Sequential Tangential Flow Filtration for purification of Plant derived nanoparticles

Sirin Korulu Koc, Francesca Loria, Jevgenia Dering, Paolo Guazzi; HansabioMed Life Science

Introduction:

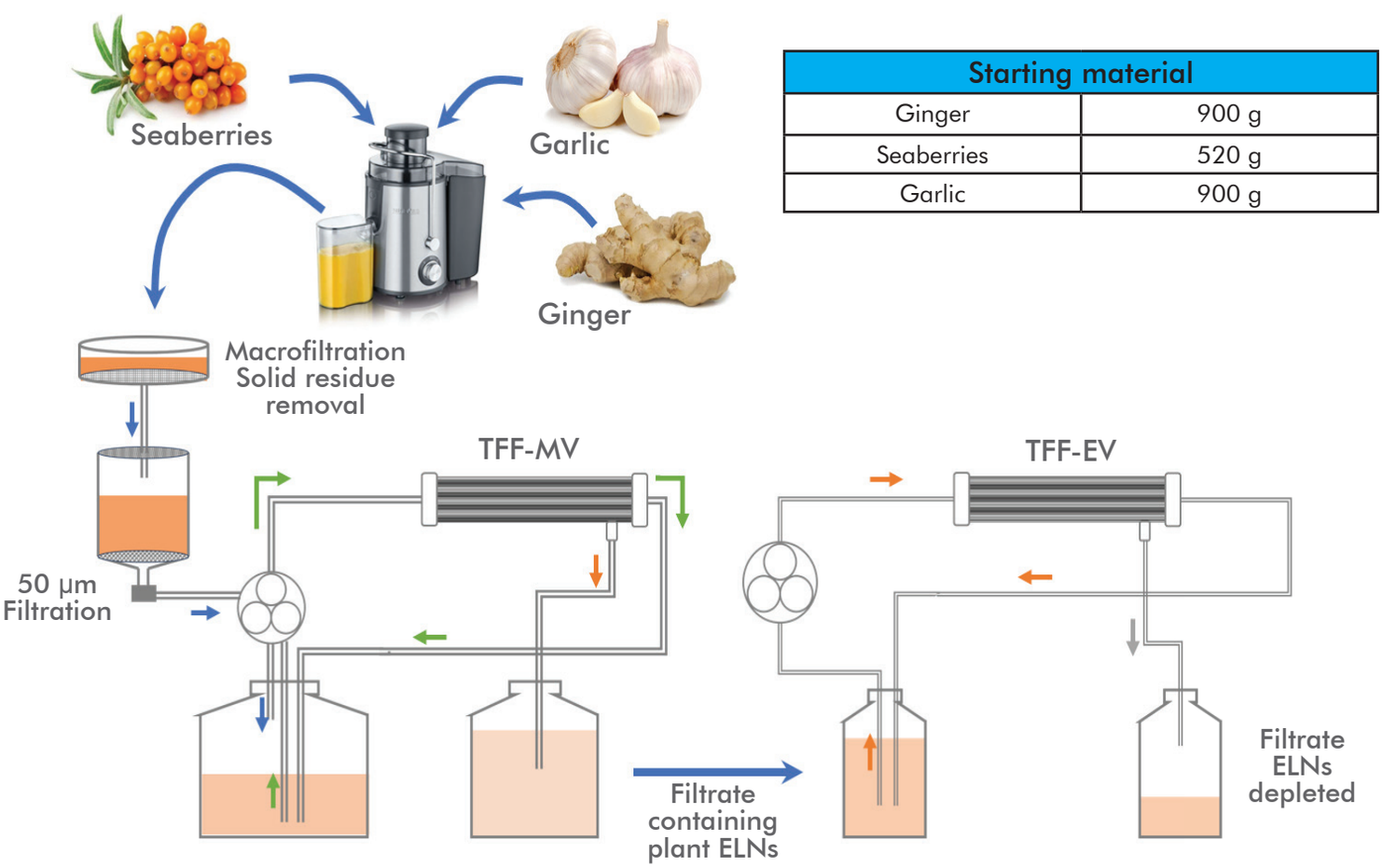
Despite the presence of nanoparticles in plants was firstly observed in late '60s, dedicated research into plant-derived nanoparticles, commonly referred to as Plant EV-Like Nanoparticles (ELN) or more frequently as Plant-EVs, began just a decade ago. Today, plant ELNs represent a new frontier for the development of a new class of therapeutics—remarkably safe and highly biocompatible. This innovation holds significant potential, particularly in the cosmetic and nutraceutical industries, and offers novel alternatives for bioactive molecule delivery. ELNs from ginger, various berries, lemons, and grapefruits have exhibited distinct anti-inflammatory [1] and antioxidant activities [2]. Furthermore, these plant ELNs have demonstrated resilience against the harsh conditions of the stomach and intestine, successfully reaching the colon and potentially influencing the microbiota [3].

