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Phytochemical Analyses and Cytotoxicity Activity Using *Artemia salina* (Brine Shrimp) Lethality Assay of the *Caladium bicolor* and *Alocasia sanderiana* Ethanolic Leaf Extract

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ABSTRACT

Even though a lot of research has previously been done on both *C. bicolor* and *A. anderiana*, more has to be done to assess the phytochemicals and cytotoxic potential (LC50 value) of their leaves. This work sought to identify the phytochemicals and cytotoxic activity of crude ethanolic leaf extracts of *C. bicolor* and *A. anderiana* using the A. salina (brine shrimp) Lethality Assay. During the phytochemical analysis, the leaves were boiled in distilled water and mixed with various reagents. The colors were examined to determine whether the various phytochemicals were present or not. A. salina cysts in saltwater were permitted to hatch in order to perform the cytotoxicity. The ten (10) nauplii were moved to various crude ethanolic leaf extract concentrations. The sixth (6th) hour death of nauplii was used to calculate the percent mortality rate. According to the results, the leaves of *C. bicolor* tested positive for flavonoids, terpenoids, tannins, and saponins. On the other hand, *C. bicolor* leaves tested negative for alkaloids and steroids. The presence of cardiac glycosides, terpenoids, and tannins in *A. anderiana* leaves was found to be positive. The leaves of *A. anderiana*, however, tested negative for alkaloids, steroids, flavonoids, and saponins. On the sixth (6th) hour, the LC50 value of *A. anderiana* crude ethanolic leaf extract was 4,712.4 µg/ml, whereas that of *C. bicolor* was 874,028.4 µg/ml. In the sixth hour, neither extract was cytotoxic. The phytochemicals and cytotoxic levels of the crude ethanolic leaf extracts of *C. bicolor* and *A. anderiana* were determined in this study. The findings can be applied to future research on the anti-inflammatory, antibacterial, antioxidant, and anti-diabetic bioactivities of *C. bicolor* and *A. anderiana*, which may yield non-cytotoxic medications.

INTRODUCTION

Over the years, medicinal plants has provided a notable edge over a variety of ailments. Phytochemicals from medicinal plants were thought to be safe, bioactive, and biodegradable. Among many other pharmacologically significant activities, laboratory-based in vitro investigations have connected these substances to antioxidant, anti-inflammatory, and anti-diabetic qualities (Mapfumari *et al.*, 2022). Even at low concentrations, phytochemicals found in medicinal plants have demonstrated varying degrees of efficacy against bacteria, making them a viable reservoir of therapeutic characteristics (Maharaj *et al.*, 2022). In order to identify different classes of phytoconstituents present in different parts of the plant for the purpose of drug discovery, phytochemical screening is a scientific process that involves analysis, examination, extraction, and experimentation. The active components can then be taken for further study and research (Sharma *et al.*, 2022).

Apart from phytochemical analysis, which was employed as a preliminary test to screen for the therapeutic properties of plants, cytotoxicity assays were employed to determine the capacity of particular chemicals or mediator cells to consume living cells. The cytotoxicity test employed in this study is the brine shrimp lethality test (BSLT). The toxicity test is based on the number of brine shrimp that die after being exposed to varying concentrations because brine shrimp are cytotoxic to a variety of chemicals and natural compounds.

In traditional medicine, *C. bicolor* and *A. anderiana* are frequently used to cure a variety of conditions, such as jaundice, boils, snake bites, and hyperglycemia (Arbain *et al.*, 2022). However, according to research by Ezea (2022), *C. bicolor*, also referred to as "Ede Umuagbara," is a wild cocoyam found in southeast Nigeria.

Numerous species of Alocasia have been found to have medicinal uses, including acute toxicity tests and anti-inflammatory, anti-diabetic, antihyperglycemic, antioxidant, antibacterial, antiparasitic, and anti-cancer effects (Arbain *et al.*, 2022).

Despite the fact that both *C. bicolor* and *A. anderiana* have been the subject of numerous investigations, more research is required to assess the phytochemicals' cytotoxicity. Nevertheless, there was a gap in determining the extracts' lethal dose. Only the widely recognized phytochemicals have been the subject of the few research that have been conducted on both plants for the phytochemical analysis. The presence of other phytochemical kinds in the plants used in this investigation has not yet been investigated.

