Cytotoxic Properties Of Two Varieties Of *Musa acuminata* Fruit: An *In-vitro* Study

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Abstract -

Background: *Musa acuminata* peels contain bioactive compounds such as flavonoids, tannins, alkaloids, glycosides, and terpenoids, which are known for their antibacterial, antihypertensive, antidiabetic, antioxidant, and anti-inflammatory properties. Despite these benefits, the cytotoxic properties of banana peels remain underexplored, particularly in dentistry. This study aims to evaluate the cytotoxic effects of banana peel extracts to determine their safe usage and potential therapeutic applications.

Materials and Methods: Six-well ELISA plates were filled with 10–12 ml of saline water, and 10 nauplii were added to each well. Banana peel extracts were introduced in concentrations of 20 µL, 40 µL, 60 µL, 80 µL, and 100 µL. The plates were incubated for 24 hours to assess the viability of the nauplii at varying concentrations.

Results: The cytotoxic activity assay demonstrated a concentration-dependent effect on nauplii viability. Lower concentrations (5 µg/ml, 10 µg/ml, 20 µg/ml, and 40 µg/ml) maintained high viability, whereas higher concentrations (80 µg/ml) significantly reduced viability. Control groups showed consistent survival rates, underscoring the potential cytotoxicity of higher concentrations.

Conclusion: The study highlights the medicinal potential of *Musa acuminata* peels, owing to their bioactive compounds. The findings reveal a concentration-dependent cytotoxic effect, emphasizing the need for cautious application in dentistry. This investigation provides valuable insights into optimizing usage levels for therapeutic purposes while minimizing risks, paving the way for further research on their applications in dental care.

Keywords: Banana peel, cytotoxicity, in vitro, dentistry.

Introduction -

Banana, scientifically known as *Musa acuminata*, extends beyond its role as a popular fruit to unveil a treasure trove of bioactive compounds within its peels. The identification of flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids in banana peels opens avenues for

exploration into their untapped medicinal potential [1]. These phytochemicals have been linked to a spectrum of biological and pharmacological effects, encompassing antibacterial, antihypertensive, antidiabetic, antioxidant, and anti-inflammatory activities [2]. Despite the acknowledgment of these properties, the full medicinal repertoire of banana peels remains largely uncharted, as the focus often centers on a plant's bioactive compounds. Given their high therapeutic potential, including antioxidant, anti-inflammatory, and antimicrobial attributes, this study endeavors to delve into the cytotoxic properties of banana peels [3].

The intricate composition of bioactive substances within banana peels positions them as a promising reservoir for novel medicinal applications. Flavonoids, recognized for their antioxidant capabilities, play a pivotal role in neutralizing free radicals and mitigating oxidative stress [4]. Concurrently, tannins and phlobatannins contribute to the astringent properties of banana peels, suggesting potential applications in wound healing and inflammation modulation. Alkaloids, glycosides, and terpenoids further enhance the therapeutic panorama, showcasing a diverse array of compounds that may exert a cumulative and synergistic effect, amplifying the overall medicinal impact of banana peels [5].

In the realm of dentistry, the multifaceted properties of banana peels hold particular intrigue. The inherent antioxidant properties could aid in combating oxidative damage to oral tissues, while anti-inflammatory attributes may contribute to managing conditions like gingivitis. Additionally, the reported antimicrobial effects of banana peel compounds raise the prospect of novel oral care formulations, potentially addressing microbial challenges within the oral environment [6]. As this study is on the cytotoxic properties of banana peels, it endeavors to bridge the existing knowledge gap, shedding light on the intricate balance between therapeutic benefits and potential adverse effects, especially concerning their application in dental contexts.

Moreover, the emphasis on cytotoxicity assessment aligns with a proactive approach to understanding the safety parameters associated with the use of banana peels in medicinal and dental applications [7]. While the bioactive compounds present offer a spectrum of benefits, a nuanced understanding of their cytotoxic effects becomes imperative for establishing guidelines on safe usage levels. This research not only pioneers exploration into the lesser-explored facets of banana peel properties but also serves as a foundation for informed decision-making regarding the integration of this natural resource into dental healthcare practices.By scrutinizing the impact on cell viability, this research aims to gauge optimal usage levels and explore potential applications in the field of dentistry, unraveling new dimensions of the banana's medicinal properties.

