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Brine Shrimp Lethality of 'Sinaw-Sinaw' *Peperomia pellucida* (Linn.) Extracts

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Abstract

Peperomia pellucida (Linn.) of Family Piperaceae, locally known as '*sinaw-sinaw*', is an annual herb that is found in tropical countries. The plant has various histories of ethno-medicinal uses depending on the region. An assessment on the toxicity of this plant extract is essential to support therapeutical claims. This study was conducted to test for *in vivo* Brine Shrimp Lethality Assay (BSLA) of the crude aqueous and ethanolic extracts of *Peperomia pellucida*. Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). Ten nauplii were added into three replicates of each concentration of the plant extract. After 6, 12, and 24 hours of incubation, the surviving brine shrimp larvae were counted and LC_{50} was determined. Results showed that the *P. pellucida* crude aqueous and ethanolic extracts displayed mild cytotoxicity against brine shrimp nauplii with LC_{50} values of 46.55 µg/mL and 74.13 µg/mL, respectively. The findings of this present study may serve as a preliminary screen for further phytochemical studies and isolation of active compounds that can possibly be used for developing new drugs of therapeutic importance.

Keywords: cytotoxicity, ethno-medicinal, larvae, phytochemical, therapeutic

Introduction

Plants have been used for human benefits immemorial. In the developing world, 70-80% of the population relies on plants for primary health care (Luitel et al., 2014). However, during the past decade, traditional systems of medicine have become increasingly important in view of their safety (Nguta et al., 2012). The medicinal value of a plant is due to the substances that it contains which produce a physiological action on the human body (Olowa & Nuñeza, 2013). Plant provides a source of active chemical compounds useful for various pharmacological uses of humans such as alkaloids tannins, flavonoids, sugars, phenols, coumarins, terpenoids, saponins, and others (Oloyede et al., 2011). However, there are still negative effects obtained in the use of plant-derived medicines which are associated with the over-dosage and lack of adequate knowledge of other detrimental by-products contain in some plants (Nwachukwu et al., 2010).

Peperomia pellucida (Linn.) of Family Piperaceae, locally known as '*sinaw-sinaw*', is an annual herb that is found in tropical countries. It develops during rainy periods and thrives in loose humid soil under shades of trees (Sheikh et al., 2013). The plant species has various histories of ethno-medicinal uses depending on the region (Oloyede et al., 2011). Solution of the fresh juice of stem and leaves is used against eye inflammation in South America (Majumder, 2011), mental disorder treatment in Bangladesh, hemorrhages treatment in Bolivia, cholesterol reduction in Brazil, renal problem and uric acid reduction in Guyana (Wei et al., 2011), and arthritis and gout treatment in the Philippines (Mahata, 2010). Its leaves and stems are also used in treating abdominal pain, abscesses, acne, boils, colic, fatigue, headache, rheumatic joint pain, and bleeding (Sheikh et al., 2013). It was also claimed by local communities that decoction of *P. pellucida* was useful in many ways like treating wounds and as joint pain reliever (Uy, 2009). According to some studies, LC_{50} (the lethal concentration required to kill 50% of the population) values of the plant extract is not significant, though the methanol, hexane, and ethyl acetate fractions were found to be toxic (Oloyede et al., 2011). However, there are only

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few cytotoxic studies for *P. pellucida* extracts using 95% ethanol along with aqueous extraction. Also, other than this research, there have been no phytochemical studies conducted for this plant taken from Ozamiz City.

For toxicity evaluation of the plant extract in this study, Brine Shrimp Lethality Assay (BSLA) with *Artemia salina* species was designated. Brine shrimp lethality test is a biological model for the preliminary testing and selection of pediculicidal components from a natural source (Vidotto et al., 2013). It is a general bioassay that appears capable of detecting a broad spectrum of bioactivity present in plant crude extract (Pisutthanan et al., 2013). The availability and ease of performing the assay makes BSLA a very useful bench top method.

