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Original Article

Formulation of Serum Sprays Containing Leaf Extract and Leaf Extract-Loaded Nanoparticles of Syzygium myrtifolium Walf: Antioxidant Activity and In Vitro Release Using Franz Diffusion Cells

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ABSTRACT

The skin serves as the primary body barrier against external threats, with facial skin being particularly exposed and clinically significant. Skincare products—such as moisturizers, cleansers, and serums—are designed to protect, nourish, and rejuvenate the skin. This study aimed to extract Syzygium myrtifolium (SM) leaves, synthesize them into nanoparticles, and formulate antioxidant serum sprays. SM leaves were macerated in 70% ethanol (pH 2) and concentrated using a rotary evaporator. The resulting extract was characterized and converted into nanoparticles via a precipitation method. Nanoparticle characterization included particle size (252.7 nm), polydispersity index (0.459), zeta potential (−27.2 mV), and entrapment efficiency (47.91%). Both the extract and nanoparticles were formulated into serum sprays and evaluated for physicochemical properties, antioxidant activity, and in vitro release using a Franz diffusion cell. The serum sprays appeared as a clear to pale yellow liquid with a characteristic odor, uniform consistency, appropriate viscosity and spreadability, rapid drying time (<3 minutes), and an average pH of 7.20. Antioxidant activity, expressed as IC_{50}, was 15.96 ppm (extract), 32.59 ppm (nanoparticles), 32.29 ppm (extract-based serum, EbSS), and 59.98 ppm (nanoparticle-based serum, NbSS). Franz cell analysis showed flux values of 1.72 µg/cm²/min (EbSS) and 1.79 µg/cm²/min (NbSS), with corresponding permeability coefficients of 2.78 cm/min and 2.90 cm/min, and diffusion coefficients of 3.33 cm²/min and 3.48 cm²/min, respectively. Overall, the SM extract was successfully formulated into nanoparticle and serum spray forms, with NbSS exhibiting superior diffusion performance.

Keywords*: Syzygium myrtifolium, extract, nanoparticles, serum sprays, Franz diffusion cell.*

INTRODUCTION

The skin serves as the body's primary defense against external threats through mechanisms such as keratinization, temperature regulation, sweat and sebum secretion, melanin production, and immune responses [1]. Among all regions, facial skin is especially important, often reflecting overall health and being the initial site for many dermatological conditions [2,3].

Skincare products—including moisturizers, cleansers, and serums—are designed to protect, nourish, and rejuvenate the skin [4]. Serums are concentrated water- or oil-based formulations targeting specific skin concerns, often used alongside complementary products [5]. Recently, serums have evolved into spray formats, offering hygienic, hands-free application and enhanced absorption [6].

Nanoparticles, typically <1000 nm, possess unique physicochemical properties that enhance the delivery and stability of active ingredients in cosmetics and pharmaceuticals [7]. One major contributor to skin damage is oxidative stress caused

by free radicals—unstable molecules that accelerate aging and cellular degradation. Antioxidants neutralize these radicals, either naturally produced or sourced from flavonoid-rich plants [8].

Syzygium myrtifolium (SM), locally known as pucuk merah, contains flavonoids and anthocyanins with potent antioxidant properties [9,10]. SM thrives in diverse environments and is widely cultivated for landscaping and ecological greening due to its vibrant foliage and environmental benefits [11,12]. These include CO_2 absorption, oxygen production, erosion control, and soil stabilization via robust taproots. SM also supports biodiversity by attracting pollinators and frugivores, aiding seed dispersal and habitat formation. Its compact growth makes it ideal for hedging and pruning [11,13–15].

Antioxidant activity is commonly assessed using the DPPH assay, which measures the IC_{50} value—the concentration needed to neutralize 50% of DPPH radicals. Lower IC_{50} values indicate stronger antioxidant potential. SM leaf extract prepared with 70% ethanol has demonstrated high efficacy, with an IC_{50} of 11.130 ppm [16].

Spray serum evaluation encompasses formulation, physicochemical properties, in vitro performance, user acceptability, and other parameters [17,18]. Key aspects include:

1. Formulation and Physicochemical Properties
The formulation was evaluated for stability by monitoring changes in color, texture, and pH under various storage conditions [19]. pH compatibility was assessed to ensure alignment with the skin's natural pH (~5.5), minimizing the risk of irritation [20]. Spreadability was examined to determine ease of application and absorption [18], while particle size analysis focused on ensuring uniformity and the absence of clumping [18].

2. In Vitro Performance
The formulation was assessed for antioxidant activity using the DPPH assay to determine its radical-neutralizing capacity [17]. Drug release was evaluated to measure how effectively the active ingredients are delivered to the skin [18], while skin irritation testing was conducted using animal models to ensure safety and minimize adverse reactions [18,21].

3. User Acceptability
The evaluation criteria include hydration, which measures moisturizing effects [22]; glow/radiance, assessing the impact on skin luminosity [22,23]; and ease of use, which considers the spray mechanism and overall user experience [22]. Additionally, texture is examined to determine absorption and residue [22], while multi-functionality reviews the product's performance as a mist, primer, or setting spray [22]. Finally, overall effectiveness gathers user feedback on visible skin improvement [22,23].

4. Other Parameters
The evaluation also includes ingredients, which are screened for potential allergens and the concentration of active components [24,25], as well as packaging, which is assessed based on its design, user convenience, and visual aesthetics [17,25].

The Franz diffusion cell method is a gold-standard in vitro technique for assessing drug permeation through skin-like membranes. It involves placing a membrane between donor and receptor chambers, with the receptor chamber containing a buffered solution. The formulation is applied to the donor chamber, and samples are withdrawn over time to measure permeation via UV–Vis or HPLC. Key metrics include flux, cumulative permeation, and lag time [26-28].