Materials and methods -

The present study does not require ethical clearance by the Institutional Human Ethical Committee as it is an invitro study. The in-vitro investigations were conducted in the Gold Lab, Saveetha Dental College and Hospitals, Chennai, India. The red banana and rasthali fruits were procured from a local fruit and vegetable market in Chennai, India.

Preparation of extract:

Before the peel was prepared for aqueous and alcoholic extracts, the unripe banana fruit of Musa acuminata (Red banana and Rasthali) was separated, cleaned, and dried. After being maintained for 48 hours at 40 degrees centigrade in a hot air oven, the peels were ground into powder. Five grams of dried peel powder from unripe red bananas and rasthali were dissolved in 25 ml of distilled water to create the aqueous extract. A similar process was used to create the alcoholic extract. Alcoholic extract was produced by heating ethanol, an organic solvent, to a temperature of 100 degrees Celsius for 30 minutes. The two extracts were then combined to produce aqueous alcoholic extract. Cotton plugs were inserted on top of the conical flasks holding the extract to stop evaporation. In an orbital shaker, the extract was shaken for 24 hours at 250 rpm. They were filtered twice once with muslin cloths and once with filter paper after being shaken all night. The resultant extracts were stored at 4°C. The preparation of the extract is depicted in **Figure 1**.

Cytotoxic activity:

Cytotoxic activity was determined by brine shrimp lethality assay. 2g of iodine free salt was weighed and dissolved in 200ml of distilled water. 6 well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (20µL,40 µL,60 µL.80 µL,100 µL). Then the banana peel were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the ELISA plates were observed and noted for number of live nauplii's present and calculated by using following formula,

number of dead nauplii/number of dead nauplii+number of live nauplii×100

Results -

In the conducted experiment, various concentrations of red banana were administered to observe their impact on the survival of nauplii over a two-day period. On Day 1, all concentrations, ranging from 5 µg/ml to 80 µg/ml, exhibited comparable results with 100 live nauplii each. However, on Day 2, a dose-dependent effect emerged, revealing a decline in the number of live nauplii at higher concentrations. Specifically, at 80 µg/ml, only 70 live nauplii were observed, indicating a potential adverse effect on nauplii survival. The control group maintained a consistent count of 100 live nauplii on both days, providing a baseline for comparison as shown in **table 1** and **figure 2&3**. These findings suggest a concentration-dependent influence on nauplii viability, warranting further investigation into the substance's impact on aquatic organisms.

The results of the cytotoxicity assay for rasthali banana demonstrate a concentration-dependent impact on the viability of nauplii over the course of two days. At lower concentrations, including 5ug/ml, 10ug/ml, 20ug/ml, and 40ug/ml, the nauplii exhibited robust viability, with 100% survival observed on both Day 1 and Day 2. However, a discernible decrease in viability emerged at 80ug/ml, revealing 100% live nauplii on Day 1 but a notable reduction to 70% by Day 2. The control group, maintained without banana peel extracts, consistently displayed 100% live nauplii throughout the experiment as shown in **table 1** and **figure 2&3**. These findings underscore a concentration-specific influence on nauplii viability, suggesting a potential cytotoxic effect at higher concentrations, while lower concentrations exhibit a more favorable impact, emphasizing

the importance of dosage considerations in exploring the biological effects of banana peel extracts.

In the experimental investigation, diverse concentrations of a 1:1 ratio of red banana and rasthali were administered to assess their impact on the survival of nauplii over a two-day period. Initially, on Day 1, all concentrations, ranging from 5 µg/ml to 80 µg/ml, exhibited uniform results with 100 live nauplii each. However, on Day 2, a concentration-dependent trend emerged. While the control group maintained a stable count of 100 live nauplii, higher concentrations demonstrated a diminishing effect on nauplii survival. Notably, at 80 µg/ml, only 70 live nauplii were observed, suggesting a potential adverse influence on nauplii viability as shown in **table 1** and **figure 2&3.** These findings underscore the importance of considering concentration-dependent effects when evaluating the impact of substances on aquatic organisms.

