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EFFECTS OF STAR APPLE (*Chrysophyllum cainito*) FRUIT AND PEEL EXTRACT USING BRINE SHRIMP (Artemia salina) LETHALITY ASSAY

John Michael F. Delay[1], Irish Kaitlyn B. Villaraiz[2], April A. Anisco [3], Elena F. Frio[4]

Colegio de la Purisima Concepcion, cresa@purisima.edu.ph
Colegio de la Purisima Concepcion, johnmichael.delay7@gmail.com

ABSTRACT

The main objectives of the study were to determine the cytotoxic activity of star apple (*Chrysophyllum cainito*) fruit extract and peel extract using brine shrimp (*Artemia salina*) effects assay. This study is limited to determine the anticancer potential of the different concentration of star apple (*Chrysophyllum cainito*) fruit extract and peel extract against the mortality rate of brine shrimp (*Artemia salina*). Using brine shrimp brine shrimp, the lethal concentration (LC) of a plant extract that results in 50% mortality (LC_{50}) is measured. Three treatments were prepared (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peel extract, Treatment C (Control) – 10 mL Distilled water) with three replicates. Ten brine shrimps were tested for their mortality rate and were recorded after 15, 30, 45, and 60 minutes of application. The different concentration of star apple (*Chrysophyllum cainito*) fruit extract and peel extract does not have anticancer potential based on the mortality of brine shrimp (*Artemia salina*) brine shrimp.

Keywords: *Star apple, Brine Shrimp, Mortality rate*

1. Introduction

Cancer has become a crucial world health concern due to its nature of uncontrolled multiplication of aberrant cells, which interfere with the normal functioning of body organs (National Cancer Institute, 2021). This is a multifaceted problem in itself because, with the many forms of cancer, treatment is rarely effective for long. This is due to several factors, such as treatment resistance, and toxicity caused by chemotherapy or radiation treatment. Furthermore, the ever-rising numbers of cancer cases implies that there is a need for higher level strategies that not only aim at curing the disease but improving health, especially through nutrition.

While the role of antioxidants has been well documented for their preventing and managing cancer effects, it is interesting to note an absence of studies looking into the treatment aspect of star apple (*Chrysophyllum cainito*) in cancer therapy. And even though the previous works illustrate the medicinal properties of star apple, especially its micronutrients such as polyphenols and flavonoids, there are no relevant studies offering a chronological specific influence of them on cancer cells. Most of the research has been based on the benefits of these antioxidants to the general health of an individual, hence the need for cancer-specific studies that seek to explore their possible cytotoxic effect to various types of cancers.

The researchers aim to investigate the anticancer properties of star apple extracts and their potential as adjunct therapies in cancer treatment. This study will contribute to the growing body of knowledge on phytotherapy in cancer management, emphasizing its potential to enhance treatment experiences and improve patient outcomes through comprehensive, integrative approaches.

Statement of the Problem

This study aimed to determine the cytotoxic activity of star apple (*Chrysophyllum cainito*) fruit extract and peel extract and using brine shrimp (*Artemia salina*) lethality assay. Specifically, this study aimed to answer the following:

1. Do star apple (*Chrysophyllum cainito*) fruit extract and peel extract (Treatment A – 10 mL star apple (Chrysophyllum cainito) fruit extract and Treatment B – 10 mL star apple (*Chrysophyllum cainito*)

peel extract, Treatment C (Control)– 10 mL Distilled water) affect the mortality of brine shrimp (*Artemia salina*)?
2. Are there significant differences in the effects of star apple (*Chrysophyllum cainito*) of different treatments of fruit extract and peel extract (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peels extract, Treatment C (Control) – 10 mL Distilled water) based on the mortality rate of brine shrimp (*Artemia salina*)?

Objectives of the Study

The main objective of the study is to determine the cytotoxic activity of star apple (*Chrysophyllum cainito*) fruit extract and peel extract using brine shrimp (*Artemia salina*) lethality assay. Specifically, this study aimed:

1. To determine if the different treatments of star apple (*Chrysophyllum cainito*) fruit extract and peel extract (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peels extract, Treatment C (Control) – 10 mL Distilled water) affect the mortality of brine shrimp (*Artemia salina*).
2. To determine if there are significant differences in the effects of star apple (*Chrysophyllum cainito*) of different treatments of fruit extract and peel extract (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peels extract, Treatment C (Control) – 10 mL Distilled water) based on the mortality rate of brine shrimp (*Artemia salina*).

