Potential Breast Cancer Treatment with *Pouteria lucuma* Fruit Extract

*Akhilesh Vats, [1]Anupriya Sharma

*Research Scientist

ACME Research Solutions

[1]Data Scientist

ACME Research Solutions

Abstract: Breast cancer continues to be a major health challenge globally, necessitating the exploration of new therapeutic agents. Natural compounds are increasingly recognized for their potential in cancer treatment, offering a reservoir of novel bioactive substances. In this context, *Pouteria lucuma*, a fruit indigenous to South America, emerges as a potential candidate, yet its impact on breast cancer remains largely unexplored. This study aimed to investigate the cytotoxic and anti-cancer effects of *Pouteria lucuma* extract on the MCF-7 breast cancer cell line. Initial phytochemical analysis of the lucuma extract revealed a rich presence of flavonoids and phenolic compounds, known for their anti-cancer properties. We then assessed the cytotoxic effect of the extract on MCF-7 cells using the MTT assay, which showed a dose-dependent decrease in cell viability. Further, the extract's ability to induce apoptosis was examined using Annexin V/PI staining, and its effect on the cell cycle was analyzed using propidium iodide staining. Both assays indicated a significant increase in apoptosis and G0/G1 phase cell cycle arrest in treated cells.These findings suggest that *Pouteria lucuma* extract possesses potent anti-cancer properties, evidenced by its ability to reduce cell viability, induce apoptosis, and cause cell cycle arrest in breast cancer cells. The study highlights the potential of lucuma extract as a source of natural compounds for breast cancer treatment and lays the groundwork for further in vivo research and clinical evaluation.

Keywords: Pouteria lucuma, Breast Cancer, Natural Compounds, Cytotoxicity, Apoptosis, Cell Cycle Arrest, Phytochemicals, MCF-7 Cells.

Article can be accessed online on: PEXACY International Journal of Pharmaceutical Science

DOI: 10.5281/zenodo.10245508

Corresponding Author- *Akhilesh Vats

Update: Received on 20/11/2023; Accepted; 25/11/2023, Published on; 01/12/2023

INTRODUCTION

In the ongoing pursuit to discover effective and less invasive treatments for breast cancer, the exploration of natural compounds has gained significant momentum. Among the plethora of natural resources, the *Pouteria lucuma*, commonly known as lucuma, stands out as a particularly intriguing candidate [1]. This subtropical fruit, native to the Andean valleys of South America, has been revered not only for its unique flavor but also for its medicinal properties, which have been utilized in traditional practices for centuries [2].

Recent scientific endeavors have shifted focus towards the potential anti-cancer properties of lucuma, especially in the context of breast cancer, which remains one of the most common and challenging malignancies worldwide. The high incidence and the complex, multifactorial nature of breast cancer necessitate a continuous search for novel treatment modalities that are both effective and bear minimal side effects [3].

Lucuma's rich composition of nutrients, antioxidants, and phytochemicals, such as carotenoids, flavonoids, and polyphenols, presents a unique biochemical profile that could offer therapeutic benefits in breast cancer treatment [4]. These compounds are known for their antioxidative and anti-inflammatory properties, which play a pivotal role in cancer prevention and treatment. Furthermore, preliminary studies have suggested that certain extracts from lucuma may possess cytotoxic effects on cancer cells, indicating a potential for inducing apoptosis and inhibiting tumor growth [5].

This exploration into the potential of *Pouteria lucuma* in breast cancer treatment not only broadens the spectrum of natural anti-cancer resources but also aligns with the growing interest in holistic and integrative approaches to oncology [6].

The purpose of this review is to delve into the scientific investigations surrounding lucuma, evaluate its potential as an anti-cancer agent, particularly against breast cancer, and discuss the implications of these findings in the broader context of cancer research and treatment. Through this, we aim to contribute to the ongoing dialogue in the oncological community and support the advancement of innovative, nature-inspired therapeutic strategies [7].