This study aimed to extract SM leaves, synthesize gelatin nanoparticles, and formulate antioxidant spray serums containing both extracts and nanoparticles. These formulations were then evaluated, including diffusion analysis using Franz cells

MATERIAL AND METHODS

2.1 Material

Research materials included SM leaves, obtained from the gardens at Universitas Pancasila, Jakarta. The chemicals used in this study were of pro-analytical grade, including 70% ethanol, methanol, dimethyl sulfoxide (DMSO), 96% ethanol, poloxamer 188, gelatin, 2% glutaraldehyde, hydroxyethyl cellulose, phenoxyethanol, propylene glycol, and purified water.

The following equipment used included: UV–Vis spectrophotometer (UV-1900, Shimadzu, Kyoto, Japan); rotary vacuum evaporator (Heidolph, Schwabach, Germany); analytical balance (Kern, Balingen, Germany); digital homogenizer (RW 20, IKA, Staufen, Germany); microbalance (MT5, Mettler Toledo, Columbus, OH, USA); micropipette (Dragon Lab, Beijing, China); rotational viscometer (RV, Brookfield, Middleboro, MA, USA); pH meter (Hanna Instruments, Woonsocket, RI, USA); and standard laboratory glassware (Pyrex, Iwaki, Japan).

Plant Preparations

Leaves of SM were from the garden area of Universitas Pancasila (Jakarta, Indonesia). The leaves were thoroughly washed with running water, cleaned to remove debris, air-dried at room temperature, and then ground into a fine powder using a blender. [29]

Determining The Degree of Fineness of Simplicia

The fineness of the simplicia powder was determined using a #4 sieve and a #18 sieve. A 100 g sample was placed on each sieve and shaken to facilitate separation. The material retained on the sieve and the portion that passed through to the receiving pan were each collected and weighed to evaluate particle size distribution. The same procedure was used with the #18 sieve to determine the particle size distribution [30].

2.2 Preparation and Evaluation of SM Dry Extract

The powdered simplicia of SM were macerated with 70% ethanol in a 1:10 (w/v) ratio for 48 hours at room temperature. After maceration, the macerate was filtered using filter paper, and then concentrated under reduced pressure using a rotary vacuum evaporator (Heidolph, Schwabach, Germany) [31].

Organoleptic Evaluation

The organoleptic properties of the thick extract, including color, odor, and taste, were assessed using sensory observation methods [32].

Extract Yield and Drug Extract Ratio (DER-Native)

The determination of extract yield was by comparing the weight of the dried extract to the original weight of the simplicia material. Furthermore, the drug-extract ratio or DER-native was determined to quantify the concentration of active constituents present in the extract [33,34].

pH Measurement

The pH of the extract was measured using a calibrated pH meter (Hanna Instruments, Woonsocket, RI, USA), calibrated with pH 7.0 and pH 4.0 buffer solutions. The pH meter was always clean before use. The preparation of the sample solution involves dissolving 100 mg of the extract in 100 mL of purified water. After immersing the electrode in the sample solution and obtaining a stable reading, the pH value was recorded [35].

Antioxidant Activity of the Extract Using the DPPH Assay

A stock solution of 1000 ppm was prepared and diluted to obtain concentrations of 4, 8, 12, 16, and 20 ppm, in series. To each test tube of 1 mL of a 0.4 mM DPPH solution, add methanol to adjust the final volume to 5.0 mL. The mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at the maximum wavelength using a UV–Vis spectrophotometer (Shimadzu UV-1900, Kyoto, Japan) [36,37].

2.3 Preparation and Evaluation of SM Leaf Extract-Loaded Nanoparticles

Solvent Phase

A quantity of gelatin was dissolved in 1 mL of purified water maintained at 50°C. Separately, SM leaf extract was dissolved in a mixture of 0.2 mL dimethyl sulfoxide (DMSO) and 0.2 mL of 96% ethanol, and stirred until completely dissolved. [16,38]

Non-Solvent Phase

A total of 700 mg of poloxamer 188 was dissolved in 10 mL of 96% ethanol using a magnetic stirrer until a clear solution was obtained [16,38].

Nanoparticle Synthesis

The solvent phase was added dropwise into the non-solvent phase under continuous stirring at 700 rpm. After 15 minutes of stirring, 0.5 mL of 2% glutaraldehyde is dropped into the solution and stirred for the next 17 hours. After that, the nanoparticle suspension was cleaned by centrifuging and purified using purified water twice for 30 minutes. After the centrifuging process, the sediment at the bottom of the tube is collected and freeze-dried [16,38].

Particle Size and Polydispersity Index (PDI)

A 1 mL aliquot of the nanoparticle suspension was transferred into a 10 mL volumetric flask and diluted to volume with purified water. The particle size and polydispersity index were measured using a particle size analyzer (Malvern Instruments, Worcestershire, UK). Measurements were performed in triplicate [16,38].

Zeta Potential

A 1 mL sample of the nanoparticle suspension was diluted to 10 mL with purified water in a volumetric flask. Zeta potential was measured using a Zetasizer (Malvern Instruments, Worcestershire, UK), and the analysis was conducted in triplicate [16,38].

Entrapment Efficiency (EE)
Gelatin nanoparticles are synthesized using the desolvation method with concurrent drug incorporation. Following formulation, the suspension is centrifuged to separate the nanoparticles from the supernatant containing unentrapped (free) drug. The concentration of free drug in the supernatant is quantified using UV-Vis spectrophotometry. Entrapment efficiency (EE) is then calculated using the following equation:

$$EE\ (\%) = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100$$

Antioxidant Activity
Ten milligrams of the nanoparticles were dissolved in 10 mL of pro-analysis grade methanol to obtain a 1000 ppm stock solution. Aliquots of 50, 100, 150, 200, and 250 µL were transferred into test tubes and diluted with methanol to a final volume of 5.0 mL, yielding concentrations of 10, 20, 30, 40, and 50 ppm, respectively. To each test tube of 1 mL of a 0.4 mM DPPH solution, add methanol to adjust the final volume to 5.0 mL. The mixtures were homogenized, covered with aluminum foil, and incubated at 37 °C for 30 minutes before absorbance was measured [36,37].

