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Harvesting Nature's Cellular Messengers: Extraction and Isolation of Plant Nanovesicles for Biomedical Applications

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OKU Sutro Excellence Day Project Cover Sheet

Project Title Harvesting Nature's Cellular Messengers: Extraction and Isolation of Plant Nanovesicles for Biomedical Applications

Full name(s) and class year(s) of all project collaborators *Example: Jane Smith, DDS 2022; John Smith, DDS 2022*
Yuchen Xie, DDS 2024; Dr. Prasanna Neelakantan; Dr. Benjamin Zeitlin

Project Category
Research awards

Enter your abstract text here (max 300 words)
Plant cells produce nanometer-sized vesicles for cellular communication, bioactive molecule transport, and modulation of the plant immune response. Extracellular nanovesicles contain proteins, RNA, and other molecules. It has been demonstrated that plant nanovesicles exert bioactive effects on human cells. Anti-inflammatory, anti-cancer, and tissue regenerative properties are significant to dentistry. Other groups have used mechanically disruptive methods to isolate plant nanovesicles. We aim to develop an isolation method for these nanoparticles from various fruits which preserves their structure and function towards their use in cell culture studies aimed at understanding the health benefits of plant nanovesicles in dental treatment and as therapeutics.

We selected species known for their potential health benefits. Uniquely, we used a cold press juicer to extract samples. The samples underwent a series of centrifugations to obtain cell- and pulp-free samples. Samples were treated to precipitate nanovesicles, then applied to size exclusion chromatography columns to isolate nanovesicles. Fractions were collected using an automatic fraction collector. The samples were then diluted in phosphate-buffered saline and analyzed by Nanosight nanoparticle tracking analysis (NTA) or Zeta-PALS particle analysis to obtain size range data. Finally the pellet was resuspended in phosphate-buffered saline and placed in -80 ˚C for long term storage.

We prepared nanovesicle enriched samples from raspberries, cranberries, limes, blackberries, blueberries, and strawberries. Analysis of blueberry samples by NTA indicate a broad range of particle sizes. Zeta-PALS analysis indicates varying ranges of particles sizes in each sample.

Our novel method for nanovesicle extraction is viable and results in vesicle enriched samples. Our use of a cold juicer instead of a blender was effective. Further research will investigate the bioactive effects of these plant-derived nanovesicles on human cells.

I would like to thank Drs. Zeitlin and Neelakantan for their mentorship on this project.

Harvesting Nature's Cellular Messengers: Extraction and Isolation of Plant Nanovesicles for Biomedical Applications

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ABSTRACT

Plant cells, like those of animals, produce nanometer-sized vesicles for cellular communication, bioactive molecule transport, and immunomodulation. It has been demonstrated that the plant nanovesicles exert bioactive effects on human cells. Of these, anti-inflammatory, anti-cancer, and tissue regenerative properties are significant to the dental field. Other groups have used mechanically disruptive methods to isolate plant nanovesicles. Our research aims to develop a nanoparticle isolation method which preserves their structure and function towards their use in cell culture studies aimed at understanding the health benefits of plant-derived nanovesicles in dental treatment and as therapeutics.

Species of fruit	*Potential health benefits*
Blackberry	antioxidants, polyphenols
Blueberry	antioxidants, polyphenols
Cranberry	antioxidant, anti-bacterial adhesion
Raspberry	antioxidant, ellagic acid
Strawberry	antioxidant, anti-inflammatory
Lime	antioxidant, antibacterial

Table 1: Fruits selected for this study and their potential health benefits

METHODS

We selected species known for their potential health benefits. 50g of each species were washed with milliQ water and processed through a cold press juicer. The pulp from the first juicing was processed again with 50 mL water. The samples were divided into conical tubes and centrifuged in a swing rotor. The samples were removed and the supernatant pipetted into high speed conical tubes to be centrifuged in a fixed position rotor. The supernatant was aliquoted into cryovials. The cell- and pulp-free samples were stored at -20˚C. An automatic fraction collector was used to collect 500 uL fractions using size exclusion chromatography to separate molecules by filtration through a gel.

This method was selected over ultracentrifugation because it reduces the pressure to which samples are exposed. Fractions 1-4 contained nanovesicles per column manufacturer calibration. 15 fractions total were collected for each sample. An inconvenience was that since the automatic fraction collector holds only 13 fractions, it was necessary to pause in the middle of collection to switch out the collection tubes. The columns are intended for mammalian cells. However, we expect satisfactory results due to structural similarities between plant and animal nanovesicles. Two preparations of each species were treated with Total Exosome Isolation reagent to precipitate nanovesicles. The samples were diluted in phosphate-buffered saline (PBS) and analyzed by Nanosight nanoparticle tracking analysis (NTA) and Zeta-PALS particle analysis (data not shown) to obtain size range data. The pellet was resuspended in PBS and placed in -80 ˚C for long term storage.