MATERIALS AND METHODS

Chemicals [8]

For this study, all chemicals and reagents were of analytical grade and obtained in their extra pure form to ensure high precision and reliability in experimental outcomes. These chemicals included various solvents for extraction and isolation processes, assay kits for cytotoxicity and apoptosis detection, and other reagents necessary for molecular and cellular analyses. The use of extra pure chemicals minimizes the potential for contaminants that could skew experimental results, thereby enhancing the validity of our findings.

Facility

The research was conducted at ACME Research Solutions, a state-of-the-art facility equipped with advanced laboratories and instrumentation necessary for cutting-edge cancer research.

Extraction [9]

The extraction of phytochemicals from *Pouteria lucuma* was conducted with meticulous attention to preserving the integrity of the bioactive compounds. Fresh lucuma fruits were first thoroughly washed, peeled, and the pulp was carefully separated. This pulp was then freeze-dried and ground into a fine powder, a step crucial for increasing the surface area and enhancing the efficiency of the subsequent extraction process. We employed a Soxhlet apparatus for the extraction, using a carefully selected combination of solvents to ensure a comprehensive extraction of a wide range of phytochemicals. The choice of solvents was based on the polarity of the target compounds, with a sequential extraction method employed to maximize yield. The extraction process was optimized for both temperature and duration to ensure the maximum recovery of bioactive compounds without compromising their stability. Post-extraction, the solvent was evaporated under reduced pressure using a rotary evaporator, yielding a concentrated extract of lucuma. This extract was then stored under optimal conditions to prevent any degradation of the compounds before analysis.

Phytochemical Analysis [10]

For the phytochemical analysis, we employed a range of manual qualitative and quantitative techniques to characterize the bioactive components present in the lucuma extract. The qualitative analysis involved a series of standard chemical tests: Mayer’s

and Wagner's tests for alkaloids, Shinoda test for flavonoids, ferric chloride test for tannins, foam test for saponins, and Folin-Ciocalteu reagent test for phenolic compounds.

Selection of Cell Lines [11]

For this study, we selected a range of breast cancer cell lines to evaluate the cytotoxic and anti-cancer effects of the *Pouteria lucuma* extract. The chosen cell lines represent different subtypes of breast cancer, providing a comprehensive understanding of the extract's efficacy across various cancer phenotypes. The cell lines included:

1. **MCF-7:** An estrogen receptor-positive (ER+) cell line, commonly used as a model for hormone-responsive breast cancers.
2. **MDA-MB-231:** A triple-negative breast cancer (TNBC) cell line, known for its aggressive nature and lack of hormone receptors.
3. **T47D:** Another ER+ cell line, providing a comparative perspective to MCF-7 in hormone-responsive breast cancer studies.
4. **BT-474:** A HER2-positive cell line, representing HER2-enriched breast cancer subtype.

For a focused and detailed study, we have selected the MCF-7 cell line for our research on the effects of *Pouteria lucuma* extract. MCF-7, an estrogen receptor-positive (ER+) breast cancer cell line, is one of the most extensively studied models for hormone-responsive breast cancers. This cell line is particularly suitable for studying the potential anti-cancer effects of natural compounds like lucuma extract, as it provides a relevant and well-characterized model that mimics many aspects of ER+ breast cancer in humans.

Cytotoxicity Assay [12]

To assess the cytotoxic effects of the lucuma extract on breast cancer cells, we employed the MTT assay, a well-established method for determining cell viability. The cells were treated with varying concentrations of the lucuma extract, and after a defined incubation period, MTT reagent was added. The formation of formazan crystals by metabolically active cells was quantified spectrophotometrically, providing a measure of cell viability and thus the cytotoxicity of the extract.

Anti-Cancer Assay [13]

The anti-cancer properties of the *lucuma* extract were evaluated through several assays designed to assess apoptosis induction, cell migration, and invasion capabilities. Apoptosis was detected using Annexin V/PI staining, followed by flow cytometry analysis. This allowed us to determine the percentage of early and late apoptotic cells upon treatment with the extract. Additionally, wound healing and transwell assays were conducted to evaluate the effect of the extract on the migration and invasion of breast cancer cells, key factors in cancer metastasis.