In this present study, crude aqueous extract and crude ethanolic extract of *P. pellucida* were tested *in vivo* for their cytotoxic effects against the brine shrimp nauplii. The findings of this present study may give baseline information on this promising plant species that could be used as a basis for the development of a new drug of great therapeutic importance.

Materials and Methods

Sample preparation

Leaves of *Peperomia pellucida* (Figure 1) was collected randomly from different areas in Ozamiz City. This plant commonly grows in the road side and readily available for study.

Extraction

a. Aqueous extraction

Fresh leaves weighing 400g were washed and put into 2L of distilled water and decocted for 30 minutes. The decoction was first filtered by cheese cloth and then by filter paper. The filtrate was subjected to freeze-drying for three days.

b. Ethanolic Extraction

The leaves were washed with water and air dried in shade for about one to two weeks. The air-dried leaves were pounded and weighed up to 25g. Pounded leaves were put into an Erlenmeyer flask containing 250 mL of 95% ethanol, soaked in the medium for three days and filtered. The filtrate was concentrated using rotavap and subsequently subjected to liquid nitrogen to obtain a dry sample.

Figure 1. Green, succulent heart-shaped leaves of the plant sample of *Peperomia pellucida*.

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Brine shrimp lethality assay

Brine shrimp eggs used in this study were obtained from Mindanao State University-Naawan. The eggs were hatched in filtered pure sea water in a large container with illumination and oxygen aerator. After 24 hours of incubation at room temperature (25°C), nauplii (larvae) were collected by a glass dropper and added into vials.

Thirty milligrams of the powdered extract from aqueous extraction was prepared up to 2mg/mL of sea water and was dissolved in dimethyl sulfoxide (DMSO). Sample concentrations (5µL, 50 µL, 250 µL and 500 µL) were made in each vial. Three replicates were prepared in every treatment. A control group was made of 0.5mL vehicle treated, DMSO with 4.5mL of brine shrimp solution without the plant extract. Ten nauplii were added into each vial. The vials were examined and the number of dead (non-motile) nauplii in each vial was counted after 6, 12, and 24 hours incubation at room temperature (25°C) with a magnifying glass and under illumination. The same procedures were conducted in the brine shrimp lethality assay treated with the air-dried leaves extract from 95% ethanolic extraction.

Statistical test

Probit analysis was used to assess the lethality concentration (LC_{50}) at 95% confidence intervals. The toxicity of the plant extract is determined using the study of Moshi et al. (2010) as a reference. Lethality concentration, LC_{50}, less than 1.0 µg/ml is highly toxic, from 1.0- 10.0 µg/ml is toxic, from above 10.0 µg/ml up to 30.0 µg/ml is moderately toxic, greater than 30 µg/ml but less than 100.0 µg/mls is mildly toxic, and greater than 100.0 µg/ml as non-toxic.

Results and Discussion

Table 1 shows the cytotoxicity of *P. pellucida* plant extracts to brine shrimp nauplii at different concentrations and incubation time intervals. The results vary with the control, aqueous and 95% ethanolic plant extracts. The lowest mortality rate was recorded at the lowest concentration (5 ul) and shortest incubation period (6 hours). The highest mortality rate is noted at the highest extract concentration (500 ul) and longest incubation period (24 hours). The percent mortality of the brine shrimp nauplii increases as the concentration of the extracts are increased. The maximum mortality at 100% was recorded in 500 ul for the ethanolic extract. The pattern is similar with the exposure time. This result corroborates with the study of Ezemonye et al. (2009) and Olowa and Nuneza (2013) that the mortality of the nauplii in sample extracts is concentration- and time exposure-dependent.

