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Toxicity of Food-Decorative Flowers: An Investigation of the Effects of Solvent Extracts of Orchid Flower on Brine Shrimp

***Pangwan Pholphirul*[1]**

[1]King Mongkut's Institute of Technology Ladkrabang International Demonstration School, King Mongkut's Institute of Technology Ladkrabang, 1 Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand. Email: pangwan211@gmail.com

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Abstracts-*Traditionally, Asian cuisine incorporates the use of decorative flowers in different dishes that are often thought of as edible. Little is known about the toxicity of such flowers. The aim of this study is to investigate the toxicity of food-decorative flowers such as the orchid. The study investigates the effects of two different concentrations (0.25mg/ml and 1.00mg/ml) of orchid flower extract on brine shrimp. An acute 72-hour toxicity assay was performed on brine shrimp with data on the number of surviving shrimps collected after 24 hours, 48 hours, and 72 hours of exposure. A control group was also used where brine shrimps were not exposed to any orchid concentration. Paired-sample independent t-tests were performed to find statistically significant differences of the number of surviving shrimps among the different concentrations with respect to the control group and each concentration. Our results show that increasing concentrations of orchid-flower extracts led to statistically and significantly higher mortality rates among the shrimp compared to the control group. The toxicity can be observed within 24 hours of exposure and intensifies with longer exposure. The statistically significant lethal effects can be observed at the concentration as little as 0.25mg/ml.*

Index Terms-Toxicity, Lethality, Orchid Flower, Food-Decorative Flowers, Brine Shrimp

[1]King Mongkut's Institute of Technology Ladkrabang International Demonstration School, King Mongkut's Institute of Technology Ladkrabang, 1 Chalong Krung Road, Ladkrabang, Bangkok 10520, Thailand. Email: pangwan211@gmail.com.

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I. Introduction

In traditional showcases of food in many cultures, especially in Asia, flowers are used as decorative additions to the extravagance of the arrangement. These flowers are often presented with traditional food as a way to highlight the authenticity of the food using the flowers' unique characteristics of being traditional flowers related to local cultures such as orchids, frangipani flowers (temple flowers),and roses. In many countries, especially in Asia and Africa, these flowers are viewed as festive and used in various occasions for celebration. Many of these flowers are common to the viewers as edible, but some may be toxic. It is also common for many households to grow these flowers where the flowers can easily be exposed to children that can mistakethe flowers as edible by their experience that the flowers are related to food in traditional food presentation. Consuming toxic flowers can be harmful to the health of people but the use of such plants has been used as alternative medicines. This research aims to shed some light on the toxicity of decorative flowers that are commonly understood as edible. The toxicity assessment is evaluated using extracts of orchid flowers under standard procedures and brine shrimp tests (Kibiti&Adfolayan, 2016; Ohikhena et al., 2016)

Orchidflowers (*Orchidaceae*) with their delicate and vibrant petals, serve as exquisite adornments for culinary creations, adding both visual appeal and a subtle, floral flavor profile.In Asian cuisine, orchid flowers are cherished not only for their visual allure but also for their symbolic significance and subtle flavor contributions. Across various Asian cultures, orchids are often incorporated into dishes as edible decorations, infusing meals with a touch of elegance and cultural heritage. In Chinese cuisine, orchid flowers are sometimes used to garnish dishes like stir-fries, soups, and salads, imparting a delicate floral aroma and a hint of sweetness. In Japanese cuisine, orchids are meticulously arranged as edible art in traditional dishes like sushi and sashimi, where their vibrant colors and intricate shapes enhance the dining experience. Similarly, in Thai cuisine, orchid blossoms are employed to adorn desserts such as mango sticky rice and coconut-based sweets, adding a visually stunning finishing touch. Throughout Asia, the use of orchid flowers as food decoration reflects a deep appreciation for beauty, flavor, and tradition, elevating culinary creations to new heights of sensory delight. However, little is known about the toxicity of orchid flowers despite its appeal asedible decorations.