Discussion -

The exploration into the medicinal potential of banana peels, as outlined in the introduction, reveals a rich array of bioactive compounds with diverse pharmacological effects. The identified flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids present in banana peels suggest a broad spectrum of biological activities, including antibacterial, antihypertensive, antidiabetic, antioxidant, and anti-inflammatory effects [8]. This study, motivated by the acknowledgment of these properties, specifically aims to delve into the cytotoxic aspects of banana peels, addressing a notable gap in existing knowledge.

The paragraph on dentistry underscores the multifaceted potential of banana peels in oral healthcare. The antioxidant, anti-inflammatory, and antimicrobial properties make banana peels a compelling candidate for applications in combating oxidative damage, managing conditions like gingivitis, and potentially formulating novel oral care products. The emphasis on cytotoxicity assessment aligns with a cautious approach, recognizing the need for a nuanced understanding of safety parameters [9].

The experiment with red banana peel extracts demonstrates a concentration-dependent impact on nauplii viability. While lower concentrations exhibit robust viability, a notable decrease is observed at higher concentrations, indicating a potential cytotoxic effect. The inclusion of a control group provides a valuable baseline for comparison. Authors often cited in this context could include those who have studied the cytotoxic activity of plant extracts, such as Muthana Hamid Hassan et al [10] and R Nithya et al [11]. The results of the cytotoxicity assay for rasthali banana further support the concentration-specific influence on nauplii viability. Lower concentrations maintain high viability, while at 80ug/ml, a discernible decrease is evident, emphasizing the need for careful dosage considerations. Authors like Mohamed E. Ibrahim et al [12] could be referenced for their contributions to understanding cytotoxic effects. The combined investigation with a 1:1 ratio of red banana and rasthali echoes the concentration-dependent trend observed individually, reinforcing the importance of considering concentration-dependent effects when evaluating the impact of substances on aquatic organisms.

In summary, this study not only expands our understanding of the potential medicinal applications of banana peels but also highlights the necessity for cautious exploration, especially concerning cytotoxic effects. The discussion incorporates existing literature and emphasizes the need for further research to establish safety guidelines and optimal usage levels, contributing to the informed integration of banana peel extracts into healthcare practices.

Conclusion -

In conclusion, the comprehensive exploration into the bioactive compounds within banana peels reveals a promising array of pharmacological potentials, spanning antibacterial, antihypertensive, antidiabetic, antioxidant, and anti-inflammatory effects [13]. The focus of this study on the cytotoxic properties of banana peels adds a crucial layer to our understanding, shedding light on potential adverse effects and safety considerations. The results consistently demonstrate a concentration-dependent impact on nauplii viability, with lower concentrations exhibiting favorable effects and higher concentrations showing a discernible decrease.

The multifaceted properties of banana peels, particularly in the realm of dentistry, showcase their potential in oral healthcare applications. The antioxidant, anti-inflammatory, and antimicrobial attributes position banana peels as promising candidates for addressing various oral conditions.

In addition to the current findings, several noteworthy studies contribute to the growing body of knowledge on plant extracts and their medicinal properties. Notable among these is the work of Avram et al., who explored the cytotoxic potential of banana plant extracts, providing insights into the diverse effects of phytochemicals on cellular viability [14]. Their research underscores the importance of understanding the concentration-specific impact of plant compounds, aligning with the trends observed in our study with banana peels.

Furthermore, the investigation by Ampasavate C et al in 2010 [15] delves into the cytotoxic properties of plant-derived compounds, offering parallels to our exploration of banana peels. Their findings strengthen the rationale for considering plant extracts, such as those from banana peels, in the development of therapeutic interventions.