In this application note, we show the efficacy and cost-effectiveness of a sequential filtration approach employing TFF-MV (200 nm pore size) and TFF-EVs (50 nm pore size) for the isolation of plant ELNs. This method ensures optimal particle yield while preserving the biological effects of these intriguing nanoparticles.

[1] Chen, Xingyi, You Zhou, and Jiujiu Yu. "Exosome-like nanoparticles from ginger rhizomes inhibited NLRP3 inflammasome activation." Molecular pharmaceutics 16.6 (2019): 2690-2699. [2] Zhang, Mingzhen, et al. "Plant derived edible nanoparticles as a new therapeutic approach against diseases." Tissue barriers 4.2 (2016): e1134415. [3] Munir, Javaria, Mihye Lee, and Seongho Ryu. "Exosomes in food: Health benefits and clinical relevance in diseases." Advances in Nutrition 11.3 (2020): 687-696.

Workflow:

1. Sample preparation and precleaning: seaberries, ginger and garlic were minced in a juicer. Following macrofiltration and the removal of the the solid residue, the juice was filtered through 50 μm filter (Biocomma).
2. Tangential flow filtration for removal of large partilces and debris: the fluid was filtered through TFF-MV, 200 nm pore size, operating with peristaltic pump Masterflex L/S 7535-04 at 80 ml/min flow velocity.
3. Plant ELNs purification was performed by Tangential Flow Filtration through the TFF-EVs filter (50 nm pore size), operating with peristaltic pump Masterflex L/S 7535-04 at 80 ml/min flow velocity. Following 3 washing steps with 50 ml of PBS 1x (Corning), the plant nanoparticles were recovered in 5 ml of PBS 1x.



Starting material	
Ginger	900 g
Seaberries	520 g
Garlic	900 g

Results:

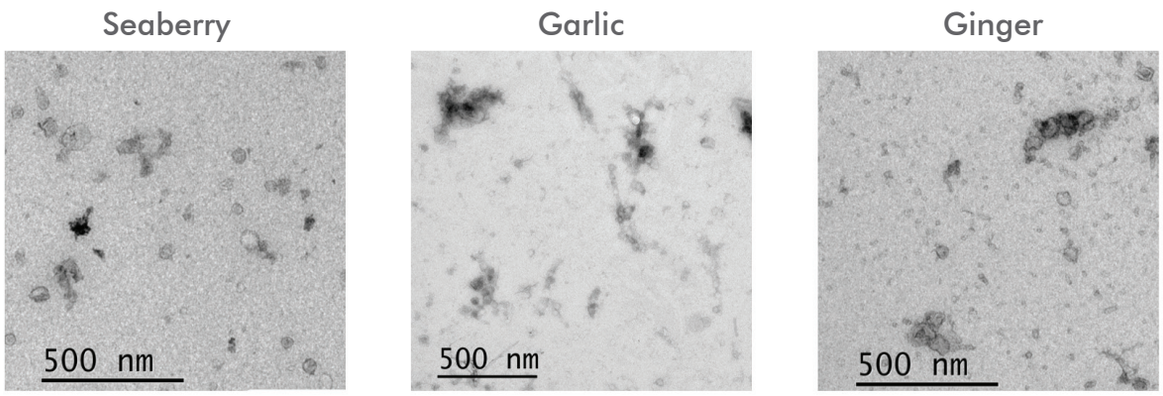
1- Nanoparticle concentration and morphology.

