
Comparison of toxic effects of *Psidium guajava* leaf and bark extracts against Brine Shrimp (*Artemia salina*)

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Abstract *Psidium guajava*, also known as guava, is a medicinal plant that mainly found in tropical or subtropical areas like the Philippines. This plant has been used in treating different diseases and is also known for its medicinal and antimicrobial properties. Despite this, there are only a few numbers of studies regarding the toxicology of *P. guajava*. In this study, Brine Shrimp Lethality Assay (BSLA) was done to identify if the leaf and bark extracts of *P. guajava* have toxic effects against *Artemia salina* (brine shrimp). Results showed bark extracts having an LC₅₀ value of 480.14 µg/mL and leaf extracts with an LC₅₀ value of 949.13 µg/mL, and the mortality rates of brine shrimps for the bark extracts were relatively higher than the leaf extracts. It revealed that *P. guajava* bark extracts were more toxic than the leaf extracts.

Keywords: guava, BSLA, LC₅₀, toxicology

Introduction

Nowadays, traditional medicines are demanded to be standardized into herbal medicines for them to be used in medical facilities. Herbal medicines are one of the main methods of remedy increasingly recognized because many countries have been extensively using it as an alternative and complementary medicine (Guintu, 2013). Medicines should be verified as safe and beneficial for them to be used in medical facilities. Security affirmation efforts are necessary, and this could be achieved through toxicity and efficacy tests (Sukandar, Kurniati and Fitria, 2014).

Psidium guajava, commonly known as guava, is an evergreen shrub or small tree growing up to 3 meters tall and is mainly found in tropical and subtropical countries. All parts of this tree including fruits, leaves, barks, and roots have been used for treating several disorders (Barbalho *et al.*, 2012). Some researches show that *P. guajava* is mainly known for its antispasmodic,

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antioxidative, and antimicrobial activities and is also been used as a hypoglycaemic agent (Gutiérrez, Mitchell and Solis, 2008).

However, only little to no information and researches are given on the issue of safety of the consumption of *P. guajava* leaves. Evidences and data regarding the toxicological properties of *P. guajava* are insufficient and lacking. Also, it has been observed that many compounds present in medicinal plants may have toxic and mutagenic effects, which may cause harm to human health (Luber *et al.*, 2015). Thus, further studies are necessary to evaluate the toxic properties of *P. guajava*.

Brine Shrimp Lethality Assay (BSLA) is a simple method, which is based on the ability to kill a simple zoological organism, used for the preliminary screening of toxicity of plant extracts, heavy metals, and medicines (Wu, 2014).

This study was designed to determine the toxic effects of *P. guajava* leaf and bark extracts against brine shrimp (*A. salina*) following the Brine Shrimp Lethality Assay (BSLA), assessing at what concentrations (LC₅₀) of the leaf and the bark extracts would be lethal for half of the brine shrimp populations.

Materials and methods

Preparation of Psidium guajava leaf and bark extracts

The sample leaves and barks of *P. guajava* were collected from the province of Bulacan. The extraction method was based from the study of Tolentino and Urdan (2016). The dried leaves were shredded into smaller pieces using a blender until they are in powder form. 20g leaf powder will be added to 200 mL of distilled water and will be stirred to produce a solution with concentration of 10%. After stirring, the solution was submerged in an 80 °C water bath. The solution will be filtered after 2 hrs of water bath. The same procedure was done for the bark extract.

Assay Proper

Artificial seawater was prepared with a concentration of 25 ppt in a container. The brine shrimp eggs were then added with the presence of light and aerator. After 24 hrs, the newly hatched eggs were siphoned and observed under a dissecting microscope. Ten hatched eggs were transferred into each of the three wells of an ELISA microplate with varying concentrations of leaf and bark extracts at 1000 µg/mL (T₁), 500 µg/mL (T₂), and 100 µg/mL (T₃), having three replicates for all the setups of both extracts. Artificial seawater was used

as the negative control (T_4). After 12 hrs, the number of dead brine shrimps were counted.

Data Analysis

Mortality rates were calculated, and the gathered data from the BSLA was analyzed using Probit analysis in order to compute for the LC_{50} values of the extracts or at which concentrations half of the brine shrimp populations were killed.

Results

Death of brine shrimp was observed after 4 hours for the leaf extract (LE) set-up with 1000 $\mu\text{g}/\text{mL}$ concentration while it took about 6 hours for the LE set-ups with concentrations of 500 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$. The computed mortality rates after 24 hour for the LE set-ups were 66.67%, 13.33%, and 16.67% for concentrations of 1000 $\mu\text{g}/\text{mL}$, 500 $\mu\text{g}/\text{mL}$, and 100 $\mu\text{g}/\text{mL}$, respectively.

For the bark extract (BE) set-ups, mortality was observed after only 3 hours for the set-up containing 1000 $\mu\text{g}/\text{mL}$ concentration. It was also observed that death occurred after 5 hours and 6 hours for the set-ups with 500 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ concentration, respectively. The mortality rate for 1000 $\mu\text{g}/\text{mL}$ concentration of bark extract is 80% while the mortality rates for both 500 $\mu\text{g}/\text{mL}$ and 100 $\mu\text{g}/\text{mL}$ are relatively lower, with values of 26.67% and 10%, respectively.