This study serves as a foundation for future research, emphasizing the need for a nuanced understanding of the intricate balance between therapeutic benefits and potential adverse effects. As the exploration of banana peel properties unfolds, it is essential to continue investigating their safety profiles and optimal usage levels. This knowledge will not only inform the integration of banana peel extracts into healthcare practices but also contribute to the broader understanding of the medicinal potential hidden within seemingly ordinary natural resources.

Figure 1: Preparation of aqueous and alcoholic extracts from the unripe banana fruit (*Musa acuminata* - Red banana and Rasthali). The banana peels were separated, cleaned, and dried in a hot air oven at 40°C for 48 hours. The dried peels were ground into powder, and 5 g of the powder was dissolved in 25 ml of distilled water for aqueous extract preparation. Similarly, for the alcoholic extract, ethanol was heated to 100°C for 30 minutes and combined with the peel powder. Both extracts were combined to produce an aqueous- alcoholic extract. The mixture was shaken in an orbital shaker at 250 rpm for 24 hours, filtered twice using muslin cloth and filter paper, and stored at 4°C.

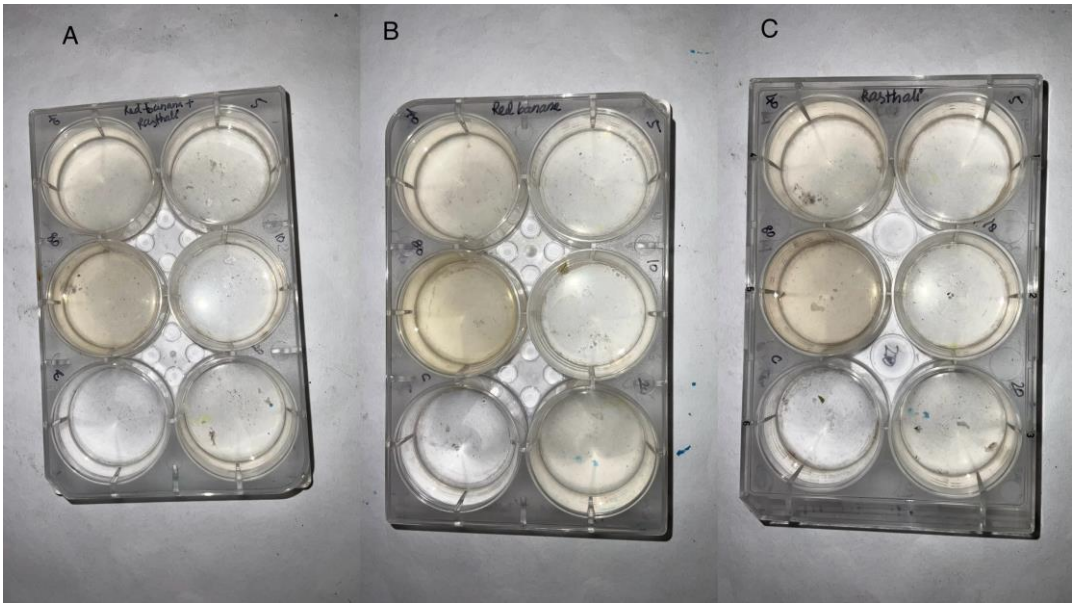

Figure 2: Cytotoxic effects of *Musa acuminata* (Red banana, Rasthali, and their 1:1 combination) peel extracts on nauplii survival over two days. The results demonstrate a concentration-dependent

impact on nauplii viability. On Day 1, all tested concentrations (5 µg/ml to 80 µg/ml) showed 100% survival across all groups. By Day 2, nauplii viability decreased significantly at higher concentrations (80 µg/ml), with survival rates dropping to 70% for Red banana, Rasthali, and their

1:1 combination extracts. In contrast, the control group consistently maintained 100% survival on both days. These findings highlight the potential cytotoxicity of higher concentrations while emphasizing the favorable viability at lower concentrations.

