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Cytotoxicity test of Betel extract using Brine Shrimp Lethality Assay

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Abstract

Piper betle, recognized for its potential antimicrobial and antifungal properties, presents an intriguing avenue for investigating its efficacy in cancer treatment. This study aims to determine the cytotoxic effects of Piper betle extract utilizing the Brine Shrimp Lethality Assay (BSLA). The results showed that when the concentration of Piper betle extract increased, the survival time of nauplii decreased in a concentration-dependent manner. Additionally, a dose-response connection was seen in the mortality rate, with greater concentrations translating into higher mortality. Significant variations in cytotoxicity between the Piper betle extract and the negative control were validated by statistical analysis. The finding is evident because it indicates that Piper betle extract has cytotoxic properties, which may have consequences for cancer research. Although more research is necessary to clarify the underlying mechanisms and pinpoint particular bioactive chemicals, the findings emphasize how critical it is to investigate Piper betle's potential for oncology therapy.

Key Words:*Piper Betle, Brine Shrimp, Lethality Assay, Mortality*

Introduction

The leaf of the Piper betle, commonly referred to as the betel vine, is the most utilized portion of this popular medicinal plant in Asia. Chewing is used traditionally according to Nayaka et al., (2021) strengthen gums, keep teeth intact, avoid bad breath, and stimulate the digestive system. Betel leaves are also used as mouthwash, vaginal douching, and dental treatments. Since the beginning of time, people have utilized plants for medical purposes. Despite being safe and effective, a few synthetic medications are helpful in the treatment of cancer. Plants, however, have shown promise in the treatment of cancer (Akhtar & Swamy, 2018). According to the study (Sakinah & Misfadhila, 2020. Piper betel is also believed to give strength to the liver and regulate blood flow. Piper betle leaf also aids in the expulsion of mucus from the respiratory tract. It has a pleasant flavor and smell that the Greeks believed would improve appetite and taste, be a tonic for the brain, heart, and liver, lessen taste, clear the throat, and purify the blood.

Piper betle shows a prospective outlook for treatment of some medical disorders like microbial and fungal infections by providing an proper understanding and undertaking relevant to the investigation of the benefits of Piper betle to cancer treatment pose huge significance to this research. The aim of the study is to determine the cytotoxicity of paper betel extract using brine shrimp lethality assay. A significant difference between different concentration of extract and negative contract will evaluate.

Subsection

Piper betle concept and importance.

The areca palm (Areca catechu), which grows in Asia, the tropical Pacific, and some regions of east Africa, yields seeds that are known as areca nuts. Because it is frequently chewed when wrapped in betel leaves, it is also known as betel nut. At least 10% of people on the planet routinely chew it, making it the fourth most popular addictive substance in the world. The International Agency for Research on Cancer declared in 2004 that chewing betel nut is a major risk factor for mouth cancer

and is carcinogenic to humans (Lechner et al., 2019). Adults and older individuals use it with smoke to increase addiction. Because of its chilly environment, nganga is wellliked in neighboring provinces, the Mountain Province, and other sections of the Cordilleras. Before struggling in rice cultivation, especially when it's going to rain, chewing moma keeps the body warm. Moma is also available to help people stay alert, such as drivers (Cosalan, 2020). According to the study (Sakinah & Misfadhila, 2020), Piper betel is also believed to give strength to the liver and regulate blood flow. Piper betle leaf also aids in the expulsion of mucus from the respiratory tract. For postpartum therapy, vaginal discharge, menstrual pain, body odor, and fertility, piper betel leaf is reputed to be effective. The multipurpose plant betel leaf includes phenolic chemicals that are cytotoxic and have antibacterial properties (Sakinah & Misfadhila, 2020).

Cytotoxicity of Piper betle using Brine Shrimp Brine shrimp (nauplii) is used as a model organism in toxicity experiments to evaluate the toxicity of active chemicals in plants using BSLA. A useful tool for toxicity assessment, Artemia salina is a significant taxon in hyper-saline biotypes and largely feeds on phytoplankton (Suneka & Manoranjan, 2021). BSLA can be used to evaluate the cytotoxicity of plants in a lab bioassay. The leaves contain pharmacologically active phytochemicals that are frequently employed in a variety of treatments (Nerdy et al., 2021).