Hypotheses of the Study

1. The different treatments of star apple (*Chrysophyllum cainito*) fruit extract and peel extract (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peels extract, Treatment C (Control) – 10 mL Distilled water) does not affect the mortality of brine shrimp (*Artemia salina*).
2. There are no significant differences in the effects of star apple (*Chrysophyllum cainito*) of different treatments of fruit extract and peel extract (Treatment A – 10 mL star apple (*Chrysophyllum cainito*) fruit extract, Treatment B – 10 mL star apple (*Chrysophyllum cainito*) peels extract, Treatment C (Control) – 10 mL Distilled water) based on the mortality rate of brine shrimp (*Artemia salina*).

Theoretical Framework of the Study

On the study of Miktas (2022), African star apples are a rich source of natural antioxidants such as flavonoids and vitamin E, C, and A. They prevent oxidative damage of cells and promote heart health. Research shows that African star apples may play an important role in preventing the growth of cancerous cells. African star apples are rich in pectin, a type of fiber, and antioxidants such as beta-carotene and vitamin E. It also has anti-inflammatory properties, which can help prevent cancer growth. On the study of Banti et al. (2021), the toxicity is then determined in terms of the mortality rate of brine shrimp larvae. Brine shrimp assay is a low cost, safe, no required feeding during the assay, while it is requiring only a small amount of the tested agent. According on the study of Islam et al. (2022), extracts from fruits and vegetables that contain fiber, vitamins, minerals, and other natural substances with antioxidant, lipid-lowering, and antiproliferative properties. Used in chemoprevention therapy, these extracts may prevent the development or recurrence of cancer.

Based on the study of Hussain et al. (2023), peels comprise several biologically active compounds, but their nutritional composition and antioxidant potential of different fruit varieties are limited. This study aimed to determine the nutritional composition and antioxidant properties of 12 peels of different fruit varieties such as apples, pomegranates, guavas, strawberries, grapes, and citrus fruits using an ultraviolet-visible (UV-Vis) spectrophotometer, an inductively coupled plasma atomic emission spectroscopy (ICP-AES), and an amino acid analyzer. Fruit variety and its peels have been distinctive variables in selecting genotypes.

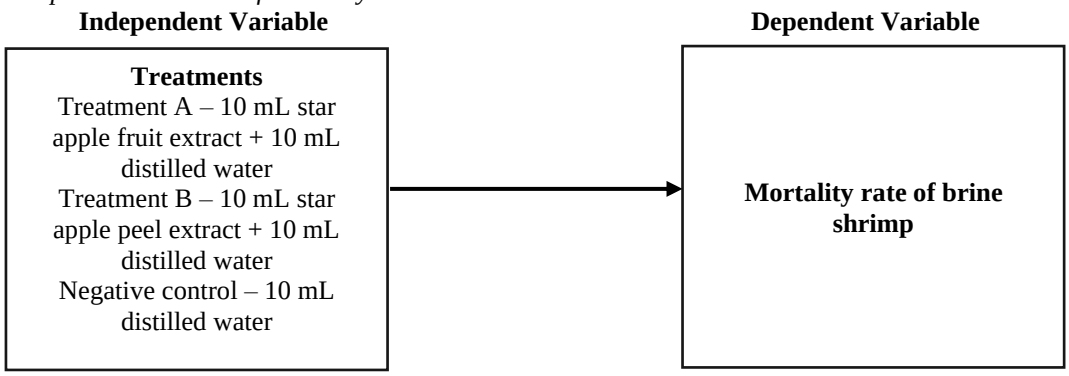
Conceptual Framework of the Study

This study examines the effect of the different treatments of star apple extracts on the mortality rate of brine shrimp. The independent variable consists of three treatment groups: Treatment A (star apple fruit extract with distilled water); Treatment B (star apple peel extract with distilled water); and a negative control group receiving only distilled water. The dependent variable is the mortality rate of brine shrimp, which serves as a bioassay to assess the potential toxicity and anticancer properties of the star apple extracts. This

framework aims to elucidate how the varying treatments impact brine shrimp mortality, providing insights into the extracts' efficacy.

Figure 1

Conceptual Framework of the Study



2.Literature Review

Local Studies and Literature. In the study conducted by Madjos et al. (2019) on the comparative cytotoxic properties of two Carica papaya leaf extract varieties from Mindanao, Philippines, researchers found a renewed interest in phytotherapy for disease treatment. The study revealed a direct relationship between extract concentration and lethality, with maximum mortality rates at 1000 µg/ml and minimum at 10 µg/ml. The 'Sunrise Solo' variety exhibited higher brine shrimp mortality and a lower LC50 value (132 µg/ml) compared to 'Cavite Special' (421 µg/ml), confirming the efficacy of the brine shrimp lethality assay for assessing medicinal plant bioactivity.