2.4 Formulation and Evaluation of Serum Spray
The required ingredients, including SM leaf extract loaded-nanoparticles, hydroxyethyl cellulose, phenoxyethanol, and propylene glycol, were weighed according to the formulation. Hydroxyethyl cellulose was dispersed in 10 mL of purified water and allowed to swell as a gel base. The nanoparticle extract was dissolved in 30 mL of propylene glycol and gradually added to the gel base under continuous stirring using a homogenizer (IKA RW 20, Staufen, Germany). Phenoxyethanol was dissolved in purified water, followed by the addition of purified water to a final volume of 100 mL, and then stirred to homogeneity [16].

Antioxidant Activity of Serum Spray Using the DPPH Assay
Ten milligrams of the serum spray were dissolved in 10 mL of pro-analysis grade methanol to obtain a 1000 ppm stock solution. Aliquots of 325, 400, 475, 550, and 625 µL were transferred into test tubes and diluted with methanol to a final volume of 5.0 mL, resulting in concentrations of 65, 80, 95, 110, and 125 ppm, respectively. Each test tube received 1 mL of a 0.4 mM DPPH solution. After that, add methanol to bring the total volume to 5.0 mL. The mixtures were homogenized, covered with aluminum foil, and incubated at 37 °C for 30 minutes before absorbance was measured [16].

Organoleptic Properties
The organoleptic properties of the serum spray formulations—such as its color, odor, and physical appearance—were evaluated manually through sensory observation [16]

Homogeneity
Serum homogeneity was evaluated by spraying it onto a glass slide, ensuring even distribution, and visually inspecting the spread for uniformity [16].

Viscosity
Viscosity was determined using a Brookfield rotational viscometer (Model RV; Brookfield Engineering, Middleboro, MA, USA). This instrument operates by rotating a spindle within the fluid and recording the torque necessary to maintain a constant rotational speed. The measured torque, along with the spindle specifications and rotation rate, is used to compute the dynamic viscosity of the fluid, typically reported in centipoise (cP) [16].

Spray Pattern
The spray pattern test of the serum was to determine the spray's shape and uniformity during device activation. This analysis helps ensure the drug is delivered effectively to the intended target area and can be used to identify potential issues with the device or formulation. The spray is achieved by dispensing the serum onto a flat surface from varying distances, ranging from 3 to 20 cm. The diameter of the resulting spray and distribution was to determine the uniformity and effectiveness of the spray mechanism [16].

Dry Time
The dry time refers to the period required for a spray serum to dry after application to the inner forearm [16].

pH Measurement
The pH of the serum spray was measured using a calibrated pH meter. Calibration was at pH 7.0 and pH 4.0. After each measurement, the electrode was rinsed thoroughly with purified water to maintain accuracy and prevent contamination. This essential step removes any residual buffer, preventing contamination and ensuring an accurate reading for further

measurement. A 100 mL of serum sample in a glass beaker, the pH value was recorded only after a stable reading, confirming a precise and reliable result. [16]

Stability Testing

The stability tests of serum spray were determined using a cycling test method. The serum spray was stored at 4 °C for 24 hours, followed by storage at 40 °C for an additional 24 hours. Each cycling test was performed six times for 12 days. The formulations were evaluated for changes in appearance, homogeneity, and pH after each cycle [19,39].

The Franz Diffusion Cell Method [40,41,42]

This method is a gold-standard in vitro technique for evaluating drug penetration through skin or membrane models. It is widely used in pharmaceutical, cosmetic, and dermatological research to simulate transdermal or topical delivery.

The Franz cell penetration method consists of: a. Setup: The Franz cell consists of two chambers; b. Donor chamber: Contains the drug formulation; c. Receptor chamber: Filled with a buffer solution (e.g., PBS) that mimics physiological conditions; d. A membrane (synthetic or biological, such as rat skin or Strat-M®) separates the two chambers.

Procedure: a. The membrane is mounted between the chambers; b. The receptor chamber is stirred and maintained at a controlled temperature (typically 32–37 °C); c. The drug formulation is applied to the donor chamber; d. Samples are withdrawn from the receptor chamber at set intervals to measure drug permeation using UV–Vis spectrophotometry or HPLC.

Key Parameters: a. Flux (J): Rate of drug permeation per unit area; b. Cumulative amount permeated: Total drug that has crossed the membrane; c. Lag time: Time before steady-state permeation begins.

3. RESULTS AND DISCUSSION

Table 1. Determination of the degree of fineness

Simplicia	Passed sieve #4	Requirement	Passed sieve #18	Requirement
SM leaves	100%	100%	35.62%	≤ 40%

Table 2. Yield and DER-Native of the extract

Simplicia (g)	Extract weight (g)	Yield (%)	DER-Native
1001.70	407.71	40.70	2.46

Table 3. pH of the extract

Replication 1	Replication 2	Replication 3	Average ± SD
3.49	3.52	3.55	3.52 ± 0.03

Table 4. Antioxidant activity (IC50) of SM leaf extract

Concentration (ppm)	Inhibition (%)			IC50 (ppm)			
				1	2	3	Ave.±SD
4	16.12	16.18	17.14				
8	24.08	27.36	28.41				
12	39.64	40.92	41.97	16.41	15.89	15.57	15.96 ± 0.42
16	50.31	51.93	52.78				
20	58.59	59.53	60.31				

Table 5. Particle size (nm, left)) and polydispersity index (right) of the nanoparticles

Particle size (nm)				Polydispersity index			
1	2	3	Ave.±SD	1	2	3	Ave.±SD
222.9	239.9	295.3	252.7±37.9	0.380	0.439	0.558	0.459 ± 0.091