The study sought to identify the phytochemicals and cytotoxicity activities using the brine shrimp lethality assay of *C. bicolor* and *A. anderiana*. The study's specific goal was to identify the phytochemicals such as tannins, saponins, terpenoids, flavonoids, steroids, and alkaloids

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found in the leaf extracts of *C. bicolor* and *A. anderiana*. The study also determined the degree of cytotoxicity of *C. bicolor* and A. sanderiana, as well as the median lethal concentration (LC50) of their crude ethanolic leaf extracts on the sixth hours.

The results of the phytochemical analyses and cytotoxicity activity are useful to potential pharmaceutical researchers that will use the plant-derived constituents of *C. bicolor* and A. sanderiana for future bioactivity screening.

MATERIALS AND METHODS

Research Design

The phytochemical analyses of the two plants' crude ethanolic leaf extracts were described using a descriptive study design. However, an experimental design was employed to use the various amounts of crude ethanolic leaf extracts to evaluate the degrees of cytotoxicity.

Research Setting

At a private institution in Iloilo City, the phytochemical and cytotoxicity investigations were carried out utilizing the phytochemical screening (color test) and brine shrimp lethality assay.

Ethical Considerations

The accredited Ethics Review Committee of a private university had reviewed the research.

Data Collection Procedures

Plant Identification and Collection: The leaves of *C. bicolor* (Figure 1.) and A. sanderiana (Figure 2) were gathered from Buhang Jaro in Iloilo City and Balabag in Pavia,

Figure 1: Photograph of *C. bicolor* Plant

Figure 2: Photograph of *A. sanderiana* Plant

respectively. For accurate identification, the Department of Agriculture identified the plant samples. The mature leaves that were gathered were pest-free and devoid of any dry or browning areas (Leong-on, 2022b).

Phytochemical Analyses

Using conventional protocols, tannins, saponins, terpenoids, cardiac glycosides, flavonoids, steroids, and alkaloids were screened. Leong-on (2020) mentioned the works of Tariq *et al.* (2012) and Tiwari *et al.* (2011), and all of the processes were taken from those works (Leong-on, 2022c).

Test for Tannins (Ferric chloride test)

A 0.5g of dry leaves were boiled for three minutes in a test tube with 20 mL of distilled water. After cooling, the mixture was filtered. Three milliliters of filtrate were then mixed with five drops of 0.1% ferric chloride. The presence of tannins was detected by the development of a brownish-green to blue-black coloring.

Test for Terpenoids (Salkowski Test)

A 0.5g of dried plant material was dissolved in 10 mL of distilled water. After cooling, the mixture was filtered. A layer was created by adding 0.75 mL of concentrated sulfuric acid after 1.25 mL of extract and 0.5 mL of chloroform had been combined. The presence of terpenoids was detected by the interface turning reddish-brown.

Test for Flavonoids (Aluminum chloride colorimetric technique)

A 0.5 gram of dried plant material was added to 20 milliliters of distilled water and brought to a boil. After cooling, the mixture was filtered. Three drops of a 1% aluminum solution were added to three milliliters of filtrate. Flavonoids were detected by the emergence of a yellow tint.

Test for Steroids (Salkowski test)

One milligram of crude plant extract was dissolved in ten milliliters of chloroform. The test tube was then filled by the sides with an equivalent volume of pure sulfuric acid. The test tube's top layer glowing red and the sulfuric acid layer changing yellow with green fluorescence were signs that steroids were present.

Test for Alkaloids (Wagner's test)

A0.5g of dried plant material was cooked in 10 mL of distilled water. After cooling, the mixture was filtered. Next, three milliliters of filtrate were mixed with three drops of Wagner's reagent. A white precipitate appeared, indicating the presence of alkaloids.

Extraction of Plant Compounds

Each plant's fresh leaves were cleaned, dried, and then pulverized into a powder. Specimen vials were then used to keep the powdered leaves. The extractant employed was ninety-five percent (95%) ethanol at room temperature. The extractant was poured until it was one inch higher than the plant material's overall height. To prevent the plant material from oxidizing, a black cloth was placed over the container holding the mixture. For 48–72 hours, the extract was homogenized, and it was stirred every 8–12 hours. A fresh cloth or filter paper was then used to filter the extract. Evaporation using a rotary evaporator set at 40–45 degrees Celsius was used to dry and remove the extractant. After that, half of the extract was put on a petri dish and covered with gauze to dry it (Leong-on, 2022a). Before being utilized for a cytotoxicity assay and a test for steroids, this was left for three to four days.