The lethality concentrations, of the aqueous and ethanolic extracts were 46.55µg/ml and 74.13µg/ml, respectively which is regarded as mildly toxic (Moshi et al., 2010). The ethanolic extracts showed higher lethality concentrations which is similar with the studies of Rajeh et al. (2010), Biswas et al. (2011), and Olowa and Nuñeza (2013) with other plant species. The observed lethality of the plant extracts to brine shrimps confirms the mild cytotoxic activities of 'sinaw-sinaw' *P. pellucida* and isolation of active compounds and thorough bioassay may further be carried out. The presence of cytotoxicity of plant extracts demands phytochemical screening to find the active components causing toxicity (Apu et al., 2010).

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Table 2. Cytotoxicity to brine shrimp nauplii of 'Sinaw-Sinaw' *Peperomia pellucida* plant extracts at different concentrations and incubation time intervals.

		6 Hours		12 Hours		24 Hours		
	Concentrations (µL)	**Number of nauplii (average)**	**Mortality rate (%)**	**Number of nauplii (average)**	**Mortality rate (%)**	**Number of nauplii (average)**	**Mortality rate (%)**	**Average mortality rate (%)**
Control	5	0	0	0	0	1.33	13.3	4.43
	50	0.67	6.7	1.33	1.33	2.33	23.3	10.44
	250	1.33	13.3	1.67	16.7	3.33	33.3	21.10
	500	1.67	16.7	1.67	16.7	4.00	40.0	24.47
Aqueous plant extract	5	0	0	0.67	6.7	2	20	8.90
	50	1.33	13.3	4.33	43.3	6.33	63.3	39.97
	250	5	50.0	9	90.0	9.67	96.7	78.90
	500	5.67	56.7	7.33	73.3	9.33	93.3	74.43
95% Ethanol plant extract	5	0	0	1	10	1.33	13.3	7.77
	50	0.33	3.3	1.67	16.7	3.33	33.3	17.77
	250	2.67	26.7	3.67	36.7	5.33	53.3	38.90
	500	3.67	36.7	5.67	56.7	10	100	64.47

Based on the individual rates, a 100% mortality rate was observed in the ethanolic plant extract (500ul). However, with the overall average of percent mortality, the crude aqueous plant extracts could produce the higher mortality rate but not the maximum rate (100%) than the control and ethanolic extracts (Figure 2). This result may indicate that water can be an effective extraction medium for 'sinaw-sinaw' to elicit its cytotoxicity. This finding is similar with the study of Ling et al. (2016) showing that water plant extracts displayed cytotoxicity. The result of this current study suggests that *P. pellucida* contains water-soluble components that exert cytoxicity. This cytotoxic activity of aqueous extracts was most pronounced at 250 ul concentration.