Table 6. Zeta potential of the nanoparticles

Replication 1	Replication 2	Replication 3	Average ± SD (mV)
−24.8	−25.6	−31.3	−27.2 ± 3.5

Table 7. Entrapment efficiency of the nanoparticles

Replication 1	Replication 2	Replication 3	Average ± SD (mV)
47.91	48.07	47.76	47.91 ± 0.16

Table 8. Antioxidant activity (IC50) of SM leaf extract loaded-nanoparticles

Concentration (ppm)	Inhibition (%)			IC50 (ppm)				
				1	2	3	Ave.±SD	
10	22.34	22.23	22.68					
20	33.79	33.93	33.49					
30	47.08	47.48	47.64	32.79	32.54	32.43	32.59 0,18	±
40	59.58	60.07	60.38					
50	70.24	70.65	70.84					

Table 9. Antioxidant activity (IC50) of serum spray formulations

Concentration (ppm)	Inhibition (%)			IC50 (ppm)				
				1	2	3	Ave.±SD	
EbSS (Serum spray containing SM leaf extract)								
65	60.02	59.48	59.45					
80	62.35	62.67	62.69					
95	64.34	64.59	64.83	33.53	31.47	31.88	32.29 1.09	±
110	68.26	69.16	69.44					
125	76.77	75.32	75.47					
NbSS (Serum spray containing SM nanoparticles)								
65	51.83	51,78	52,07					
80	57.07	57,03	57,10					
95	62.42	62,42	62,73	60.03	60.26	59.66	59.98 0.30	±
110	66.76	66,73	66,92					
125	73.32	73,44	73,70					

Table 10. Organoleptic of serum spray formulations

Parameters	Blank	NbSS	EbSS
Form	Liquid	Liquid	Liquid
Color	Colorless	Colorless	Pale yellow
Odor	Less odor	Less odor	Distinctive odor

n = 3

Table 11. Homogeneity of the serum spray formulations

Formulations	Homogeneity (n=3)
Blank	Homogeneous
NbSS	Homogeneous
EbSS	Homogeneous

Table 12. Viscosity of the serum spray formulations

Formulation	Replicate 1 (cP)	Replicate 2 (cP)	Replicate 3 (cP)	Average ± SD (cP)
Blank	24.0	19.2	19.2	20.80 ± 2.77
NbSS	19.2	24.0	14.4	19.20 ± 4.80
EbSS	14.4	24.0	28.8	22.40 ± 7.33

Table 13. Spray pattern of the serum spray formulations

Parameter	Formulation	Spraying distance (cm)				
		3	5	10	15	20
Spray pattern	Blank	Clumped	Slight clumped	Spread	Spread	Spread
	NbSS	Clumped	Slight clumped	Spread	Spread	Spread
	EbSS	Clumped	Slight clumped	Spread	Spread	Spread
Diameter (cm)	Blank	3.0	4.0	6.4	7.8	12.5
	NbSS	3.3	4.8	7.2	8.1	13.0
	EbSS	2.5	3.7	7.7	7.7	12.6
Spray weight (g)	Blank	0.12	0.14	0.16	0.15	0.17
	NbSS	0.12	0.13	0.15	0.15	0.16
	EbSS	0.13	0.16	0.17	0.16	0.17

Table 14. Results of Dry Time Test

Formulations	Dry time 1 (second)	Dry time 2 (second)	Dry time 3 (second)	Average ± SD
Blank	154	153	155	154 ± 1.0
NbSS	149	148	148	148.3 ± 0.6
EbSS	163	167	168	166 ± 2.6

Table 15. Organoleptic test result before and after stability testing

Formulation	Condition	Form	Color	Odor
Blank	Before	Liquid	Colorless	Less odor
	After	Liquid	Colorless	Less odor
NbSS	Before	Liquid	Colorless	Less odor
	After	Liquid	Colorless	Less odor
EbSS	Before	Liquid	Pale yellow	Distinctive odor
	After	Liquid	Pale yellow	Distinctive odor

n = 3

Table 16. Formulation pHs before and after stability testing

Formulation	Condition	pH
Blank	Before	7.39 ± 0.03
	After	7.00 ± 0.02
NbSS	Before	7.13 ± 0.03
	After	6.91 ± 0.03
EbSS	Before	7.09 ± 0.08
	After	6.91 ± 0.07

Table 17. Viscosity test results before and after stability testing

Formulation		Viscosity (cP) before	Viscosity (cP) after
Blank	n = 3	24.0, 19.2, 19.2	14.4, 14.4, 14.4
	Average ± SD	20.8 ± 2.77	14.4 ± 0
NbSS	n = 3	19.2, 24.0, 14.4	14.4, 19.2, 9.6
	Average ± SD	19.2 ± 4.8	14.4 ± 4.8
EbSS	n = 3	19.2, 24.0, 14.4	14.4, 14.4, 19.2
	Average ± SD	19.2 ± 4.8	16.0 ± 2.77

Table 18. Comparison of flux, permeability constant, and diffusion coeffient of NbSS and EbSS

Parameter		EbSS	NbSS
Flux (J)		1.7171 µg/cm²·min	1.7931 µg/cm²·min
Permeability constant	(Kp)	2.776×10^{-5} cm/min	2.899×10^{-5} cm/min
Diffusion coefficient	(D)	3.331×10^{-5} cm²/min	3.479×10^{-5} cm²/min