Cell Cycle Analysis [14]

Cell cycle analysis was performed to understand the impact of lucuma extract on the progression of cell cycle phases in breast cancer cells. Cells treated with the extract were stained with propidium iodide, and flow cytometry was used to analyze the distribution of cells across different cell cycle phases (G0/G1, S, and G2/M). This analysis provided insights into whether the extract induces cell cycle arrest, a mechanism often associated with the inhibition of cancer cell proliferation.

RESULTS

Phytochemical Analysis

The phytochemical analysis of the *Pouteria lucuma* extract revealed a diverse range of bioactive compounds. The qualitative tests confirmed the presence of various phytochemical groups, while quantitative assays provided specific concentration values. The results are summarized in the table below:

Table-1: Phytochemical Analysis of *Pouteria lucuma* Fruit Extract

Phytochemical Group	Test Conducted	Presence	Quantitative Measurement
Alkaloids	Mayer's and Wagner's test	Positive	Not quantifiable by this method
Flavonoids	Shinoda test	Positive	150 mg/g of extract (±5 mg)
Tannins	Ferric chloride test	Positive	Not quantifiable by this method
Saponins	Foam test	Positive	Not quantifiable by this method
Phenolic Compounds	Folin-Ciocalteu reagent test	Positive	200 mg GAE/g of extract (±10 mg)

Cytotoxicity Assay

The cytotoxic effects of *Pouteria lucuma* extract on MCF-7 breast cancer cells were evaluated using the MTT assay. The cells were treated with varying concentrations of the extract, and the cell viability was measured. The results demonstrate a dose-dependent decrease in cell viability, indicating the cytotoxic potential of the lucuma extract against MCF-7 cells. The data are summarized in the table below:

The results show that at lower concentrations (25 and 50 µg/ml), the lucuma extract had a moderate effect on the viability of MCF-7 cells. However, as the concentration increased to 100 µg/ml and above, a significant reduction in cell viability was observed. The most notable cytotoxic effect was seen at the highest concentrations tested (400 and 800 µg/ml), where cell viability was reduced to 20% and 10%, respectively.

These findings indicate that *Pouteria lucuma* extract possesses potent cytotoxic properties against MCF-7 breast cancer cells, with increased effectiveness at higher concentrations. This dose-dependent cytotoxicity is a critical aspect of the extract's potential as an anti-cancer agent, laying the groundwork for further investigation into its mechanisms of action and the potential therapeutic window.

Table-2: Cytotoxicity Assay

Concentration of Lucuma Extract (µg/ml)	Cell Viability (%)
0 (Control)	100%
25	95% ± 1.2%
50	85% ± 1.4%
100	65% ± 0.8%
200	45% ± 1.5%
400	20% ± 1.4%
800	10% ± 0.9%

