7'TH INTERNATIONAL CONFERENCE
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Journal homepage: https://ojs.universitastelogorejo.ac.id/index.php/ichpr

Biosurfactant Screening for Green-Stabilized *Wolffia arrhiza* Nanosuspensions

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ARTICLE INFO

Article history:
Received 6 November 2025
Accepted 12 December 2025

Keywords:
Wollfia arrhiza
Biosurfactant
Green nanotechnology
Nanosuspensions
High pressure homogenization

ABSTRACT

Problem. Developing stable nanosuspensions from plant-based materials such as *Wolffia arrhiza* requires effective stabilizers to prevent aggregation and achieve uniform particle dispersion. Conventional synthetic surfactants may raise environmental and safety concerns, underscoring the need for biocompatible biosurfactant alternatives. **Purpose.** This study aimed to screen several biosurfactants for their ability to stabilize *W. arrhiza* nanosuspensions, based on particle size and polydispersity index (PI) measured after key processing stages. **Methods.** Nanosuspensions were prepared using 5,000 mg of *W. arrhiza* dispersed in aqua demineralisata and stabilized with 0.5% (w/v) of one of seven biosurfactants: Emulsan, Liposan, Rhamnolipid, Trehaloselipid, Fengisin, Viscosin, and Fosfolipid. The process included (1) initial dispersion using Ultra-Turrax at 15,000 rpm for 10 minutes, followed by (2) premilling via high-pressure homogenization (HPH) at 300 bar (1 cycle) and 500 bar (2 cycles). Particle size and PI were assessed after both stages using dynamic light scattering. **Main findings.** After premilling, Fosfolipid produced the smallest particle size (841.4 nm± 1.24) and lowest PI (0.335±0,01), indicating superior stabilization. Trehaloselipid also showed favorable performance, generating particles below 1,000 nm with moderate PI values. In contrast, Viscosin resulted in the largest particle size (1050.3 nm± 1.56) and highest PI (0.610± 0.06). Overall, biosurfactants with phospholipid and glycolipid characteristics outperformed polymeric or lipoprotein-based stabilizers. **Conlusions.** Fosfolipid emerged as the most effective stabilizer for producing stable and uniformly dispersed *W. arrhiza* nanosuspensions. These findings support the potential of biosurfactant-assisted green processing for nanoformulations, providing a sustainable alternative to synthetic stabilizers while maintaining nanosuspension quality.

INTRODUCTION

Wolffia arrhiza is one of the smallest aquatic plants (Park et al., 2021), rich in protein and amino acids(Sree et al., 2023), micronutrients, and bioactive phytochemicals (Das et al., 2023), making it a promising candidate for functional foods, nutraceuticals(Liao et al., 2022), and health-oriented formulations (Romano & Aronne, 2021; Sree et al., 2019). Despite its potential, its application in nanoscale delivery systems remains challenging due to poor dispersibility(Kaialy & Al Shafiee, 2016), high aggregation tendency(Puss et al., 2023), and limited stability in aqueous systems (Teja et al., 2022; Yang et al., 2020). Nanosuspension technology offers a strategy to improve solubility(Samanta et al., 2022), bioavailability(Bayda et al., 2020), and functional performance of plant-based materials (Jacob et al., 2020); however, the effectiveness of this approach depends heavily on appropriate stabilizers (Wang & Staufenbiel, 2024). Conventional surfactants used in nanosuspension production(Verma et al., 2011), such as synthetic polymers and detergents(Wu et al., 2011), may pose ecological and toxicity concerns (Elmowafy et al., 2021; Soisuwan et al., 2019). In contrast, biosurfactants—naturally derived amphiphilic molecules produced by microorganisms—offer biocompatibility (Al-Sakkaf & Onaizi, 2022), biodegradability (Knoth et al., 2019), and suitability for green nanotechnology approaches (Knoth et al., 2019; Markande et al., 2021; Vieira et al., 2021). Selecting an effective biosurfactant stabilizer is therefore essential to ensure successful particle size reduction, prevent aggregation(Markande et al., 2021), and produce uniform nanosuspension dispersions (Knoth et al., 2021; Tian et al., 2020). This study aimed to screen seven biosurfactants for their ability to stabilize *Wolffia arrhiza* nanosuspensions produced using a top-down High Pressure Homogenization (HPH) process. The screening focused on evaluating particle size and polydispersity index (PI), which represent key indicators of nanosuspension stability and homogeneity.

METHODS

Materials

Wolffia arrhiza powder was obtained from the cultivation place of Azolla Popongan in Popongan district, Central Java. It was dried and milled to a fine powder (100-200 µm). The biosurfactants Emulsan, Liposan, Rhamnolipid, Trehaloselipid, Fengisin, Viscosin, and Fosfolipid were purchased from Sigma-Aldrich US. All other reagents were of analytical grade.