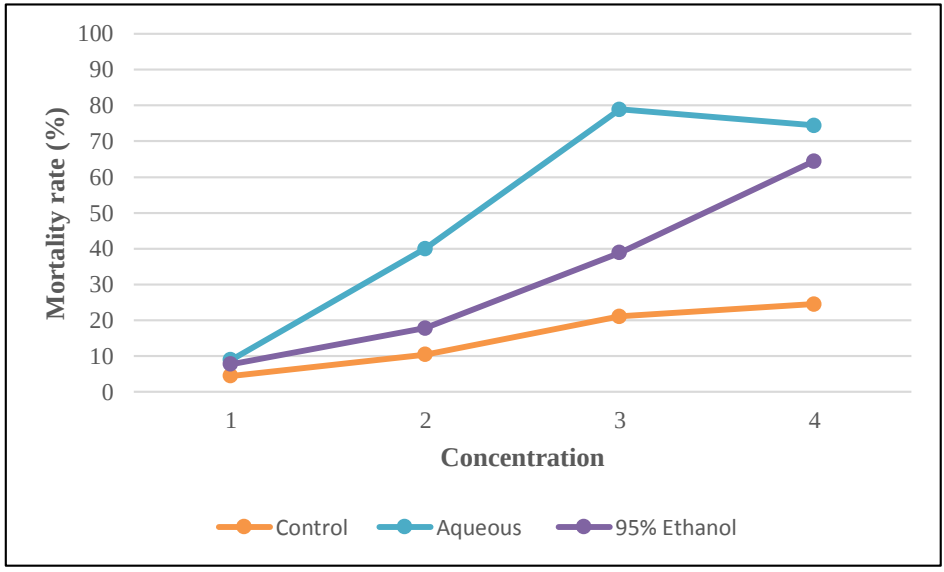

Figure 2. Overall mortality rate of the brine shrimp nauplii with the control group, aqueous and 95% ethanol extracts at different concentrations [(5µl (1), 50µl (2), 250µl (3), 500µl (4)].

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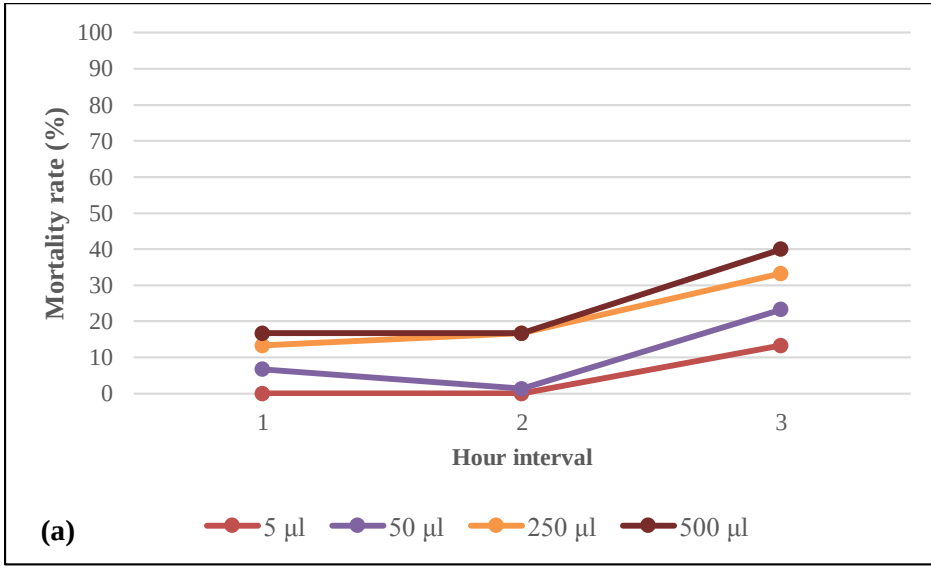
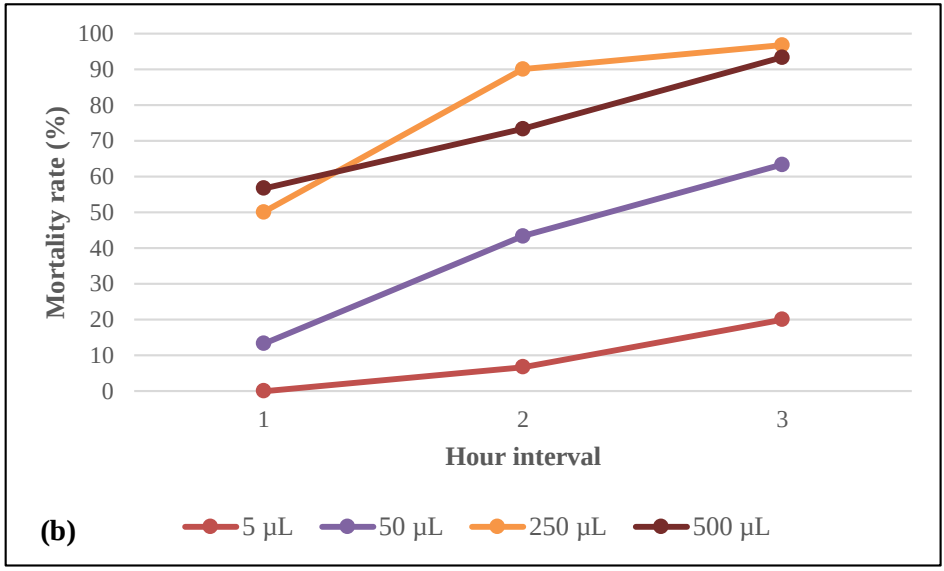
Figure 3 shows the mortality rates of the brine shrimp nauplii at different plant extract concentrations and incubation time with the control group, aqueous and 95% ethanol extracts, respectively. The control exerted lowest mortality rates that do not confirm cytotoxicity. However, this study shows that both crude aqueous and crude ethanolic plant extracts displayed cytotoxic effects to brine shrimp nauplii, but the maximum effect (100% mortality) was at the highest concentration of ethanolic extract after 24 hours of incubation. While this study supports the finding of Ling et al. (2016) using different plant species, the toxicity results of the extracts obtained from of *P. pellucida* in the study of Oloyede et al. (2011) showed that the methanolic fractions are toxic to brine shrimp larvae at different lethal concentrations while the aqueous fractions were not toxic. Pappachen and Chacko (2013) also did not show that *P. pellucida* crude methanolic extract is toxic. Hence, it is important to conduct a phytochemical analysis of this plant species.