A. Nanoparticle Tracking Analysis (NTA), performed with Zetaview analyzer (Particle Metrix).

Sample	Particle Concentration (Part/ml)	Mean Size (nm)	Volume (ml)	Total Particles
Garlic	$5.4x10^{11}$	82.5 +/- 6.6	5	$2.7x10^{12}$
Seaberry	$5.6x10^{11}$	117.8 +/- 11.4	5	$2.8x10^{12}$
Ginger	$4.0x10^{11}$	101.3 +/- 5.6	5	$2.0x10^{12}$

Instrument settings:
Camera sensitivity 85
Shutter 100
Temperature 25°C.

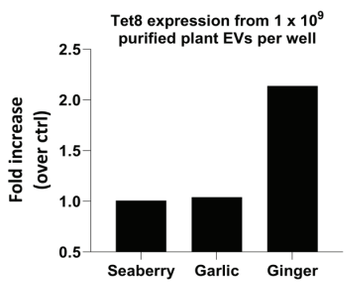
B. Transmission Electron Microscopy analysis (TEM).

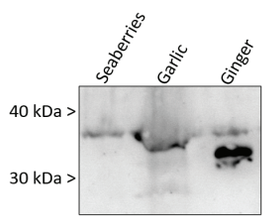


Nanoparticles imaged with transmission EM using Hitachi HT7800, operating at 100 KV. Images taken with Gatan Rio9, model 1809 (Gatan Inc).

2- Particle characterization and biologic activity.

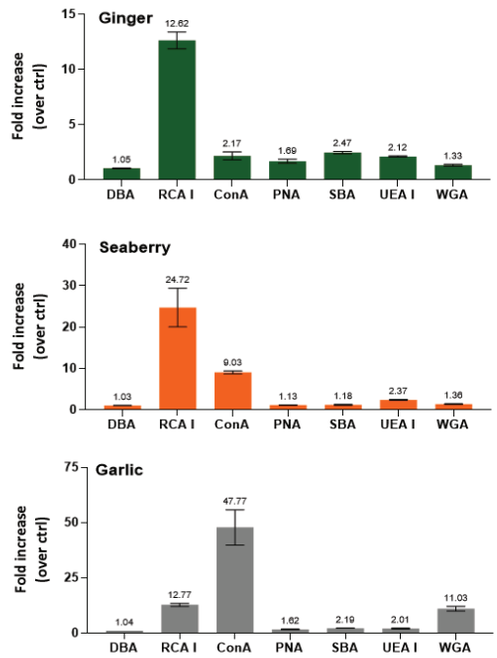
A. TET8 marker expression in purified ELNs



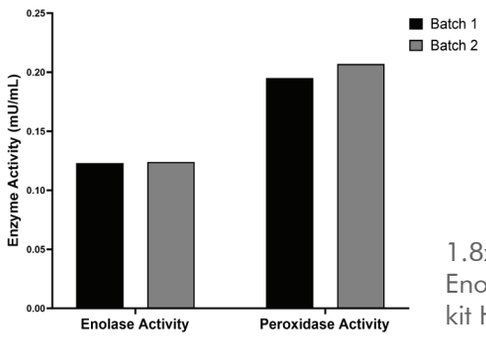


ELISA and WB performed using $1x10^9$ purified plant ELNs per sample. anti-TET8 (Phito AB).

B. Lectin profiling in purified ELNs



C. Enzymatic activity assays in ginger ELNs



$1.8x10^{10}$ nanoparticle/well Enolase and Peroxidase activity kit HansaBioMed Life Sciences

Lectin profiling performed using HB black plates HansaBioMed Life Sciences and Lectin kit I Fluorescein (Vector Lab)

Conclusion:

Combination of TFF-MV and TFF-EVs allows fast, scalable and cost effective purification of plant derived nanoparticles (ELNs). Purified particles are suitable for functional assays and biomarker profiling.

HansaBioMed Life Sciences

HansaBioMed Life Sciences LTD
Mäealuse 2/1,12618 Tallinn, Estonia
+372 6561996
info@hansabiomed.eu