Formulation Design

Nanosuspensions were prepared using a fixed concentration of *W. arrhiza* (5,000 mg) and biosurfactant (0.5% w/v) (**Table 1**). The formulations were categorized based on biosurfactant molecular weight: high (Emulsan, Liposan) and low (Rhamnolipid, Trehaloselipid, Fengisin, Viscosin, Fosfolipid).

Table 1. Composition of wolffia nanosuspension

Biosurfactants	Formulation (%)						
	A	B	C	D	E	F	G
Wolffia powder	5	5	5	5	5	5	5
Emulsan	0,5	-	-	-	-	-	-
Liposan	-	0,5	-	-	-	-	-
Rhamnolipid	-	-	0,5	-	-	-	-
Phospolipid	-	-	-	0,5	-	-	-
Trehaloselipid	-	-	-	-	0,5	-	-
Fengisin	-	-	-	-	-	0,5	-
Viscosin	-	-	-	-	-	-	0,5
Water	94,5	94,5	94,5	94,5	94,5	94,5	94,5

Preparation of Nanosuspensions

For each formulation, 5,000 mg of *W. arrhiza* was dispersed in 100 mL demineralized water containing 0.5% (w/v) of one biosurfactant. The nanosuspension was prepared using a two-step top-down approach. **Dispersion stage:** The mixture was processed using an Ultra-Turrax homogenizer (IKA T25) at 15,000 rpm for 10 minutes to ensure complete wetting and preliminary size reduction. **Premilling stage:** The coarse suspension was further processed using a High-Pressure Homogenizer (HPH, Avestin Emulsifex C5, Avestin) at 300 bar for 1 cycle, followed by 500 bar for 2 cycles.

Characterization

Particle size (Z-average, nm) and polydispersity index (PI) were measured via dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instrument, UK), after both dispersion and premilling stages. Lower size and PI values indicate better colloidal stability and monodispersity.

RESULT AND DISCUSSION

Result

Particle Size and PI After Dispersion Stage

During the dispersion stage, phospholipid exhibited the best wetting and initial size reduction, yielding particles of 1107.1 nm ± 2.12 (PI 0.547±0.07). Trehaloselipid (1211.3nm ± 2.14) and Fengisin (1209.8 nm ± 1.36) also performed favorably (**Fig 1).**

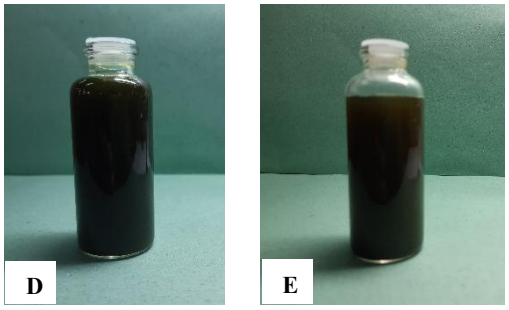

Figure 1. Appearance of wolffia suspensions stabilized with phospholipid (D) and trehaloselipid (E) after the initial dispersion stage (Ultra-Turrax, 15,000 rpm, 10 min).

Biosurfactants such as Liposan and Emulsan produced larger particle sizes (>1500 nm), while Viscosin generated the largest particles (1804.7 nm ± 2.05; PI 0.668 ± 0.08), suggesting weaker stabilization during early dispersion (**Table 2**). These initial findings indicate that biosurfactants with glycolipid or phospholipid structures may interact more effectively with *W. arrhiza* particles, improving wettability and preventing early aggregation.

Table 2. Particle size and PI after dispersion stage

Formula code	Parameters	
	Z-average (nm± SD)	**PI (±SD)**
A	1550,2 ± 2.28	0,512 ± 0.02
B	1572,5 ± 3.35	0,649 ± 0.05
C	1422,3 ± 1.31	0,577 ± 0.04
D	1107,1 ± 2.12	0,547 ± 0.07
E	1201,3 ± 2.14	0,532 ± 0.03
F	1269,8 ± 1.36	0,523 ± 0.06
G	1804,7 ± 2.05	0,668 ± 0.08

Particle Size and PI After Premilling (HPH) Stage

After premilling, all formulations demonstrated significant size reduction (approximately 20–35%), confirming the efficiency of HPH. Fosfolipid remained the most effective stabilizer, producing the smallest particle size (841.4 nm± 1.24) and lowest PI (0.335± 0.01). Trehaloselipid (919.3 nm± 1.94; PI 0.417± 0.08) and Fengisin (1007.1 nm± 2.31; PI 0.522± 0.02) also showed improved stability and narrower distribution (**Fig 2**). The suspension stabilized with phospholipid appears more visually uniform, with minimal upper-layer color gradation, reflecting a narrower particle size distribution and the lowest PI value observed in the screening experiment (841.4 nm; PI 0.435).