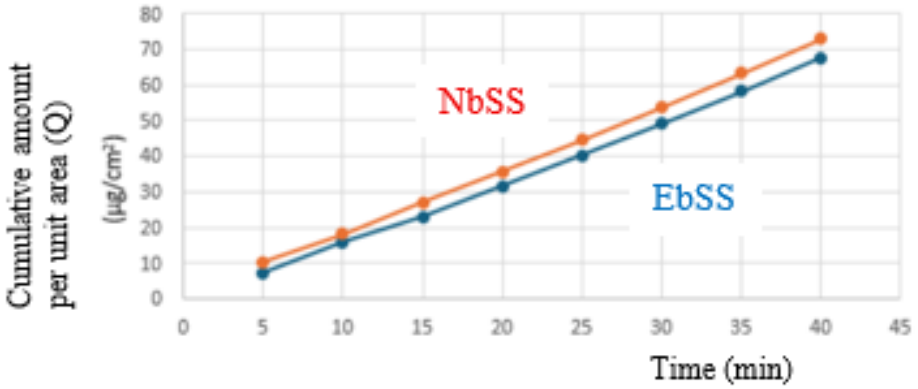

Figure 1. Graph of the relationship between time and Q

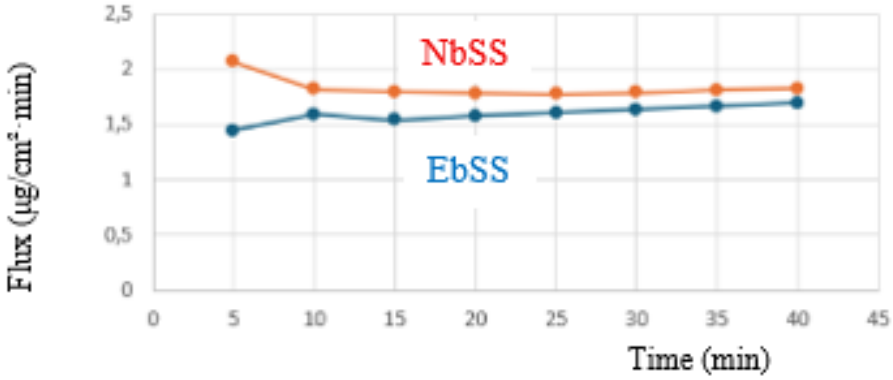

Figure 2. Graph of the relationship between time and flux

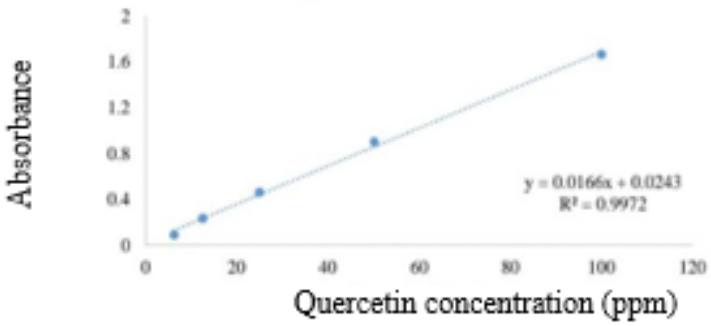

Figure 3. Graph of calibration curve of quercetin at 430 nm

3. DISCUSSION

3.1 Plant Preparation

Determining the Degree of Fineness of Simplicia

Determining the degree of fineness of simplicia powder is a critical step in ensuring optimal extraction efficiency and formulation performance. Particle size directly influences the surface area available for solvent interaction, which affects the diffusion rate of analytes during extraction. If the powder is too fine, it may clog filters and slow down the filtration process, leading to operational inefficiencies. Conversely, overly coarse powder reduces the contact surface area, resulting in suboptimal diffusion and lower yield of active constituents. According to standard herbal pharmacognosy practices, a fineness degree of 4/18 is acceptable for many plant-based materials. This specification requires that 100% of the powder passes through sieve no. 4 (4.75 mm), ensuring uniformity, while no more than 40% passes through sieve no. 18 (1.0 mm), preventing excessive fineness that could hinder processing. In this study, the powder from SM leaves met the required specifications, with 100% passing sieve no. 4 and 35.62% passing sieve no. 18, indicating a suitable particle size distribution for further extraction and formulation (Table 1). [30,31]

3.2 Preparation and Evaluation of SM Dry Extract

The extraction of SM leaf simplicia was by the maceration method with 70% ethanol. This solvent concentration was selected for its optimal polarity, enabling the selective extraction of bioactive compounds such as flavonoids, tannins, and phenolics, while minimizing the co-extraction of non-target constituents. Ethanol at 70% has been shown to yield extracts with superior purity and enhanced biological activity due to its balanced polarity and selective solubility for key phytochemicals. [30,43-45] Maceration was chosen for its simplicity, cost-effectiveness, and suitability for thermolabile compounds, as it avoids thermal degradation by operating at ambient temperatures. This method is widely applied in phytochemical research to preserve the structural integrity of sensitive secondary metabolites, including flavonoids and phenolics. [29,45] After 48 hours of soaking, the mixture is filtered through filter paper to separate the liquid extract from the plant residue. The resulting filtrate was dried using a rotary vacuum evaporator at 40 °C and 200 rpm, allowing for gentle solvent removal without compromising the stability of heat-sensitive compounds. [44]

Organoleptic Examination

Organoleptic examination of the thick extract of SM leaves was conducted by evaluating its visual appearance (color), olfactory characteristics (odor), and physical consistency (texture). The extract had a dark red hue, a distinctive tea-like aroma, and a viscous, semi-solid consistency, indicative of the presence of polyphenolic compounds such as flavonoids and tannins, which are known to impart such sensory attributes. [45,46]

Extract Yield and Drug Extract Ratio (DER-Native)