Similarly, Lumogdang (2021) explored the cytotoxic potential of ethanolic extracts of Chrysophyllum cainito (star apple) using the brine shrimp lethality bioassay. The Philippines' rich plant biodiversity includes star apples, known for their ethno-medical properties. Extracts were tested at concentrations of 10, 100, 500, and 1000 µg/ml. Results indicated that all extracts exhibited greater activity than the decoction, particularly those using absolute ethanol and a 50:50 ethanol-water mixture, highlighting the potential of C. cainito as an alternative medicine. Further research is needed to identify the specific bioactive compounds responsible for these effects.

Foreign Studies and Literature. The study by Sarah et al. (2017) focused on the brine shrimp lethality assay, a significant tool for the preliminary cytotoxic assay of plant extracts, which determines the effectiveness of various substances in killing laboratory-cultured brine shrimp. Each of the five test tubes was labeled 1–5, containing 10 brine shrimp, 1 mL of seawater, and 1 mL of a prepared solution. Serial dilutions were made from a stock solution with concentrations of 1 mg/mL, 100 µg/mL, 10 µg/mL, and 1 µg/mL. After 24 hours, mortality rates were observed and calculated. The percent lethality of the brine shrimp for each concentration and the control was determined, noting the number of live brine shrimp and overall mortality.

In an investigation by Mahboob (2015) on the cytotoxic activity of hydroalcoholic extracts of medicinal plants using *Artemia salina*, the brine shrimp lethality assay successfully screened for biological activity. After exposing the brine shrimp to various treatments for 24 hours, larvicidal activity was assessed based on percentage larval mortality. Hydroalcoholic extracts from different plant parts were evaluated at concentrations ranging from 1–5000 µg/mL. Out of 17 plants tested, *Veratrum album* Bernh, *Capsicum annuum* L, *Atropa belladonna* L, *Toxicodendron pubescens* Mill, and *Berberis vulgaris* L exhibited potent lethality with LC50 values of 400, 420, 430, 610, and 612 µg/mL, respectively.

Based on research by Konan et al. (2022), the phytochemical screening and toxicity assessment of *Imperata cylindrica* (L.) P. Beauv. (Poaceae) raw extracts used the brine shrimp lethality assay. This study highlighted the importance of assessing herbal medicinal preparations as dietary supplements for disease prevention and alternative medicine, noting that some medicinal plants can be toxic. The study indicated a lack of comprehensive toxicological data available for these plants.

In their study, Doan and Le (2020) reported on *Chrysophyllum cainito*, a tropical fruit tree with numerous health benefits. This species demonstrates strong antioxidant properties both in vitro and in vivo. The

extract exhibits anti-inflammatory responses, enhances wound healing speed, and aids in regulating fat uptake. Additionally, the fruit shows anticancer activity against osteosarcoma. Overall, the aerial parts of *C. cainito* provide substantial health benefits.

Chel-Guerrero et al. (2018) conducted research on the phytochemical profile, toxicity, and pharmacological potential of peels from four tropical fruit species, including *Annona squamosa* and *C. cainito*. Their findings revealed that all extracts contained flavonoids, anthraquinones, and triterpenoids. The two species exhibited the highest total phenolic content, while *M. bijugatus* showed the highest total sugar content. The antioxidant capacities were also notable, indicating the potential of these fruit peels as sources of bioactive compounds.

Oranusi et al. (2015) investigated the antimicrobial activities and chemical compositions of *Chrysophyllum cainito* (star apple) fruit. They identified pathogenic microorganisms present in both pulp and seed, including species from *Bacillus*, *Corynebacterium*, and *Staphylococcus*. The study also noted significant levels of vitamins A and C, along with essential minerals, emphasizing the nutritional value of *C. cainito*.

Asare (2015) analyzed the nutritional and phytochemical constituents of *Chrysophyllum albidum* G. Don. The results indicated varying carbohydrate and protein levels between the peel and pulp, alongside significant phenolic and flavonoid contents. This study contributed valuable data on the fruit's mineral composition and potential health benefits. Arana-Argáez (2017) studied the immunosuppressive effects of methanolic extracts of *C. cainito* leaves on macrophage functions. The results showed that the extract significantly inhibited phagocytosis and decreased the production of pro-inflammatory cytokines in a concentration-dependent manner, suggesting potential immunomodulatory properties.