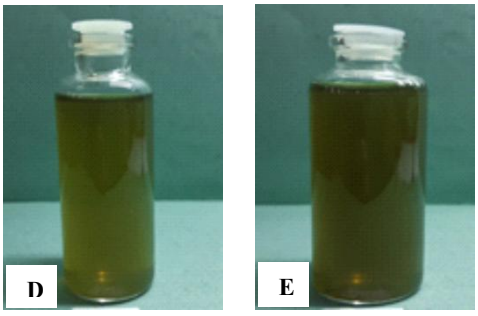

Figure 2. Visual characteristics of wolffia nanosuspensions stabilized with phospholipid (D) and trehaloselipid (E) after premilling stage (HPH 1 cycle at 300 bar, and 2 cycle at 500).

In contrast, Viscosin continued to show the least favorable performance (1050.3 nm± 1.56; PI 0.610± 0.06), reflecting insufficient steric or electrostatic stabilization under high shear and pressure conditions (**Table 3**).

Table 3. Particle size and PI after premilling stage

Formula code	Parameters	
	Z-average (nm± SD)	**PI (±SD)**
A	1012,2± 3.06	0,533± 0.09
B	1019,8± 2.17	0,575± 0.07
C	1070,3± 3.22	0,529± 0.08
D	841,4± 1.24	0,335± 0.01
E	919,3± 1.94	0,417± 0.08
F	1007,1± 2.31	0,522± 0.02
G	1050,3± 1.56	0,610± 0.06

Discussion

The fabrication of *W. arrhiza* nanosuspensions via a two-stage top-down approach—comprising an initial dispersion stage followed by a premilling stage—is a strategically designed process that effectively transitions a coarse, heterogeneous mixture into a stabilized, sub-micron colloidal system. Each stage fulfills a distinct and critical function in the overall particle size reduction and stabilization pathway. The primary objective of the dispersion stage is not to achieve nano-scale dimensions, but to ensure complete wetting of the hydrophobic *W. arrhiza* powder and to break apart large, loose agglomerates(Tian et al., 2020). The premilling stage employs High-Pressure Homogenization (HPH) to achieve the targeted nano-scale particle size through controlled, high-energy fragmentation. The premilling HPH stage is the high-energy, precision step responsible for achieving the

target nanoscale dimensions and a narrow PSD(Parmar et al., 2021). The visual behavior of the *Wolffia arrhiza* suspensions at the initial dispersion stage reflects the differing stabilizing capacities of the biosurfactants prior to high-pressure size reduction. The phospholipid-stabilized sample displays a more even color distribution, suggesting superior wetting efficiency and early steric stabilization during high-speed mixing. This observation aligns with its smaller initial particle size compared with trehaloselipid. In contrast, the trehaloselipid-stabilized suspension shows a slightly more prominent color gradient at the top portion of the bottle, indicating modest variation in particle distribution and partial flocculation during the first dispersion phase. However, the absence of sedimentation in both samples indicates that both phospholipid and trehaloselipid effectively maintain short-term colloidal stability even before HPH processing. These qualitative features are consistent with the DLS data obtained at the dispersion stage and support the conclusion that phospholipid provides more efficient initial stabilization. The superior performance of phospolipid can be attributed to its structure, which provides strong electrostatic and steric stabilization, preventing particle aggregation during HPH (Markande et al., 2021). Glycolipids such as Trehaloselipid also performed well, likely due to their hydrophilic sugar groups enhancing interfacial adsorptio(Vieira et al., 2021). In contrast, high-molecular-weight polymeric biosurfactants like Viscosin and Emulsan may form viscous layers that impede efficient size reduction, resulting in larger particles and broader size distribution(Elmowafy et al., 2021). These findings align with previous studies indicating that low-molecular-weight biosurfactants with amphiphilic structures are more effective in nanosuspension stabilization. The significant reduction in PI after premilling for all formulations confirms the effectiveness of HPH in achieving a more homogeneous particle distribution. The results underscore the potential of biosurfactants as sustainable stabilizers in green nanotechnology, particularly for sensitive bioactive materials like *W. arrhiza*.

CONCLUSION

This study demonstrates that biosurfactant selection critically influences the physical stability of *Wolffia arrhiza* nanosuspensions. Fosfolipid emerged as the most effective stabilizer, yielding the smallest particle size (841.4 nm± 1.24) and lowest PI (0.335± 0.01) after HPH processing. Glycolipid- and lipopeptide-type biosurfactants also showed promising performance, whereas polymeric biosurfactants were less efficient. The results validate a green, biosurfactant-assisted top-down approach for producing stable plant-based nanosuspensions, offering a viable alternative to synthetic stabilizers in nutraceutical and pharmaceutical applications.

ACKNOWLEDGEMENT (if any)

The authors gratefully acknowledge Pharmaceuticals Research Group, School of Pharmacy ITB for technical support and laboratory facilities. We also thank the Ministry of Higher Education, Science and Technology of the Republic of Indonesia for funding this work under the National Research Grant Scheme (PDD Grant No. 015/C3/DT.05.00/PL2025).

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