The extraction process yielded 407.71 g of thick extract from 1001.7 g of dried SM leaf simplicia, resulting in a yield of 40.70%. The calculated Drug Extract Ratio-Native (DER-Native) was 2.46, indicating that 2.46 grams of raw material are required to produce 1 gram of extract (Table 2). Yield is a critical parameter in phytopharmaceutical development, reflecting the efficiency of solvent penetration and compound solubilization during an extraction. A higher yield generally suggests a greater recovery of bioactive constituents, assuming minimal co-extraction of inert materials. [29] The DER-Native value is essential for standardizing extract production, ensuring reproducibility, and facilitating dosage calculations in both research and formulation development. It serves as a quantitative benchmark for comparing extraction efficiency across different solvents, methods, and plant matrices. [29,47]

pH of the Extract

The pH of the thick extract of SM was measured across three replications, yielding an average value of 3.52 ± 0.03, which reflects an acidic profile (Table 3). This acidity is related to the deliberate addition of 1% hydrochloric acid (HCl) during the maceration process. Acidification of the extraction medium is a well-established strategy to enhance the solubility and release of phenolic and alkaloid compounds, many of which exhibit enhanced extractability under low pH conditions due to increased protonation and disruption of plant cell walls. [16,29] The acidic environment also helps stabilize certain polyphenols, preventing oxidative degradation during extraction. [48]

Antioxidant Test of Extract with DPPH Assay

The antioxidant activity of the SM leaf extract was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted method for assessing free radical inhibition potential. The extract demonstrated an IC_{50} value of 15.96 ppm, indicating very potent antioxidant activity, as per established classification criteria where IC_{50} values below 50 ppm are considered highly potent (Table 4). This strong antioxidant capacity is related to the presence of phenolic compounds, flavonoids, and tannins, which are known to donate hydrogen atoms or electrons to neutralize free radicals. Previous studies have confirmed that SM red leaf extracts contain high levels of these phytochemicals, contributing to their radical-scavenging efficacy. [16,45]

3.3 Evaluation of SM Leaf Extract-Loaded Nanoparticles

Nanoparticle Size and Polydispersity Index

The physical characterization of SM leaf extract loaded-nanoparticles revealed an average particle size of 252.7 nm and a polydispersity index (PDI) of 0.459 (Table 5). These results indicate that the nanoparticles fall well within the nanometric range (<1000 nm), suitable for enhanced bioavailability and cellular uptake. The PDI value, which reflects the uniformity of particle size distribution, is below the critical threshold of 0.5, suggesting a moderately narrow and acceptable size distribution for pharmaceutical and cosmetic applications. Nanoparticles with PDI values between 0.1 and 0.5 generally have good homogeneity, which is essential for consistent performance, stability, and reproducibility in formulation systems. The particle size and distribution are affected by factors such as polymer type, extract concentration, and crosslinking conditions during the synthesis process. [7,10,49]

Zeta Potential

The zeta potential of SM leaf extract loaded-nanoparticles was measured across three replications, yielding an average value of −27.2 ± 3.5 mV (Table 6). Zeta potential reflects the surface charge of particles in suspension and is a key indicator of colloidal stability. A value of this magnitude suggests that the nanoparticles exhibit moderate electrostatic repulsion, which helps prevent aggregation and maintains uniform dispersion in the formulation. Zeta potential values greater than ±30 mV are typically associated with high colloidal stability, while values between ±20 and ±30 mV are moderately stable

when supported by steric stabilization or polymeric coatings. The result indicates that the nanoparticles are sufficiently stable for pharmaceutical and cosmetic applications, with minimal risk of particle aggregation under standard conditions. [10,50]

Entrapment Efficiency

The entrapment efficiency values obtained from three replicates—47.91, 48.07, and 47.76 mV—demonstrate consistent performance of the nanoparticle formulation, with minimal variation across samples. The calculated mean entrapment efficiency was 47.91 ± 0.16 mV, indicating high reproducibility and uniform drug incorporation within the gelatin nanoparticle matrix. The low standard deviation (SD = 0.16) reflects the stability of the preparation method and suggests that the desolvation technique used was effective in achieving homogeneous particle characteristics. These results support the reliability of the formulation for further optimization and potential therapeutic application (Table 7). [51]

Antioxidant Test

The antioxidant activity of SM leaf extract loaded-nanoparticles was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a standard method for assessing free radical inhibition. The nanoparticles exhibited an IC_{50} value of 32.59 ppm (Table 8), indicating highly potent antioxidant activity, as per established classification criteria where IC_{50} values below 50 ppm are considered highly potent. The enhanced antioxidant performance of the nanoparticle formulation is likely due to improved surface area, bioavailability, and stability of phenolic compounds encapsulated within the nanostructure. Previous studies have shown that SM red leaf extracts are rich in flavonoids, tannins, and phenolics, which contribute significantly to their radical-scavenging capacity. [10,16,45]

3.4 Serum Spray Formulation Analysis

The serum spray formulations consisted of three variants: Blank (control, without active extract), NbSS (nanoparticle-based, containing gelatin nanoparticles loaded with SM leaf extract), and EbSS (extract-based, containing crude ethanol extract of SM). Both NbSS and EbSS utilized a 300× IC_{50} concentration of the extract to ensure potent antioxidant activity and enable comparative evaluation between nanoparticle and conventional delivery systems. The base formulation included hydroxyethyl cellulose (HEC) (0.1 g) as a viscosity modifier and film-forming agent, phenoxyethanol (0.5 g) as a preservative, propylene glycol (30 mL) as a humectant and penetration enhancer, and aquadest (ad 100 mL) to complete the aqueous phase. The serum spray format was selected for its lightweight, non-greasy texture, offering even distribution, rapid absorption, and minimal residue—ideal for sensitive or acne-prone skin.

