that *S. saman* pod can be a source of antioxidants.

In a previous study, the chloroform and hexane soluble fraction of *S. Saman* have showed IC_{50} value of 12µg/ml and 14µg/ml respectively in scavenging DPPH radical while the reference butylated hydroxytoluene showed an IC50 value of 10µg/ml [ferdous]. In a separate study, the antioxidant activities of the plant was tested using alcoholic extract, wherein 70 percent of the extracts have shown reducing power and nitric oxide radical scavenging activity [18]. It has also been reported that around 10-15mg of the *S. saman* extracts gave 68% DPPH scavenging activity by reducing the DPPH radical to corresponding hydrazine when its reacts with hydrogen donors in antioxidant principles [19].

Antioxidants play a substantial role in human health. Recent developments in the medical field reveal a number of diseases associated with free radicals which can be scavenged by antioxidants [20]. Compounds with antioxidant properties also inhibit lipid peroxidation and other free radical-mediated processes, thus, protecting the human body from several diseases caused by reactions of radicals [21].

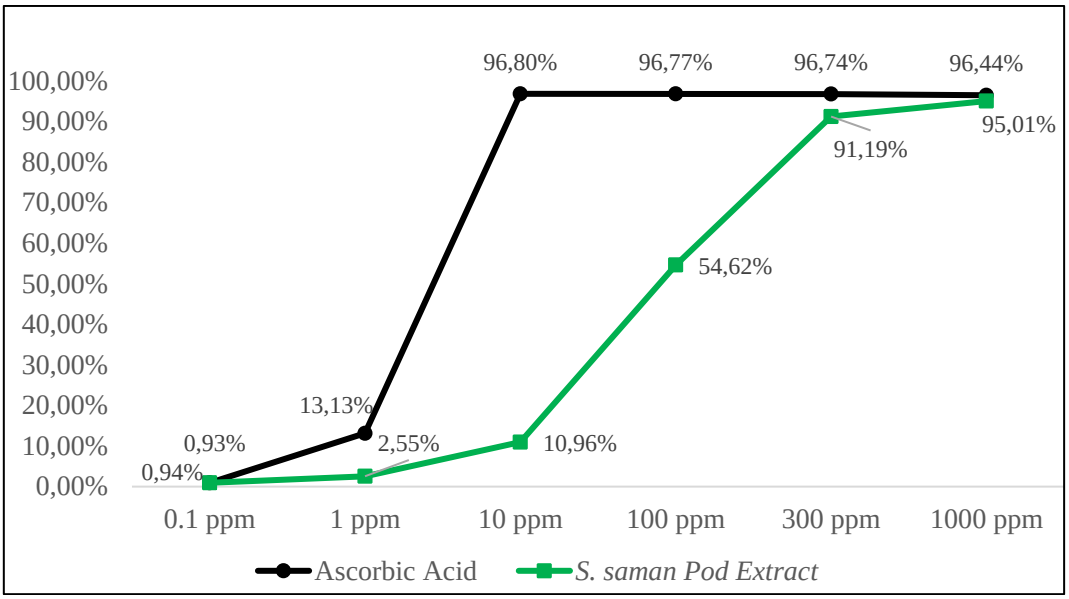


Figure 3. *DPPH radical scavenging activity of S. saman ethanolic extracts*

CONCLUSION

In this study, *Samanea saman* pod extract has shown the toxicity against brine shrimp nauplii and zebrafish embryo. It was also revealed that the pod extract can be potential source of novel products with antioxidant properties. Results of the study showed that despite its potential medicinal and nutritional values, *S. saman* may also pose toxic effects to certain organisms. This may also be applicable to other medicinal plants or herbs.

Hence, prior to their utilization, toxicity assessment should be thoroughly carried out to establish the safety of such products.

Conflict of Interest. The authors declare no potential conflicts of interest with regard to this research, authorship, and/or publication of this article.

Authorship Contributions. Concept: J.K.J., J.P., H.M.A, Design: J.P., H.M.A., Data Collection or Processing: J.K.S., H.M.A., Analysis or Interpretation: J.K.J., J.P., H.M.A, Literature Search: J.K.J., R.P.C., Writing: J.K.J., R.P.C.

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