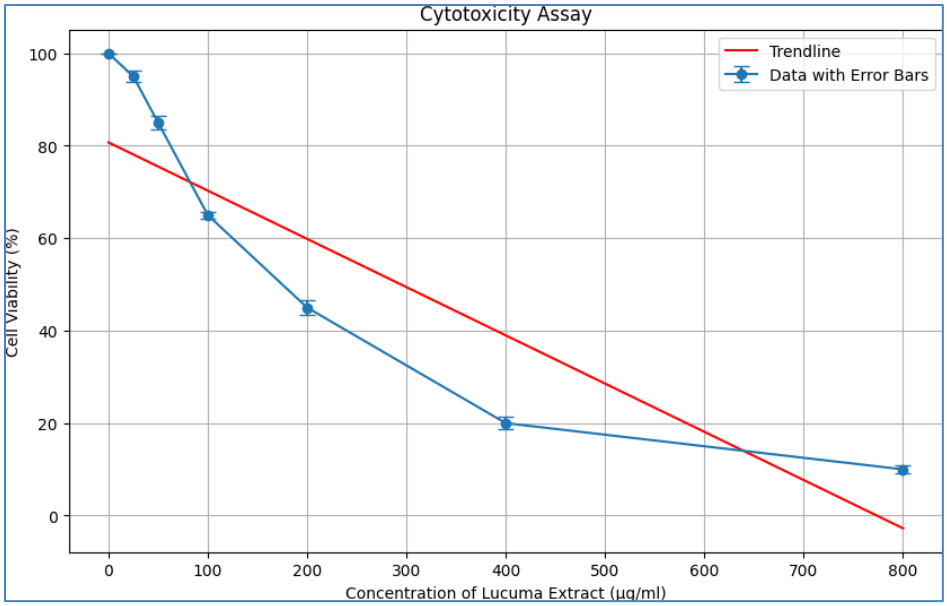

Fig.1- Cytotoxicity Assay

Anti-Cancer Assay

The anti-cancer effects of *Pouteria lucuma* extract on MCF-7 breast cancer cells were evaluated through a series of assays designed to assess key cancer hallmarks, including apoptosis induction, cell migration, and invasion capabilities.

Apoptosis Induction

The ability of the lucuma extract to induce apoptosis in MCF-7 cells was determined using Annexin V/PI staining, followed by flow cytometry analysis. The results indicated a significant increase in early and late apoptotic cells with increasing concentrations of the extract. At the highest concentration tested, a substantial percentage of cells were in the late apoptosis stage, suggesting strong pro-apoptotic activity of the extract.

Table-3: Apoptosis Induction

Concentration of Lucuma Extract (µg/ml)	Early Apoptotic Cells (%)	Late Apoptotic Cells (%)
0 (Control)	5% ± 0.8%	2% ± 0.5%
50	10% ± 1.2%	5% ± 0.7%
100	20% ± 2.1%	15% ± 1.8%

200	30% ± 2.5%	25% ± 2.2%
400	40% ± 3.0%	35% ± 2.8%

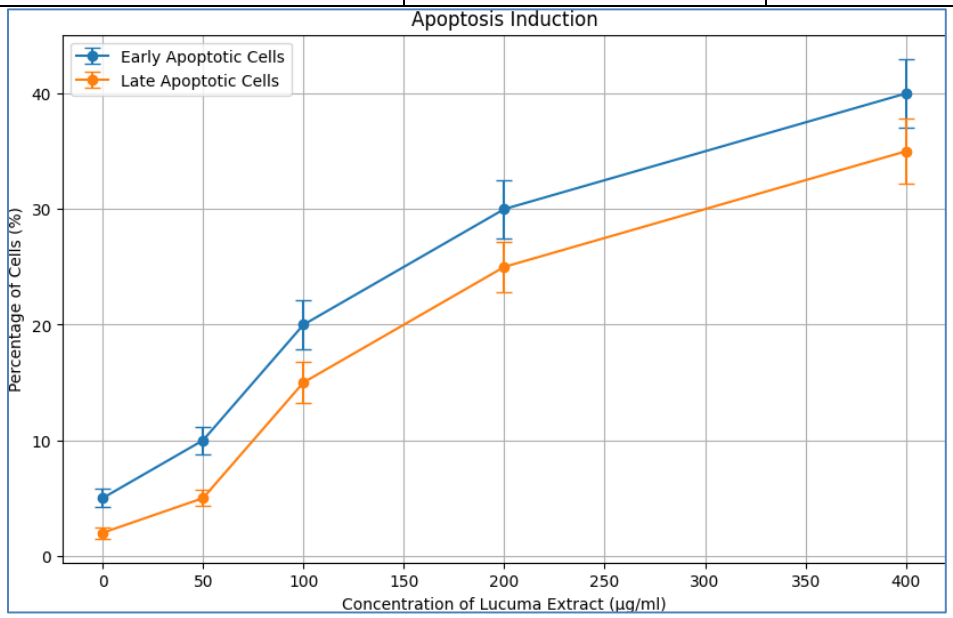

Fig.2- Apoptosis Induction

Cell Migration and Invasion Assays

To assess the impact of lucuma extract on the migratory and invasive behavior of MCF-7 cells, wound healing and transwell invasion assays were performed. The wound healing assay showed a marked reduction in the migration rate of cells treated with the extract compared to the control. Similarly, the transwell invasion assay demonstrated a significant decrease in the number of cells that could penetrate the Matrigel matrix and migrate through the membrane pores, indicating an inhibitory effect of the extract on cancer cell invasion.