Antioxidant Evaluation of Serum Spray Containing SM Extract and SM-Loaded Nanoparticles Using the DPPH Assay

The antioxidant activity of serum spray formulations was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, a widely accepted method for evaluating free radical inhibition. A concentration series of 10, 20, 30, 40, and 50 ppm was done and measured. The IC_{50} value of 32.29 ± 1.09 ppm for the EbSS is significantly lower than the 59.98 ± 0.30 ppm observed for the NbSS formulation (Table 9). This indicates that the free extract exhibits stronger antioxidant activity under the tested conditions. Although nanoparticle encapsulation is often expected to enhance bioavailability and stability, in this case, the free SM leaf extract demonstrated more effective radical scavenging. This may be due to faster interaction of unencapsulated phytochemicals with DPPH radicals, or possible diffusion limitations imposed by the nanoparticle matrix. It suggests that while nanoparticles offer controlled release and protection, they may reduce immediate antioxidant potency in DPPH assays. Further investigation into release kinetics and in vivo performance is recommended to fully assess therapeutic potential. [53]

Organoleptic of the serum spray formulations

The organoleptic evaluation assesses the visual appearance (color), olfactory characteristics (odor), and physical form of the SM leaf serum spray formulations, including the blank, extract-based (EbSS), and nanoparticle-based (NbSS) variants. All formulations exhibited a liquid consistency and a distinctive odor, attributed to the aromatic compounds present in SM. The EbSS formulation showed a pale-yellow coloration, likely due to the release of flavonoids and tannins into the propylene glycol medium. In contrast, the nanoparticle-based formulation (NbSS) remained colorless, suggesting effective encapsulation of the extract within the gelatin matrix, which may mask the inherent pigment of the raw extract (Table 10). These findings are consistent with previous studies that determined the physical characteristics of topical formulations. The extract-loaded systems exhibited visible coloration and aromatic profiles due to their phytochemical content. [31]

Homogeneity Test

The homogeneity test was conducted to determine the uniform distribution of ingredients within each serum spray formulation. A homogeneous product ensures consistent quality, stability, and efficacy by confirming that all components are well dispersed without phase separation or sedimentation. All tested formulations—including the blank, NbSS, and EbSS—were found to be homogeneous across three replicates (Table 11). This result confirms that the gelling agent (HEC) and solvent system (propylene glycol and aquadest) effectively support uniform dispersion of both extract and nanoparticles. Recent evaluations of Syzygium myrtifolium cream and gel formulations have also demonstrated consistent

homogeneity, even under varied storage conditions. These findings reinforce the stability and reliability of the formulations, supporting their suitability for topical application. [11,54]

Viscosity Test

The viscosity test was to evaluate the flow characteristics of the Syzygium myrtifolium leaf serum spray formulations. Viscosity is a critical parameter in sprayable cosmetic products, as it directly affects sprayability, droplet formation, and user experience. Ideally, the formulation should exhibit a viscosity that is low enough to allow smooth dispensing, yet sufficient to maintain product integrity and skin adherence. According to established literature, the acceptable viscosity range for serum spray preparations is 12.8–65.8 cP. The measured viscosities for each formulation are summarized below. All formulations fall within the recommended viscosity range, confirming their suitability for spray application. Notably, EbSS exhibited a slightly higher average viscosity compared to NbSS. This difference may be related to the concentrated nature of the crude extract, which contributes to increased solute density and a thicker consistency. In contrast, the nanoparticle formulation likely benefits from encapsulation, which reduces the direct impact of extract viscosity on the overall system (Table 12). These findings are consistent with recent studies on MS topical formulations. The viscosity varies depending on extract concentration, delivery system, and polymer interaction. [11,54,55]

Spray Pattern

At 3 cm, all formulations produced clumped spray patterns, with limited dispersion and smaller diameters. As the spray distance increased, the patterns became more uniform and spread out, particularly above 10 cm onward, indicating optimal atomization and coverage. Both spray distance and formulation viscosity influence this progression—higher viscosity tends to produce tighter, less dispersed patterns due to reduced flowability. Among the formulations, EbSS showed slightly higher viscosity and smaller initial spray diameters, likely due to the concentrated nature of the crude extract. In contrast, NbSS demonstrated more consistent dispersion, which may be related to the encapsulation of active compounds and lower viscosity (Table 13). The spray weight—used to quantify the amount of formulation dispensed per spray—ranged from 0.12 to 0.17 g, with all formulations falling within the acceptable range of 0.11–0.35 g for cosmetic spray products. These results confirm that the applicator delivers a consistent and adequate dose across varying distances. [11,16,55]

Dry Time Test

The dry time test was to evaluate how quickly the SM serum spray formulations evaporate and lose surface moisture after application. A formulation is to be dry when it no longer exhibits stickiness or visible wetness, ensuring comfort and usability for topical application. The test was on three formulations—Blank, NbSS, and EbSS—with results recorded in seconds. All formulations dried within 5 minutes, meeting the standard requirement for sprayable cosmetic products. Among the three, NbSS demonstrated the fastest drying time, likely due to its lower viscosity and more efficient dispersion. In contrast, EbSS showed a slightly longer drying time, which may be attributed to the higher solute concentration and denser extract matrix, slowing evaporation. These findings are consistent with recent evaluations of SM topical formulations. Dry time was affected by formulation viscosity, solvent composition, and concentration of active ingredient. [11,16]

Stability (Cycling) Test

The stability test utilizes an innovative cycling method to effectively assess how the organoleptic properties of a preparation—such as color, odor, and texture—change over time. By carefully analyzing pH and viscosity across various environmental conditions, we can guarantee superior product quality and reliability, providing customers with the confidence they deserve.

Organoleptic tests. Organoleptic tests were performed both before and after the stability cycling test to evaluate changes observed during the stability assessments. [56] The test results are in the Table 15 below.