Moreover, Li (2015) explored the effects of the polyphenolic fraction of *C. cainito* extract on osteosarcoma cells, revealing a high polyphenol content and antioxidant capacity. The extract influenced reactive oxygen species generation and exhibited cytotoxic effects, further emphasizing the potential therapeutic applications of *C. cainito*.

Makinde et al. (2019) investigated the impact of different processing methods on nutrients and anti-nutrient compositions in African star apple kernels. Their findings indicated that certain vitamins were significantly affected by processing methods, while anti-nutritional factors were present in notable amounts.

Lastly, the research by Adekanmi and Olowofoyeku (2020) highlighted the potential and applications of African star apple in Nigeria. The study emphasized the importance of underutilized plants in food and pharmaceuticals, noting the traditional therapeutic uses of various parts of the plant, including leaves, stem, roots, and fruits.

3. RESEARCH METHOD

Research Design

The researchers used a completely randomized design to assign treatments to be applied. A completely randomized design is a research design where the treatments are entirely appointed at random so that each experimental unit has the same chance of receiving any one treatment. Simple random sampling will be used to determine which treatment a cup will be subjected to.

Collection and Identification of Star Apple Plant

The star apple fruit were bought from Bagong Lipunan Public Market 1626 San Roque Ext, Roxas City, Capiz, Philippines. The plants were brought to a plant identification test at the Department of Agriculture and was identified by Mr. Audie B. Belargo, Senior Aquaculturist of the Department of Agriculture of Roxas City.

Preparation of Brine Shrimp

Brine shrimp (*Artemia salina*) cysts were hatched in a shallow rectangular dish (25 cm x 33 cm) half-filled with sea water and was easily hatched from their cyst. An aluminum foil divider with several 0.7 mm holes were used to make unequal compartments. The larger chamber was darkened and covered with aluminum foil and a pinch of cysts sprinkled within. The smaller compartment was exposed to light. And after 48 hours, the phototrophic brine shrimp was collected using pipette from the lighted side.

Figure 2 *Brine Shrimp Eggs*

Preparation of the Extract and Treatments for Experimentation

The collected star apple fruit were washed with tap water and rinsed with distilled water. The star apple fruit were peeled. The extracted star apple fruit were put into a sterile beaker after the star apple fruit has been mixed in a blender. The star apple fruit and peels extract were filtered using a filter paper.

Assessment of Cytotoxic Activity

Small sterile cups are used as container for each sample. Each containing 5 mL of sea water and a drop of yeast suspension (Gloripan, 3 mg in 5mL of sea water) as food. Using a sterilized plastic spoon, 10 Brine Shrimp brine shrimp were transferred into each cup. Using syringe (5mL), different concentrations of star apple fruit extract were added to each cup containing Brine shrimp brine shrimp. The cups were maintained under the exposure of light. The dead brine shrimp were counted with the aid of 3x magnifying glass after 15, 30, 45, and 60 minutes of application. The percent mortality was determined and subjected to statistical analysis.

Data Gathering Process

Using a magnifying glass, the number of dead brine shrimp were counted after 15, 30, 45, and 60 minutes of treatment. The mortality rate was determined and were statistically examined.

Analysis of Data

The Statistical Package for Social Sciences was used to analyze the data. Percentage was computed to determine the mortality rate of the brine shrimp when applied with the different treatments. One-way Analysis of Variance was used to determine if there are significant differences among the results.