Image 1: The use of orchids as decoration in food is widespread in Asian cultures.

II. Materials and Methods

Several studies in scientific literature have demonstrated the efficacy of brine shrimp bioassays in evaluating the toxicity of herbal products. For instance, research published in the Journal of Ethnopharmacology titled "Evaluation of the toxicity and antileishmanial activity of extracts from Brazilian sponges Aplysinafulva and Petromicacitrina" (Almeida et al.,

2006) utilized brine shrimp assays to assess the toxicity of extracts from Brazilian sponges. Similarly, a study published in the journal Natural Product Research titled "Toxicity assessment of the essential oil of Plectranthusamboinicus and its major constituents towards Artemiasalina" (Tao et al., 2017) employed brine shrimp bioassays to evaluate the toxicity of essential oils derived from Plectranthusamboinicus. These studies illustrate the utility of brine shrimp bioassays as a preliminary screening method for assessing the toxicity of herbal preparations, providing valuable insights into their safety profiles.

A. Plant materials:

The orchid plant materials used in this study were collected from Bangkok, Thailand, with careful consideration to exclude any potential contamination from insecticides, thus ensuring the reliability of toxicity assessments. To prepare the flowers for analysis, a meticulous washing process involving tap water and salt was employed to remove dirt and contaminants, repeated the process to guarantee cleanliness 3 times. Following washing, the flowers were cut into small pieces and subjected to drying at 90 degrees Celsius for two hours in a convection drying oven to eliminate moisture. The dried flowers were powdered using a mortar and pestle, yielding a homogeneous powder suitable for further experimentation.

B. Extract preparation:

The powdered orchid flowers were extracted using distilled water. with a ratio of 2:1 water to powder by volume, within a laboratory flask covered with foil paper. The mixture was then left refrigerated for three days to allow thorough extraction. Following this, the resulting extract underwent filtration using filtered papers to remove solid residues. The filtrate was transferred to an evaporation dish and exposed to a laboratory hot water bath set to 90 degrees Celsius to evaporate the water, resulting in 2560mg. concentrated dried flower extract. After completed evaporation, 64ml. of sea water was added to dissolve the dried extract (water that brine shrimps lived in to prevent other factors that may influence the outcome), resulting in a crude extract concentration of 40mg/ml. A two-fold serial dilution was carried out to obtain a test solution in two concentrations of the flower extract (0.25 mg/ml and 1.00 mg/ml) using the dilution equation (C1)(V1)=(C2)(V2), where C1 is the concentration of the starting solution. V1 is the volume of the starting solution. C2 is the concentration of the final solution. V2 is the volume of the final solution.

C. Brine Shrimp Lethality Assay:

This research uses brine shrimps to test for toxicity of orchid flowers. Brine shrimp, commonly known as Artemia, are frequently employed in toxicity testing to evaluate the safety of herbs and herbal extracts(Almeida et al., 2006), (Tao et al., 2017). This method, also known as the brine shrimp lethality assay (BSLA), relies on the sensitivity of brine shrimp larvae to various substances, making it a cost-effective and rapid screening tool for assessing potential toxicity. The Brine Shrimp Lethality Assay was conducted based on the assay described by Kibit&Adfolayan (2016). Brine Shrimp (Artemia Salina) in their early juvenile stage, all born within the same period. The assay involved assessing the lethality of orchid extract following a standard procedure. Twelve 50ml. beakers were prepared and categorized into three groups: four for the control group, four for the 0.25mg/ml orchid extract concentration assay, and four for the 1.00mg/ml orchid extract concentration assay, each appropriately labeled. Each beaker contained 15 brine shrimps (Artemia Salina) throughout the experiment. In the control group, 10ml of sea water from the same batch was added to each beaker. For the 0.25mg/ml concentration (Con0.25), 9.9375ml of water and 0.0625ml of crude extract were added, while for the 1.00mg/ml concentration (Con1.00), 9.75ml of water and 0.25ml of crude extract were added. The beakers were observed, and the number of

deceased larvae in each beaker was recorded after 24, 48, and 72 hours. The total shrimp count per beaker was also recorded. The experiment was then replicated a second time to confirm the results.