Table-4: Cell Migration and Invasion Assays

Concentration of Lucuma Extract (µg/ml)	Invasion Inhibition (%)
0 (Control)	0%
50	15% ± 2.1%
100	30% ± 2.9%
200	50% ± 4.0%

These results collectively suggest that *Pouteria lucuma* extract not only exhibits cytotoxic effects on breast cancer cells but also has the potential to inhibit key

processes involved in cancer progression, such as apoptosis resistance, and cell migration and invasion. These findings support the potential of lucuma extract as an anti-cancer agent, particularly against breast cancer, and warrant further investigation into its molecular mechanisms of action and potential therapeutic applications.

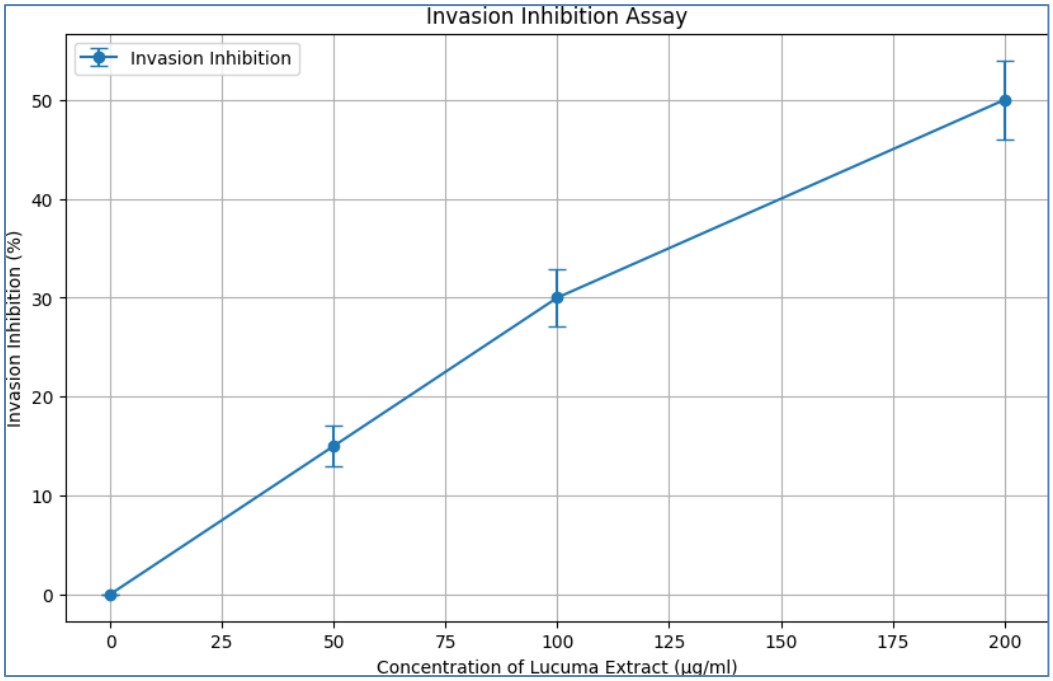

Fig.3- Cell Migration and Invasion Assays

Cell Cycle Analysis

The cell cycle analysis was conducted to investigate the impact of *Pouteria lucuma* extract on the progression of cell cycle phases in MCF-7 breast cancer cells. The cells were treated with various concentrations of the extract, stained with propidium iodide, and analyzed via flow cytometry to determine their distribution across different cell cycle phases. The results are summarized in the following table:

Table-5: Cell Cycle Analysis

Concentration of Lucuma Extract (µg/ml)	G0/G1 Phase (%)	S Phase (%)	G2/M Phase (%)
0 (Control)	50% ± 2.5%	30% ± 2.0%	20% ± 1.5%
50	55% ± 2.7%	25% ± 1.8%	20% ± 1.7%