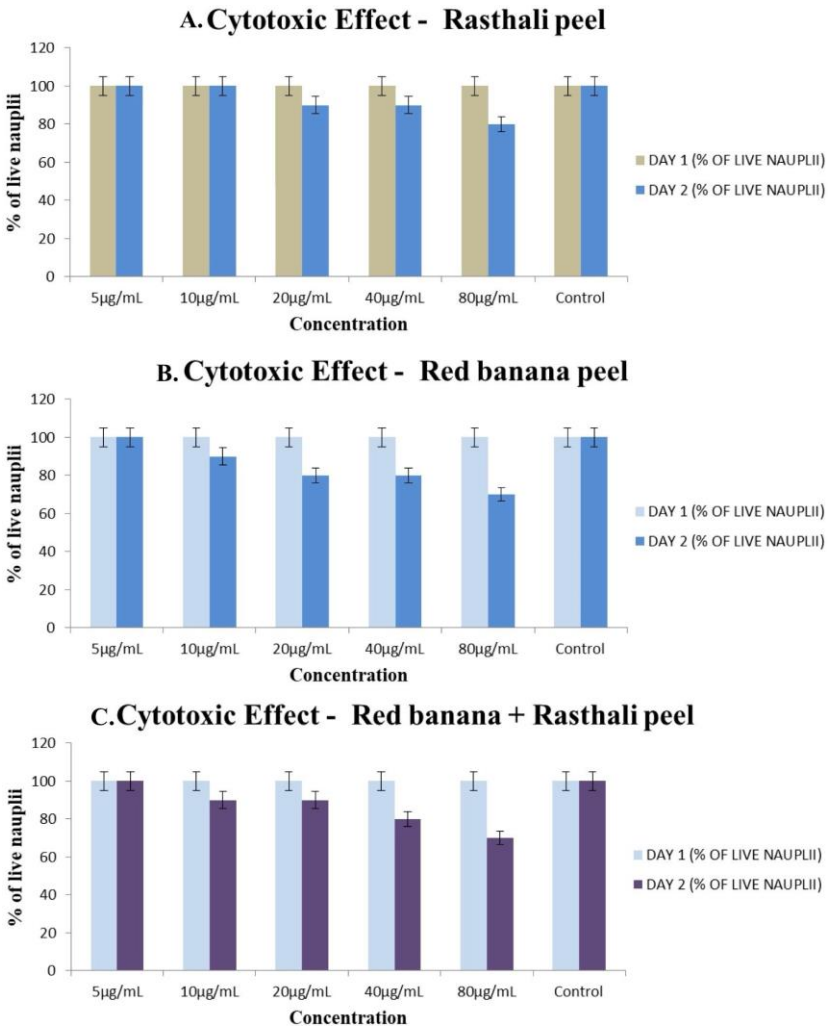

Figure 3: Cytotoxic effects of *Musa acuminata* (Red banana, Rasthali, and their 1:1 combination) peel extracts on nauplii survival over two days. The results demonstrate a concentration-dependent impact on nauplii viability. On Day 1, all tested concentrations (5 µg/ml to 80 µg/ml) showed 100% survival across all groups. By Day 2, nauplii viability decreased significantly at higher concentrations (80 µg/ml), with survival rates dropping to 70% for Red banana, Rasthali, and their 1:1 combination extracts. In contrast, the control group consistently maintained 100% survival on both days. These findings highlight the potential cytotoxicity of higher concentrations while emphasizing the favorable viability at lower concentrations.

Concentration	Red banana		Rasthali		1:1 ratio	
	Day 1(no. of live nauplii)	Day 2(no. Of live nauplii)	Day 1(no.of live nauplii)	Day 2(no. Of live nauplii)	Day 1(no.of live nauplii)	Day 2(no. Of live nauplii)
5ug/ml	100	100	100	100	100	100
10ug/ml	100	90	100	100	100	90
20ug/ml	100	80	100	90	100	90
40ug/ml	100	80	100	90	100	80
80ug/ml	100	70	100	80	100	70
Control	100	100	100	100	100	100

Table 1: The results of the cytotoxicity assay for rasthali banana demonstrate a concentration-dependent impact on the viability of nauplii over the course of two days.

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