D. Statistical Analysis

The results were expressed as the mean value of the number of survivingshrimps' comparisons.Paired tests are performed using an Independent Sample t-test. Significant differences between control and each experimental concentration group were assessed by the statistical software. A probability level ofp-value < 0.05 (confidence level of 95 percent) was considered to indicate the statistically significant difference between the groups.

III. Results

The Box Plots for the number of brine shrimps that survived in the control group (Control) and the concentrations of 0.25mg/ml (Con0.25) and 1.00mg/ml (Con1.00) after 24 hours, 48 hours, and 72 hours in both experiments are presented in Figure 1.

Figure 1: Box Plots of the number of brine shrimps that survived for Control, Con0.25, and Con1.00 lethal concentration.

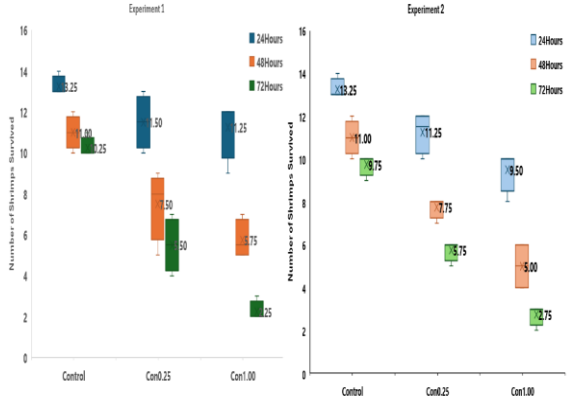

Figure 1 exhibits the average number of brine shrimps that survived for Control, Con0.25, and Con1.00 after 24 hours, 48 hours, and 72 hours respectively.Con0.25 beakers had a significantly lower number of surviving shrimps compared to those in the Control beakers. Con0.25 had a significantly lower number of surviving shrimps compared to those in the Control beakers (p-value of 0.048 during the first 24 hours, 0.0106 at 48 hours, and 0.0005 during at 72 hours). Con1.00 beakers also had a significantly lower number of surviving shrimps compared to those in the Control beakers, in a greater extent than Con0.25 (p value of 0.0447 at 24 hours, 0.0002 at 48 hours, and 0.000 at 72 hours). The results were consistent in the second experiment (where Con0.25 beakers survived shrimps had the p-value compared to those in the Control beakers of 0.0100 at 14 hours, 0.0005 at 48 hours, and 0.0000 at 72 hours. Con1.0 beakers survived shrimps had a p-value of 0.0005 at 24 hours, 0.0001 at 48 hours, and 0.000 at 72 hours. The result confirmed that the higher the lethal concentration, the more likely that we observed the lethality of brine shrimps. The results are consistent for all time durations in both experiments. (Table 1)

Our results clearly show differences in the survival of brine shrimp in Control, Con0.25, and Con1.00 after 24 hours, 48 hours, and 72 hours It was observed that after 24 hours, the average of 1.75 shrimps died in the Control group, while3.5 and 3.25 shrimps died in the Con0.25 group, suggesting that the lethality might start to have the effect on brine shrimp within 24 hours. The lethality was more severe when the shrimps are exposed to lethal concentration for a longer period. After 72 hours, the average number of surviving shrimps was reduced to 2.25 to 2.75 in the first and second experimentsfor Con1.00 concentration,

while the number of survived shrimps in the control group stayed atrelatively high at 10.25 and 9.75 in the first and the second experiments. Moreover, the number of surviving shrimps after 24 hours, 48 hours, and 72 hours seemed to reduce in a linear form by time, suggesting that time exposure to toxic solvent of orchid flower may matter in lethality of brine shrimp.