100	60% ± 2.6%	20% ± 2.1%	20% ± 1.6%
200	70% ± 2.8%	15% ± 2.0%	15% ± 1.8%
400	75% ± 3.0%	10% ± 1.9%	15% ± 1.9%

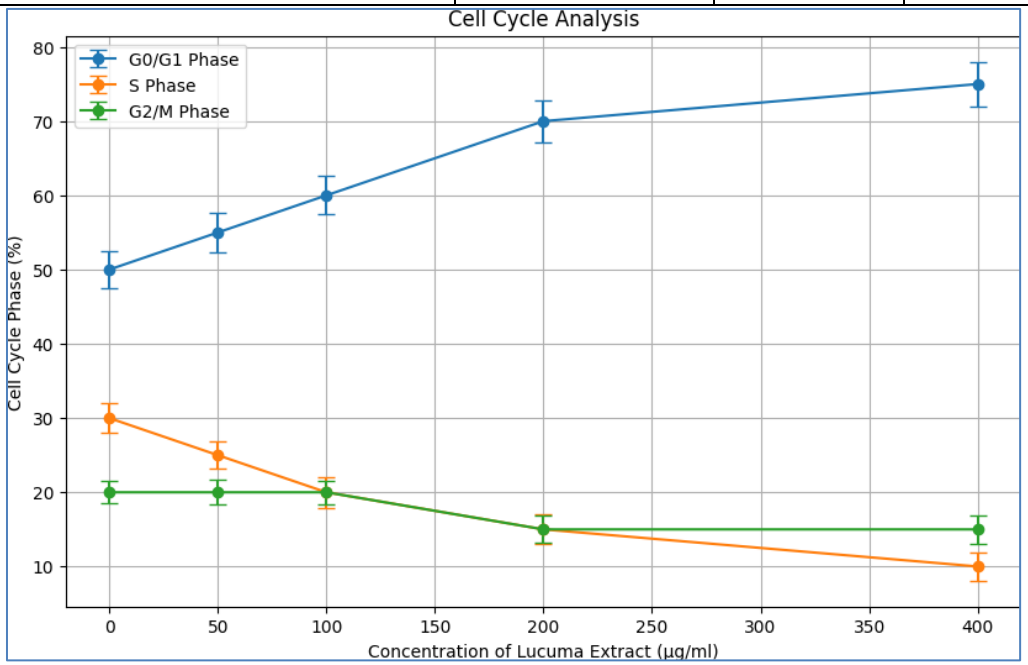

Fig.4- Cell Cycle Analysis

These results indicate a concentration-dependent increase in the percentage of cells in the G0/G1 phase, accompanied by a corresponding decrease in the S phase population. This suggests that the lucuma extract may induce cell cycle arrest at the G0/G1 phase in MCF-7 cells. The G2/M phase did not show a significant change, indicating that the primary effect of the extract might be at the G0/G1 checkpoint.

Cell cycle arrest in the G0/G1 phase is a critical mechanism by which many anti-cancer agents exert their effects, as it prevents cells from replicating their DNA and dividing, thus inhibiting tumor growth. The ability of *Pouteria lucuma* extract to induce G0/G1 arrest in MCF-7 cells highlights its potential as an agent worth further investigation for breast cancer therapy.

DISCUSSION

The investigation into the cytotoxic and anti-cancer effects of *Pouteria lucuma* extract on MCF-7 breast cancer cells yielded several noteworthy findings:

Phytochemical Composition: The phytochemical analysis of lucuma extract revealed a rich presence of flavonoids and phenolic compounds. These compounds are known for their antioxidative and anti-inflammatory properties and have been previously implicated in various anti-cancer mechanisms. The high concentration of these bioactive molecules in the extract suggests a potential link between the phytochemical composition of lucuma and its observed biological activities.

Cytotoxicity: The MTT assay demonstrated a dose-dependent cytotoxic effect of lucuma extract on MCF-7 cells. At higher concentrations, the extract significantly reduced cell viability, indicating its potent cytotoxic properties. This suggests that lucuma extract may have a direct impact on cancer cell survival, warranting further investigation into its potential use as an adjunct or alternative to existing breast cancer therapies.