Table 1. Mortality rate at 1000 $\mu\text{g}/\text{mL}$ (T_1), 500 $\mu\text{g}/\text{mL}$ (T_2), and 100 $\mu\text{g}/\text{mL}$ (T_3) of leaf and bark extract

	Mortality Rate	
	Leaf Extract	Bark Extract
T_1	66.67%	80%
T_2	13.33%	26.67%
T_3	16.67%	10%

The LC_{50} , the concentration of the extract that will cause death for 50% of the population, was computed using probit analysis. As seen in Table 3, the

acquired LC₅₀ value for the leaf extract was 949.13 µg/mL and 480.14 µg/mL for the bark extract.

Table 2. Data used for computation of LC₅₀

	Leaf Extract	Bark Extract
Slope	1.04621	1.94130
Intercept	1.88506	-0.20535
LC₅₀ (µg/mL)	949.13	480.14
Correlation Coefficient	0.58437	0.92097

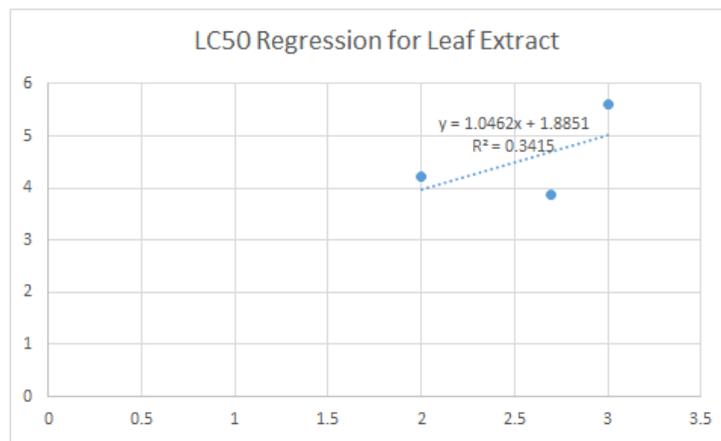


Figure 1. Linear Regression for Leaf Extract in Percent death (in Probit) vs log₁₀ (Concentration)

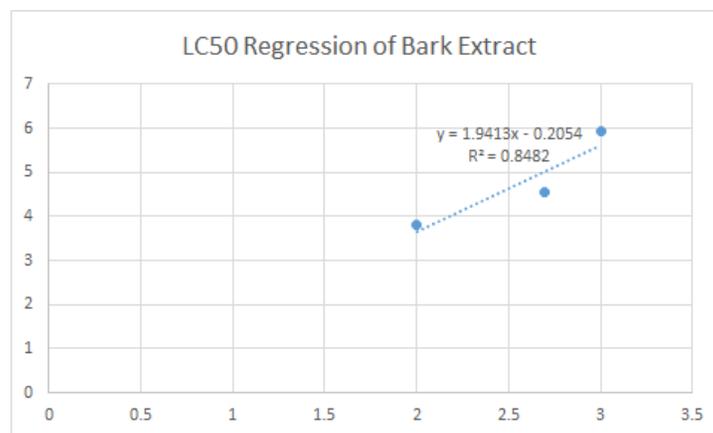


Figure 2. Linear Regression for Bark Extract in Percent death (in Probit) vs log₁₀ (Concentration)

Table 3. Probit analysis of different treatments of the two extracts

Concentration ($\mu\text{g/mL}$)	$\log_{10}(\text{concentration})$	% death		Probit		LC_{50} ($\mu\text{g/mL}$)	
		LE	BE	LE	BE	LE	BE
1000	3	66.67%	80%	5.61	5.95		
500	2.69897	13.33%	26.67%	3.87	4.56	949.13	480.14
100	2	16.67%	10%	4.23	3.82		

Discussion

Based on Meyer's toxicity index, *P. guajava* leaf and bark extracts are considered to be toxic due to them having LC_{50} values less than 1000 $\mu\text{g/ml}$ (949.13 $\mu\text{g/mL}$ for leaf and 480.14 $\mu\text{g/mL}$ for bark). While for Clarkson's toxicity criterion, *P. guajava* leaf extracts are considered as low toxic due to the LC_{50} value of 500 – 1000 $\mu\text{g/ml}$, and the bark extracts with LC_{50} value of 100 - 500 $\mu\text{g/ml}$ are medium toxic. Thus, this implies that bark extracts are more lethal or toxic than the leaf extracts.

Phytochemical studies have revealed the presence of compounds such as flavonoids, tannins, terpenoids, phenols, and glycosides (Biswas, 2013), which exhibit medical and pharmacological activities, including cytotoxicity. Flavonoids are dietary compounds that were found to possess toxic effects in high doses (Skibola and Smith, 2000). Phenolic compounds have a great potential of exhibiting pharmacological activities, and the components including tannins are considered to be toxic in high concentrations. Terpenoid, also known as isoprenoid, is a compound that is significant in the essential oils of most medicinal plants. The compound phenol is a common components of disinfectants used in households. Derived from plants as a drug or poison, glycoside plays an important role in plants since some chemicals are being stored as an inactive glycoside.

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