Table 1 presents the results of statistical tests using paired t-tests for independent samples for the number of surviving shrimps in Control, Con0.25, and Con1.00 concentrations from Experimental 1 and 2 respectively. With the exception of Con0.25 after 24 hours, all paired test for both Con0.25 and Con1.00 with respect to the control group exhibits statistically significant differences between the numbers of surviving shrimps in the experiment groups after 24 hours, 48 hours, and 72 hours of exposure (Table 1). The statistical significance was measured at p-value of less than 0.05, with some paired tests that hada significance level at a p-value less than 0.01. The second experiment firmly confirmed the toxicity of orchid-flower lethal concentration on brine shrimp. Moreover, the results suggest thetoxicity of orchid flower lethal concentration may start at as little as 0.25 mg/l.

IV. Conclusion and Discussion

This study attempts to investigate the toxic effects of popular food-decorative flowers. The study uses orchidflowers to demonstrate its toxic effects on brine shrimp. Our results suggest that orchids, indeed, can be toxicand acute toxicity assay. Increasing concentrations of orchid flower extracts led to statistically and significantly higher mortality rates among the shrimpcompared to the control group. The toxicity begins within 24 hours of exposure and intensifies with longer exposure. The results of our initial assay were replicated with our follow-up study to confirm these results, suggesting also that the lethal effects may start at a concentration of as little as0.25 mg/l.These results have shown that raised orchid flowerspose toxic effects on brineshrimps in small doses. More research is need on decorative flowers, especially those use on Asian cuisine.Our results raise awareness of the potential risk associated with placing flowers on food as a decoration and further research is needed in order to test more plants in the aim of minimizing the risk of consuming decorative flowers.

Table 1: Independent sample t-test for the average number of surviving brine shrimps in Control, Con0.25, and Con1.00 of lethal concentration of different pairs

	24 Hours		48 Hours		72 Hours	
Paired Test 1	**Mean**	**p-value**	**Mean**	**p-value**	**Mean**	**p-value**
Con0.25	11.5	0.0448*	7.5	0.0106*	5.5	0.0005**
Control	13.25		11		10.25	
Con1.00	11.25	0.0447*	5.75	0.0002**	2.25	0.0000**
Control	13.25		11		10.25	
Con0.25	11.5	0.809	7.5	0.1274	5.5	0.0033**
Con1.00	11.25		5.75		2.25	
Paired Test 2						
Con0.25	11.25	0.0100**	7.75	0.0005**	5.75	0.0000**
Control	13.25		11		9.75	
Con1.00	9.5	0.0005**	5	0.0001**	2.75	0.0000**
Control	13.25		11		9.75	
Con0.25	11.25	0.0448*	7.75	0.0047**	5.75	0.0001**
Con1.00	9.5		5		2.75	

Note: * Indicates statistical significance at $P < 0.05$ and ** Indicates statistical significance at $P < 0.01$

V. References

Almeida, J.R.G.S., Souza, G.R., Silva, J.C.E., Silva, J.G.E., Aguiar, E.V., Nascimento, S.C., & Ribeiro, R.M. (2006). Evaluation of the toxicity and antileishmanial activity of extracts from Brazilian sponges Aplysinafulva and Petromica citrine, *Journal of Ethnopharmacology*, 107(2), 278-282.

Anthoons, B., Lagiotis, G., Drouzas, A.D., de Boer, H., &Madesis, P. (2022). Barcoding high resolution melting (bar-hrm) enables the discrimination between toxic plants and edible vegetables prior to consumption and after digestion, *Journal of Food Science*, 87(9), 4221–4232.

Kibiti, C.M. &Adfolayan, A.J. (2016). Antifungal activity and brine shrimp toxicity assessment of Bulbineabyssinica used in the folk medicine in the Eastern Cape Province, South Africa. *Bangladesh Journal of Pharmacology*, 11(2), 469-477.

Ohikhena, F.U., Wintola, O.A., &Adfolayan, A.J. (2016). Toxicity Assessment of Different Solvent Extracts of the Medicinal Plant, Phragmantheracapitate (Sprengel) Balle on Brine Shrimp (Artemiasalina). *International Journal of Pharmacology*, 12(7), 701-710.