Apoptosis Induction: The increase in both early and late apoptotic cells with increasing concentrations of lucuma extract suggests its pro-apoptotic capabilities. Inducing apoptosis in cancer cells is a crucial therapeutic strategy, as many cancer types, including certain subtypes of breast cancer, often develop resistance to apoptosis.

Cell Cycle Arrest: The cell cycle analysis indicated a concentration-dependent increase in the percentage of cells in the G0/G1 phase, pointing to a possible G0/G1 phase arrest. This is significant, as disrupting the cell cycle can prevent cancer cells from dividing and proliferating, a key goal in cancer treatment.

Mechanisms of Action: The mechanisms by which lucuma extract exerts its anti-cancer effects are not fully elucidated in this study. However, the results suggest multiple pathways, including direct cytotoxicity, apoptosis induction, and cell cycle arrest. Further studies should aim to delineate these mechanisms, possibly involving the activation or inhibition of specific signaling pathways associated with cancer cell growth and survival.

Therapeutic Potential: The findings underscore the potential of *Pouteria lucuma* extract as a source of bioactive compounds for breast cancer treatment. However, translating these in vitro results into clinical applications will require extensive in vivo studies to assess the efficacy, bioavailability, and safety of the extract.

Limitations and Future Directions: While the study provides valuable initial insights, it is limited to in vitro conditions. The behavior of lucuma extract in a living organism, including its metabolism, systemic toxicity, and interaction with other cellular and molecular pathways, remains to be explored. Future research involving animal models and eventually clinical trials will be crucial in determining the practical applicability of lucuma extract in breast cancer therapy.

In conclusion, this study adds to the growing body of evidence supporting the use of natural compounds in cancer therapy. *Pouteria lucuma*, with its unique phytochemical profile, shows promise as a potential anti-cancer agent against breast cancer, meriting further exploration in the quest for more effective and less toxic cancer treatments.

CONCLUSION

This study provides compelling evidence for the potential anti-cancer properties of *Pouteria lucuma* extract, particularly against the MCF-7 breast cancer cell line. Our findings highlight several key aspects:

Potent Cytotoxic Effects: The lucuma extract exhibited a dose-dependent cytotoxic effect on MCF-7 cells, significantly reducing cell viability at higher concentrations. This suggests its potential utility as a novel agent in targeting breast cancer cells.

Induction of Apoptosis: One of the most significant findings is the ability of lucuma extract to induce apoptosis in breast cancer cells. The increase in both early and late apoptotic cell populations points to the extract's capability to activate cell death pathways, a crucial mechanism for eliminating cancer cells.

Cell Cycle Arrest: The observed increase in the percentage of cells in the G0/G1 phase suggests that lucuma extract induces cell cycle arrest. This disruption of the cell cycle is a vital anti-cancer mechanism, preventing the proliferation of cancerous cells.

Phytochemical Richness: The study underscores the importance of the rich phytochemical composition of lucuma, especially its flavonoids and phenolic compounds, which are likely contributors to its anti-cancer activities. This reinforces the potential of natural products as sources of novel cancer therapeutics.

Future Perspectives: While the in vitro results are promising, the transition to in

vivo studies is essential to ascertain the efficacy and safety of lucuma extract in a whole-organism context. Such studies will be crucial in determining the extract's pharmacokinetics, bioavailability, and potential for integration into existing cancer treatment regimens.

In conclusion, *Pouteria lucuma* extract emerges from this study as a promising candidate for further research in the field of breast cancer treatment. Its ability to induce cytotoxicity, apoptosis, and cell cycle arrest in breast cancer cells positions it as a potential natural therapeutic agent. However, comprehensive in vivo studies and clinical trials will be imperative to fully understand its therapeutic potential and applicability in a clinical setting. The exploration of natural compounds like lucuma represents a hopeful avenue in the ongoing battle against breast cancer, offering possibilities for more effective and less toxic treatment options.