Procedural Design

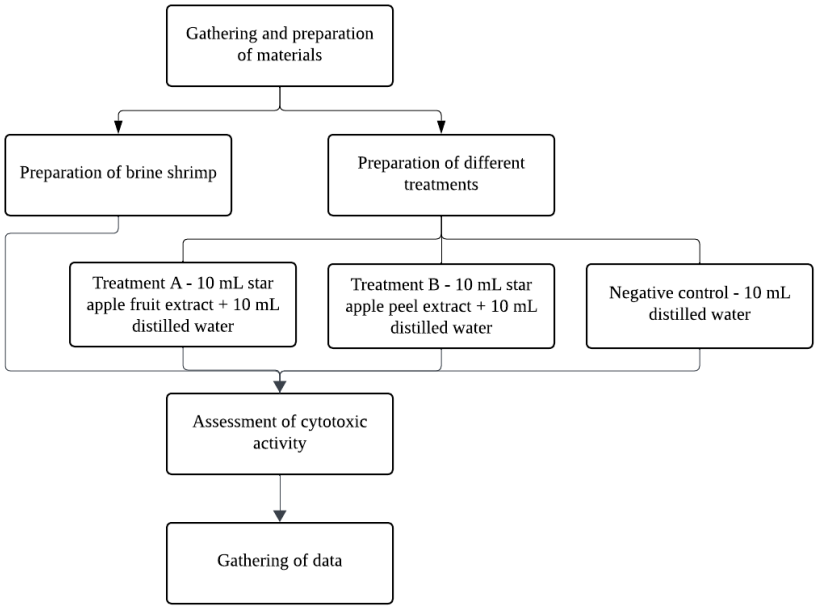


Figure 4 *Procedural Design of the Study*

4. Results

Mortality Rate of Brine shrimp After 15 Minutes of Application of the Different Treatments

Table 1 shows that the brine shrimp applied with treatment B (10 mL star apple peel extract) has the highest average mortality rate of 10%, followed by treatment A (10 mL star apple fruit extract), with an average mortality rate of 6.67%. While with treatment C (10 mL distilled water), the average mortality rate is 0%.

Table 1. ***Mortality Rate of Brine shrimp After 15 Minutes of Application of the Different Treatments***

Treatment	Mortality Rate of Brine shrimp			Average Mortality Rate of Brine shrimp
	R1	R2	R3	
A (10 mL star apple fruit extract)	1 (10%)	0 (0%)	1 (10%)	6.67%
B (10 mL star apple peel extract)	2 (20%)	0 (0%)	1 (10%)	10%
C (10 mL distilled water)	0 (0%)	0 (0%)	0 (0%)	0%

Legend: Treatment A - 10 mL star apple fruit extract; Treatment B -10 mL star apple peel extract; Treatment C – 10 mL distilled water

Mortality Rate of Brine shrimp After 30 Minutes of Application of the Different Treatments

Table 2 shows that the brine shrimp applied with treatment B (10 mL star apple peel extract) has the highest average mortality rate of 13.33%, followed by treatment A (10 mL star apple fruit extract), with an average mortality rate of 10%. While with treatment C (10 mL distilled water), the average mortality rate is 0%.

Table 2. ***Mortality Rate of Brine shrimp After 30 Minutes of Application of the Different Treatments***

Treatment	Mortality Rate of Brine shrimp			Average Mortality Rate of Brine shrimp
	R1	R2	R3	
A (10 mL star apple fruit extract)	1 (10%)	1 (10%)	1 (10%)	10%
B (10 mL star apple peel extract)	3 (30%)	0 (0%)	1 (10%)	13.33%
C (10 mL distilled water)	0 (0%)	0 (0%)	0 (0%)	0%

Legend: Treatment A - 10 mL star apple fruit extract; Treatment B -10 mL star apple peel extract; Treatment C – 10 mL distilled water

Mortality Rate of Brine shrimp After 45 Minutes of Application of the Different Treatments

Table 3 shows that the brine shrimp applied with treatment B (10 mL star apple peel extract) has the highest average mortality rate of 13.33%, followed by treatment A (10 mL star apple fruit extract), with an average mortality rate of 10%. While with treatment C (10 mL distilled water), the average mortality rate is 0%.

Table 3 .*Mortality Rate of Brine shrimp After 45 Minutes of Application of the Different Treatments*

Treatment	Mortality Rate of Brine shrimp			Average Mortality Rate of Brine shrimp
	R1	R2	R3	
A (10 mL star apple fruit extract)	1 (10%)	2 (20%)	1 (10%)	16.66%
B (10 mL star apple peel extract)	3 (30%)	2 (20%)	3 (30%)	26.67%
C (10 mL distilled water)	0 (0%)	0 (0%)	0 (0%)	0%

Legend: Treatment A - 10 mL star apple fruit extract; Treatment B -10 mL star apple peel extract; Treatment C – 10 mL distilled water

Mortality Rate of Brine shrimp After 60 Minutes of Application of the Different Treatments

Table 4 shows that the brine shrimp applied with treatment B (10 mL star apple peel extract) has the highest average mortality rate of 26.67%, followed by treatment A (10 mL star apple fruit extract), with an average mortality rate of 16.66%. While with treatment C (10 mL distilled water), the average mortality rate is 0%. According to the study of Lumogdang (2021), the mortality rates of the brine shrimp were observed after 6 and 24 hours. The results showed that all the prepared extracts exhibited active biological activities with the ethanolic and hydro-ethanolic extracts exhibiting greater activities compared to the decoction.