Cytotoxic Analyses Using Brine Shrimp Lethality Assay

To ascertain the plants' potential for cytotoxicity, they were analyzed using the brine shrimp lethality assay. Compared to more complex and costly in-vivo and in-vitro experiments, this bioassay was able to detect a wide range of bioactivity that was present in the extract.

Preparation of Seawater

Thirty-eight grams (38 g) of rock salt was dissolved in 1 L of distilled water.

Hatching of Brine Shrimps

For this technique, brine shrimp eggs, also known as Artemia salina leach, were gathered. The prepared seawater was poured into a shallow rectangular dish, filling it 34% of the way. The jar was filled with fifty milligrams (50 mg) of brine shrimp cysts. Black cartolina paper was used to darken a section of the container. For two days, the nauplii cysts were let to hatch in the seawater tank and grow into nauplii-like adults. The nauplii without eggshells were gathered in the lit section of the tiny tank after the freshly born shrimp were attracted to a light source. To make the fresh, pure saltwater more visible, these were pipetted and filtered. For every sample concentration, ten (10) nauplii were employed (Suryawanshi *et al.*, 2020; Leong-on, 2022).

Preparation of Test Samples

Every sample concentration was measured using small container cups. After adding 100 mg of the sample to 5 mL of the produced saltwater, various concentrations of crude ethanolic leaf extracts, ranging from 0 to 10 mg, were added to the cups. One drop of seawater was added to the container for 0 mg. Each addition contained 1 mg of the plant material, and the solution was utilized for the remaining concentrations (Leong-on, 2022).

Cytotoxicity Assay

Five milliliters of prepared saltwater were added to the cup with the dry extract and swirled. By pouring a drop of seawater to a white spoon and counting the brine shrimp nauplii until there were ten, ten were added to each cup containing the plant extract. After removing superfluous water, two drops containing ten brine shrimp nauplii were introduced to the cup containing the ten nauplii together with five milliliters of seawater and extract. Their food, 5 mL of saltwater, was mixed with 3 mg of measured yeast in a different container. A single drop of the suspension was added to each cup. The light was used to preserve these cups. The nauplii were fed using the yeast solution. After six hours, the number of dead shrimp was counted under a magnifying lens, and the percentage of mortality was then calculated.

Recording of Observations

Every six hours, the quantity of dead brine shrimp was tallied, and the subsequent observations were contrasted with the test control. The brine shrimp's mortality percentage was determined by counting the number of dead shrimp each hour (Kale *et al.*, 2019).

% mortality = (number of dead nauplii)/(number of live nauplii)

Using brine shrimp lethality assays of *C. bicolor* and A. sanderiana crude ethanolic leaf extracts, this study created three (3) trials and five (5) replicates to guarantee the validity and reliability of the procedures to ascertain the phytochemical and cytotoxicity analyses. The expert validated the results.

Statistical Tool

After calculating the percent mortality (%), the median lethal concentration (LC50) was determined six hours later using the cytotoxicity bioassay instrument. The LC50 value was found using the probit analysis.

Waste Disposal

Toxic materials or residues were disposed of in receptacles designated for hazardous chemical wastes and tightly sealed to prevent leaks. Dry infectious garbage was placed in a red container, while wet infectious waste was placed in a yellow bin (Nandy *et al.*, 2022).

RESULTS AND DISCUSSIONS

The results indicated that the presence of tannins with a brownish-green solution and saponins with a hazy appearance was detected. There were also flavonoids with a light yellow solution, and terpenoids with a light red-brown solution. Nevertheless, *C. bicolor* tested negative in the test for alkaloids, which had a clear, light brown solution, or steroids, which had a dark green solution (Table 1).

Regarding A. sanderiana leaves, it showed positive results for the presence of terpenoids with a light red solution, and tannins with a brownish-green solution. With a clear, light brown solution devoid of foam, however, it tested negative for saponins. A clear, purple solution was produced, and the results were negative for flavonoids as well. Alkaloids have a clear, light brown solution and steroids have a dark green solution, hence those tests are negative (Table 1).

Table 1: Phytochemical analyses of the *C. bicolor* and A. sanderiana Leaves

Plant	Tannins	Saponins	Terpenoids	Flavonoids	Steroids	Alkaloids
C. bicolor	Positive	Positive	Positive	Positive	Negative	Negative
A. sanderiana	Positive	Negative	Positive	Negative	Negative	Negative
Negative Control	Negative	Negative	Negative	Negative	Negative	Negative

The *C. bicolor*'s LC50 value was 874,028.4 µg/ml whereas the A. sanderiana was 4,712.4 µg/ml (Table 1), according to A. salina nauplii death rates. At the sixth hour, both the ethanolic leaf extracts were not cytotoxic.