Methods

In quantitative research, it helps in data collection for better decision-making and establishing study facts (Sirisilla, 2023). This study aims to comprehend the significance of the cytotoxicity test of Betel extract using Brine Shrimp Lethality Assay. Collection of specimens 1. Fresh Piper betle stems were gathered from Quiapo, Metro Manila. 2 Extraction of Piper betle stems.The Piper betle stems were gathered, washed with tap water, allowing to dry with sunlight, and then ground into powder by a mortar and pestle. Then, stored in a closed plastic container at room temperature. Prepare concentration of Piper betle consisting of 180 grams of powder in a solvent of water consisting of 1,500 ml of distilled water. Then, place the powdered Piper betle stems in the solvent of water.Stir the mixture vigorously to enhance the extraction process. Filter the mixture The researchers found a related study to have a guide with the concentration of Piper betle with solvent of water. From the extract of Piper betle, the researchers were going to get the dilution. 25 ppm, 50 ppm, 100 ppm, 200 ppm (Suneka & Manoranjan, 2021). For the live nauplii, count the nauplii that show movement. Calculate the mortality rate for each concentration. Then, compute the LC50. The LC50 value is the concentration of the extract required to kill 50% of the brine shrimp. A lower LC50 value indicates higher toxicity. Brine shrimp cysts are hatched following standard procedures, and the newly hatched nauplii are transferred into separate petri dishes with aeration. Subsequently, we expose the nauplii to Piper betle extract solutions of different concentrations, ensuring that each container contains an equal number of nauplii for consistent exposure. The exposure duration ranges from 24 hours while maintaining appropriate environmental conditions. After each exposure period, we meticulously count the number of survived and deceased brine shrimp nauplii in each petri dish, recording the mortality rate for each concentration and exposure duration. Statistical analyses, such as Probit analysis, are then applied to determine LC50 values for each condition.

Results

The researchers computed the time of efficacy per dilution of extracts. The researchers gather the data for trials 1 and 2. For the 0.025 ml and 0.05 ml dilutions, the average time of the hatched nauplii is 3 hours. The next dilution is 0.1 ml, which results in an average of 2.5 hours. The last dilution

that the researchers have is 0.2 ml, which has an average of 2 hours. Therefore, the fastest hour of the death nauplii is 2 hours, with a dilution of 0.2 ml.

Cytotoxicity level of Piper betle using BSLA based on time efficiency.

Dilutions (ppm)	Trial 1	Trial 2	Average
0.025 ml	3 hours	3 hours	3 hours
0.05 ml	3 hours	3 hours	3 hours
0.1 ml	2 hours	3 hours	2.5 hours
0.2 ml	2 hours	2 hours	2 hours

The researchers computed the mortality rate based on the dilution of extracts using the formula % mortality = (no. of dead nauplii / initial no. of live nauplii) x 100. The researchers gather the data for trials 1 and 2. For the 0.025 ml dilution, the average percentage of dead nauplii is 75.87 %. At a 0.05 ml dilution, the average percentage of death of the hatched nauplii is 86.67 %. The next dilution is 0.1 ml, which results in an average percentage of 91.11 %. The last solution that the researchers have is 0.2 ml, which has an average percentage of 94.45 %. Therefore, the most deaths of nauplii are at 0.2 ml dilution, with an average percentage of 94.45%.

Mortality rate of brine shrimp using BSLA based on the dilution of extract.

Dilutions (ppm)	Trial 1	Trial 2	Average
0.025 ml	80 %	71.74 %	75.87 %
0.05 ml	86.67 %	86.67 %	86.67 %
0.1 ml	93.33 %	88.89 %	91.11 %
0.2 ml	95.56 %	93.33 %	94.45 %

The researchers computed the p-value using a two-way ANOVA. The computed value using two-way ANOVA is 0.8247 lower than the p-value of 0.5611 at the 0.05 level of significance, then the hypothesis is ACCEPTED. Therefore, there's no significant difference in the cytotoxic level of Piper betle. The computed value using two-way ANOVA is 395.7415 lower than the p-value of 0.0002776 at the 0.05 level of significance, so the hypothesis is REJECTED.

Cytotoxicity level of Piper betle

Dilutions (ppm)	Time efficacy	Mortality rate (%)	F-ratio	P-value	Level of Significance	Decision
0.025 ml	3 hours	75.87 %	0.8247	0.5611	0.05	Accepted
0.05 ml	3 hours	86.67 %				
0.1 ml	2.5 hours	91.11 %	395.7415	0.0002776		Rejected
0.2 ml	2 hours	94.45 %				
Ave:	2.625 hours	87.025	Ave: 44.825			

Therefore, there's a significant difference in the cytotoxic level of Piper betle. According to the study of Anjur et al. (2022), titled "Antibacterial Activity and Toxicity Study of Selected Piper Leaf Extracts Against the Fish Pathogen (Aeromonas hydrophila)," the three indigenous Piper plants—Piper betle, Piper sarmentosum, and Piper nigrum—were used in the study to test the antibacterial qualities of raw water extracts.

Cytotoxicity level using BSLA between Piper betle extract and negative control.

	Dilutions (ppm)	Negative control	F-ratio	P-value	Level of Significance	Decision
Time efficacy	3	1	4.5918	0.278	0.05	Accepted
Mortality rate	14	5	2.4694	0.3608		
Ave:	8.5	3	Ave: 5.75			

Discussion

The mortality rate and deaths found in each trial of the nauplii are significantly related to the presence of the Piper betle stem extract in each of the trials. 2. There is a correlation between the strengths of dosages and the number of deaths in the trials. 3. The researchers conclude that the life expectancy of brine shrimp in the trials decreases as the presence of Piper betle stem extract increases.

The Piper betle stem extract has cytotoxic qualities that may aid in helping people further create better medicine to aid in cancer research

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