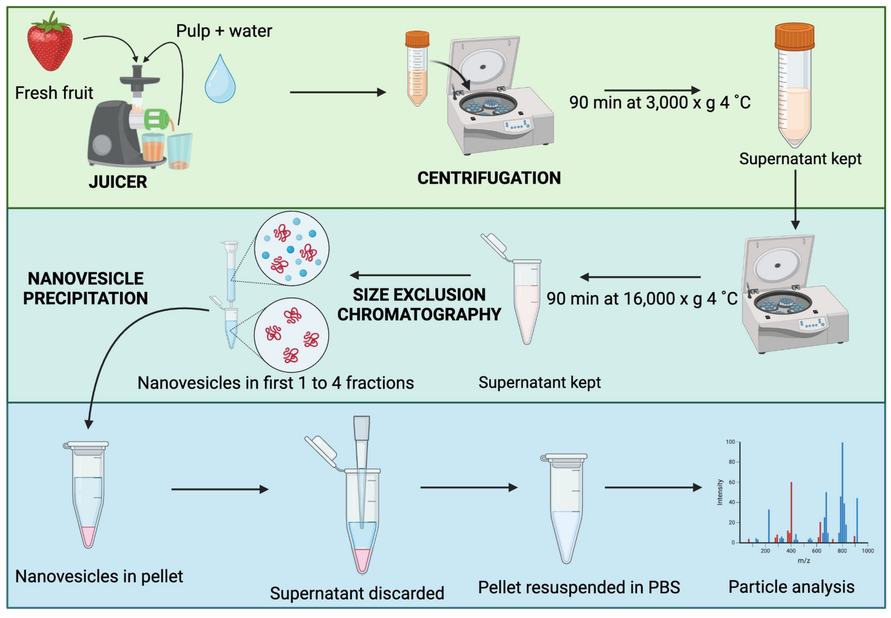

Figure 1: Diagram depicting novel workflow for extraction and isolation of plant nanovesicles

RESULTS

We prepared nanovesicle enriched samples from raspberries, cranberries, limes, blackberries, blueberries, and strawberries. Analysis of blueberry samples by NTA indicate particles in the 30 to 500 nm. Zeta-PALS analysis indicates particles of 15 nm to 1 um. These size ranges are consistent with those expected for plant nanovesicles.

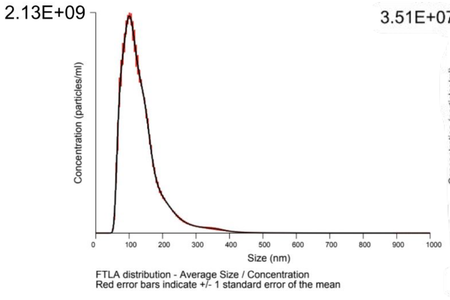

Figure 2: Particle size range and concentration of blueberry juice before size exclusion chromatography

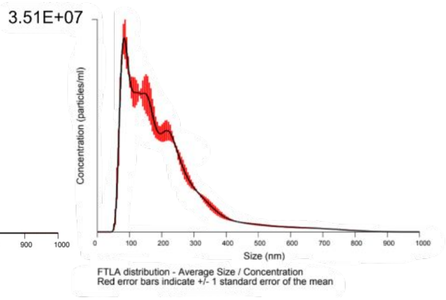

Figure 3: Particle size range and concentration of blueberry sample after size exclusion chromatography

CONCLUSION

- Our novel method for nanovesicle extraction results in vesicle-enriched samples with less chance of artifact creation.
- The use of a cold juicer instead of a blender was effective in preserving expected range of plant vesicle size.
- Size exclusion chromatography is appropriate for enrichment of plant vesicle samples but may result in loss of material.
- Precipitation using Total Exosome Isolation Reagent was effective in concentration of plant nanovesicles.
- The nanovesicles produced from our isolation method are likely to retain their endogenous effects.

FUTURE DIRECTIONS

Studies indicate that plant nanovesicles can be utilized in the treatment of periodontitis through the regulation of mRNA translation. The stability of plant nanovesicles in the oral cavity enables efficient long lasting delivery of drugs. The specificity of the bioactive effects of nanovesicles can be altered by modifying their surface proteins. Further research will investigate the bioactive effects of these plant nanovesicles on human cells and their potential role in dental treatment.

ACKNOWLEDGEMENTS
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REFERENCES
Zhang, Z., Yu, Y., Zhu, G., Zeng, L., Xu, S., Cheng, H., Ouyang, Z., et al. (2022). The Emerging Role of Plant-Derived Exosomes-Like Nanoparticles in Immune Regulation and Periodontitis Treatment. Frontiers in Immunology, 13, 896745.