Table 4. ***Mortality Rate of Brine shrimp After 60 Minutes of Application of the Different Treatments***

Treatment	Mortality Rate of Brine shrimp			Average Mortality Rate of Brine shrimp
	R1	R2	R3	
A (10 mL star apple fruit extract)	1 (10%)	2 (20%)	1 (10%)	16.66%
B (10 mL star apple peel extract)	3 (30%)	2 (20%)	3 (30%)	26.67%
C (10 mL distilled water)	0 (0%)	0 (0%)	0 (0%)	0%

Legend: Treatment A - 10 mL star apple fruit extract; Treatment B -10 mL star apple peel extract; Treatment C – 10 mL distilled water

Significant Difference in the Mortality Rate of Brine shrimp After Application of the Different Treatments

Table 5 shows that there are no significant differences ($p > 0.05$) in the mortality rate of brine shrimp after 15 minutes and 30 minutes of application of star apple (*Chrysophyllum cainito*) fruit extract, peel extract, and distilled water. On the other hand, there are significant differences ($p < 0.05$) in the mortality rate of brine shrimp after 45 minutes and 60 minutes of application.

Table 5. ***Summary Table of the One-Factor ANOVA of the Effects of Star Apple (Chrysophyllum cainito) Fruit Extract, Peel extract, and Distilled Water in 15 Minutes, 30 Minutes, 45 Minutes, and 60 Minutes***

	Sum of Squares	df	Mean Square	F	Sig.
After 15 minutes					
Between Groups	155.56	2	77.78	1.75	.25
Within Groups	266.67	6	44.44		
Total	422.22	8			
After 30 minutes					
Between Groups	288.89	2	144.44	1.86	.23
Within Groups	466.67	6	77.78		
Total	755.56	8			
After 45 minutes					
Between Groups	1066.67	2	533.33	24.00	.001*
Within Groups	133.33	6	22.22		
Total	1200.00	8			
After 60 minutes					
Between Groups	1066.67	2	533.33	24.00	.001*
Within Groups	133.33	6	22.22		
Total	1200.00	8			

* With significant difference at 5% level of significance

DISCUSSION

The results of this research shed light on the cytotoxicity of *Chrysophyllum cainito* extracts, notably the peel extract, against brine shrimp. The mortality rates observed with both the peel and fruit extracts, though low, are demonstrative of some biological activity, especially when one considers the control (distilled

water), where no mortality was recorded. These findings imply that the star apple peel can be an excellent source of bioactive compounds that can be exploited in medicine or pharmacy. Even though the mortality rates are far from the 50% lethality (LC50) limit, showing that the extracts are safe and not very toxic, the time-dependent increase in mortality suggests that the bioactive agents contained within the extracts have some possible lasting effects.

The potential insights that can be gained from this research can be considered in how it evaluated mortality at several different time stages A clearer way of contrast between the treatment groups is made possible by utilizing distilled water in the control groups. Debates are suggested by some of the factors which constrain the study, including low mortality rates which cast doubt on the usefulness of the star apple extracts for high toxicity uses. There may be restrictions in terms of how much change can be applied to the extraction method and the power of the concentrations used.

This study found out that, even though the fruit and peel extracts of *Chrysophyllum cainito* demonstrate cytotoxicity, their low therapeutic index may indicate that they are suitable for medicinal application. The contributions in this study are therefore filling the gap of assertions on the extracts of tropical fruits, as well as stressing on the extractive processes in the provision of active assays. Lastly, future studies should aim at increasing extraction effectiveness and investigating alternative bioassays in order to exploit these compounds more therapeutically.

5. Conclusions

The results of this study show that the different treatments of *Chrysophyllum cainito* fruit and peel extracts do not affect brine shrimp mortality, and that there are no significant differences between the treatments. While mortality rates were observed with both the fruit and peel extracts, none reached the 50% LC threshold, indicating that the extracts are not highly cytotoxic. Treatment B (peel extract) consistently showed a higher mortality rate than Treatment A (fruit extract), and there were significant differences in mortality after 45 and 60 minutes, suggesting that time and extract composition may influence cytotoxic activity. However, the low mortality rates overall suggest limited effectiveness of these extracts in inducing significant lethality in Artemia salina.

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