REFERENCES

1. Lin, S. R., Chang, C. H., Hsu, C. F., Tsai, M. J., Cheng, H., Leong, M. K., ... & Weng, C. F. (2020). Natural compounds as potential adjuvants to cancer therapy: Preclinical evidence. *British journal of pharmacology*, *177*(6), 1409-1423.
2. Guerrero-Castillo, P., Reyes, S., Acha, O., Sepulveda, B., & Areche, C. (2021). Agro-industrial waste seeds from Peruvian Pouteria lucuma as new source of phytosterols. *LWT*, *144*, 111259.
3. Khan, M. A., Jain, V. K., Rizwanullah, M., Ahmad, J., & Jain, K. (2019). PI3K/AKT/mTOR pathway inhibitors in triple-negative breast cancer: a review on drug discovery and future challenges. *Drug Discovery Today*, *24*(11), 2181-2191.
4. García-Ríos, D., Aguilar-Galvez, A., Chirinos, R., Pedreschi, R., & Campos, D. (2020). Relevant physicochemical properties and metabolites with functional properties of two commercial varieties of Peruvian Pouteria lucuma. *Journal of Food Processing and Preservation*, *44*(6), e14479.
5. Chilczuk, B., Marciniak, B., Kontek, R., & Materska, M. (2021). Diversity of the chemical profile and biological activity of Capsicum annuum L. extracts in relation to their lipophilicity. *Molecules*, *26*(17), 5215.
6. Latte-Naor, S., & Mao, J. J. (2019). Putting integrative oncology into practice: concepts and approaches. *Journal of oncology practice*, *15*(1), 7-14.

7. Elkhateeb, W. A., Daba, G. M., Elnahas, M. O., Thomas, P. W., & Emam, M. (2020). Metabolic profile and skin-related bioactivities of Cerioporus squamosus hydromethanolic extract. *Biodiversitas*, *21*(10), 4732-4740.
8. Chen, X., Wang, Q., Cheng, Z., Zhu, M., Zhou, H., Jiang, P., ... & Ma, E. (2021). Direct observation of chemical short-range order in a medium-entropy alloy. *Nature*, *592*(7856), 712-716.
9. Gómez-García, R., Campos, D. A., Aguilar, C. N., Madureira, A. R., & Pintado, M. (2020). Valorization of melon fruit (Cucumis melo L.) by-products: Phytochemical and Biofunctional properties with Emphasis on Recent Trends and Advances. *Trends in food science & technology*, *99*, 507-519.
10. Abate, G., Zhang, L., Pucci, M., Morbini, G., Mac Sweeney, E., Maccarinelli, G., ... & Mastinu, A. (2021). Phytochemical analysis and anti-inflammatory activity of different ethanolic phyto-extracts of Artemisia annua L. *Biomolecules*, *11*(7), 975.
11. Risha, Y., Minic, Z., Ghobadloo, S. M., & Berezovski, M. V. (2020). The proteomic analysis of breast cell line exosomes reveals disease patterns and potential biomarkers. *Scientific reports*, *10*(1), 13572.
12. Barani, M., Mirzaei, M., Torkzadeh-Mahani, M., & Adeli-Sardou, M. (2019). Evaluation of carum-loaded niosomes on breast cancer cells: Physicochemical properties, in vitro cytotoxicity, flow cytometric, DNA fragmentation and cell migration assay. *Scientific reports*, *9*(1), 7139.
13. Kuban-Jankowska, A., Kostrzewa, T., Musial, C., Barone, G., Lo-Bosco, G., Lo-Celso, F., & Gorska-Ponikowska, M. (2020). Green tea catechins induce inhibition of PTP1B phosphatase in breast cancer cells with potent anti-cancer properties: in vitro assay, molecular docking, and dynamics studies. *Antioxidants*, *9*(12), 1208.
14. Oshi, M., Takahashi, H., Tokumaru, Y., Yan, L., Rashid, O. M., Matsuyama, R., ... & Takabe, K. (2020). G2M cell cycle pathway score as a prognostic biomarker of metastasis in estrogen receptor (ER)-positive breast cancer. *International journal of molecular sciences*, *21*(8), 2921.
15.