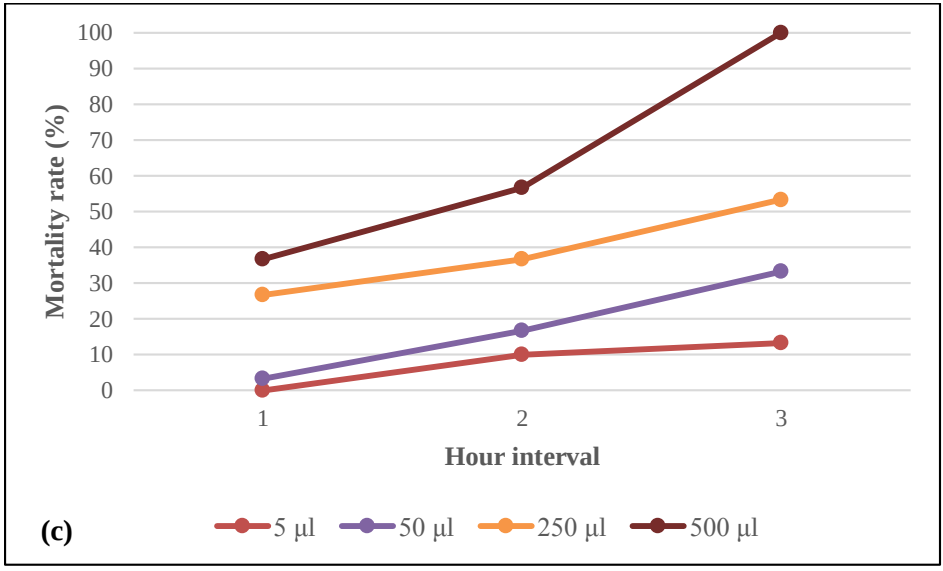


Figure 3. Mortality rate of the brine shrimp nauplii with the control group (a), *P. pellucida* aqueous extracts (b) and 95% ethanolic

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extracts (c) at different concentrations and incubation hour intervals [6 hours (1); 12 hours (2); 24 hours (3)].

Conclusion and Recommendation

The leaf extracts of *P. pellucida* from Ozamiz City exhibited mild toxicity against the brine shrimp nauplii. Both water and ethanolic extracts displayed cytotoxic activity but the maximum mortality rate was at the highest concentration of ethanolic extract and at the longest time exposure. The brine shrimp lethality assay is limited to providing preliminary screen only, hence, phytochemical analysis of *P. pellucida* is recommended to provide confirmatory results of the presence of active compounds. Isolation of these active components can pave way to the development of useful drugs of therapeutic importance.

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