Table 2: Cytotoxicity of Crude Ethanolic of *C. bicolor* and A. sanderiana Leaf Extract Using Brine Shrimp (A. salina) Assay

Concentrations (mg/5ml)	% Mortality of *A. salina* in *C. bicolor* (mean+SD)	% Mortality of *A. salina* in *A. sanderiana* (mean+SD)
0	0.0 + 0.00	1.6 + 0.08
1	1.0 + 0.00	2.2 + 0.06
2	1.0 + 0.00	2.2 + 0.12
3	1.0 + 0.00	3.2 + 0.18
4	1.2 + 0.45	3.0 + 0.10
5	1.4 + 0.55	3.6 + 0.03
6	1.2 + 0.45	3.8 + 0.12
7	1.4 + 0.55	3.2 + 0.12
8	1.2 + 0.45	3.6 + 0.08
9	1.4 + 0.55	3.8 + 0.06
10	1.6 + 0.55	4.2 + 0.06
LC 50	874,028.4 µg/ml	4,712.4 µg/ml

Both *C. bicolor* and A. sanderiana have saponins and tannins. These compounds from Wrightia tinctoria, Euphorbia hirta, Thespesia populnea, and Cassia alata were demonstrated efficacy against Bacillus subtilis and Pseudomonas aeruginosa at a dose of 0.5 µg (Rji *et al.*, 2019).

Both *C. bicolor* and A. sanderiana contain terpenoids. These compounds are known to possess antiviral, antifungal, antimicrobial, and antiparasitic qualities. They also have anti-inflammatory, antiviral, and antioxidant lower the risk of vascular diseases (Ullah, 2020) and cancer chemopreventive effects (Ramteke *et al.*, 2021).

Both *C. bicolor* and A. sanderiana have been found to contain cardiac glycosides. This compound had been shown to increase cardiac output.

It was discovered that *C. bicolor* contains flavonoids. Flavonoids are effective in preventing the growth of tumor cells and inducing the activation of several vital detoxification enzymes.

The cytotoxicity of plants varies according to their constituent parts and the extractant used. When the LC50 value was 500–1000 µg/ml, the cytotoxic activity was deemed mild. When the LC50 result falls between 100 and 500 µg/ml, it indicates moderate toxicity; when it falls between 0 and 100 µg/ml, it indicates strong toxicity. When the LC50 value exceeds 1000 µg/ml, it is classified as non-cytotoxic (Nguta *et al.*, 2013). Strongly poisonous plants may provide new scaffolding for the development of anti-cancer medications. Conversely, those with low toxicity might be excellent candidates for the creation of food supplements, nutraceuticals, or herbal medications.

C. bicolor exhibited less cytotoxic activity than A. sanderiana. However, both crude ethanolic leaf extracts were non-cytotoxic because their LC50 values were greater than 1000 µg/ml. Non-cytotoxic medications may be derived from plants such as *C. bicolor* and A. sanderiana. When compared to chemically manufactured drugs, natural products may have fewer adverse effects or be non-cytotoxic, which could explain their growing popularity.

The study's results were accurate only when the crude ethanolic leaf extracts of *C. bicolor* and A. sanderiana death rate was calculated at the sixth hour and LC50 values were determined. The mature leaves were gathered from San Isidro, Jaro, and Balabag, Pavia, Iloilo City, Philippines, at 6:00 in the morning. This investigation served as the foundation for additional bioactivity testing of crude ethanolic leaf extracts of *A. sanderiana* and *C. bicolor*.

CONCLUSIONS

The phytochemicals found in *C. bicolor* leaves included flavonoids, terpenoids, cardiac glycosides, tannins, and saponins; alkaloids and steroids did not test positive. Tannins,and terpenoids are found in A. sanderiana leaves. Alkaloids, steroids, flavonoids, and saponins all had negative results. The LC50 value for *C. bicolor* was 874,028.4 µg/ml but the value for A. sanderiana was 4,712.4 µg/ml,. This outcome makes it clear that on the sixth (6th) hour, neither the crude ethanolic leaf extract of *C. bicolor* nor A. sanderiana was cytotoxic. The study's findings can be applied to future research on *C. bicolor* and A. sanderiana's various bioactivities, including their anti-inflammatory, antioxidant, antibacterial, and anti-diabetic properties.

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