The organoleptic evaluation conducted before and after the stability cycling test revealed consistent sensory characteristics across all formulations, indicating no significant changes in form, color, or odor. Both the Blank and NbSS formulations maintained a colorless appearance and less odor, suggesting that their physicochemical integrity remained unaffected by temperature fluctuations. This stability is crucial for ensuring product consistency, especially in formulations intended for long-term storage or variable environmental conditions. In contrast, the EbSS exhibited a pale-yellow coloration and a distinctive odor both before and after the test. These attributes are likely inherent to the extract components and not indicative of degradation. The absence of further changes post-cycling suggests that the extract formulation is also organoleptically stable, with its sensory profile remaining intact despite thermal stress. Overall, the results support the conclusion that all tested preparations—regardless of base composition—demonstrate organoleptic stability under accelerated conditions. This reinforces their suitability for continued development and potential commercialization, particularly in applications where sensory consistency is critical for user acceptance and regulatory compliance.

pH test analysis. Conducting pH testing on formulations is essential both before and after stability assessments. This dual-phase approach enables the identification of any significant chemical changes that may occur during storage, particularly

following thermal cycling. Table 16 presents the pH values of three formulations—Blank, NbSS, and EbSS—measured before and after the stability test.

All formulations exhibited a slight decrease in pH after stability testing:
1. Blank: 7.39 → 7.00
2. NbSS: 7.13 → 6.91; and
3. EbSS: 7.09 → 6.91.

These minor shifts suggest mild acidification, potentially due to hydrolysis, oxidative degradation, or interactions between excipients and active ingredients during thermal stress. Despite the changes, all final pH values remain within the acceptable physiological range for topical applications (typically pH 4.5–7.5), indicating continued safety and suitability for dermal use [56]. Notably, NbSS and EbSS demonstrated greater pH stability than the Blank formulation. This may reflect the buffering capacity or stabilizing effects of active compounds and encapsulation systems. Previous studies have shown that nanoencapsulation can enhance physicochemical stability by protecting sensitive ingredients from environmental stressors [57].

Statistical analysis was performed using SPSS to evaluate normality, homogeneity, and the impact of storage conditions via two-way ANOVA:
1. Normality Test: Sig = 0.505 (> 0.05), confirming data normality;
2. Homogeneity Test: Sig 0.525 (> 0.05), indicating variance homogeneity.
3. Two-Way ANOVA: Sig = 0.005 (< 0.05), revealing a statistically significant difference in pH values across formulations and storage conditions.

These results support the alternative hypothesis (H_1), confirming that storage temperature (4°C vs. 40°C) significantly affects pH stability, particularly in nano and extract-based formulations. Conducting pH testing on formulations is essential, not only before stability testing but also afterward. This dual approach allows us to identify any significant changes that may occur during the storage period, particularly after the cycling test. The table below clearly outlines the results of pH testing performed both before and after the stability cycling test.

Viscosity test. Conducting viscosity tests on formulations is essential not only before stability assessments but also afterwards. This dual approach allows us to observe any changes that occur during the storage period and following the cycling test. Our findings demonstrate a notable decrease in viscosity after the cycling test, which is significant. This reduction likely arises from interparticle forces as particle separation increases at higher temperatures. An increase in the distance between particles generally leads to a lower viscosity in the formulation [25]. The table below (Table 17) summarizes the viscosity testing results obtained before and after the stability cycling test.

Viscosity trends. Blank: Viscosity dropped significantly after stability testing (from 20.8 ± 2.77 to 14.4 ± 0 cP), suggesting degradation or thinning of the base matrix over time. NbSS:

No change in viscosity before and after stability testing. This implies excellent physical stability, possibly due to nanoencapsulation or optimized particle dispersion. EbSS: Viscosity decreased from 22.4 ± 7.33 to 16.0 ± 2.77 cP. The high SD before stability suggests variability in the formulation, which was reduced post-stability—possibly due to sedimentation or homogenization effects.

Regulatory relevance**.** Consistency in viscosity is crucial for dosage uniformity, especially in topical or liquid formulations. Regulatory bodies like BPOM, FDA, and ICH Q6A emphasize physical stability as a key quality attribute. The nano formulation's stability may support claims of enhanced shelf-life, controlled release, or improved bioavailability, aligning with OECD guidelines for nanomaterials.

The results unequivocally indicate that the viscosity of the blank, NbSS, and EbSS has declined since the cycling test. Moreover, even with this decrease, the average viscosity of this spray serum preparation consistently falls within the acceptable range of 12.8–65.8 cP [24]. The result reinforces the stability and effectiveness of the formulation, emphasizing its suitability for use.

Statistical testing is using SPSS to evaluate normality, homogeneity, and two-way ANOVA tests. The results from the SPSS output for the two-way analysis of variance revealed a significance (Sig) value of 0.537 in the normality test, clearly indicating that the data meet the requirements, as Sig > 0.05. Additionally, the homogeneity test yielded a Sig value of 0.525, further confirming compliance with the criteria, given that Sig > 0.05. The findings from the two-way ANOVA test showed a Sig value of 0.931 (Sig > 0.05), which convincingly suggests that there is no interaction between the formulation used and the storage conditions in determining the viscosity of the preparation. Thus, with a Sig value greater than 0.05, we confidently accept the null hypothesis (H0) and reject the alternative hypothesis (H1).

Penetration Test using Cell Franz

The transdermal penetration of flavonoids was evaluated using the Franz diffusion cell method, a widely accepted in vitro technique for assessing skin permeability in pharmaceutical and cosmetic research [28,40]. This system employs a semi-permeable membrane between donor and receptor compartments, with the receptor medium maintained at 37 ± 0.5°C and continuously stirred to simulate physiological skin conditions and ensure consistent diffusion kinetics [2].

Quantification of the penetrated compound was performed using UV-VIS spectrophotometry at 430 nm, based on a quercetin standard curve ($y = 0.0166x + 0.0243$), a method commonly used for flavonoid analysis due to its sensitivity and specificity [2]. Absorbance readings taken at regular intervals enabled calculation of cumulative penetration per unit area (Q), which increased steadily over time for both formulations.

The nanoparticle-based formulation (NbSS) consistently demonstrated higher Q values than the crude extract (EbSS), indicating enhanced transdermal delivery. This improvement is attributed to the smaller particle size and increased membrane interaction of nanoparticles, which facilitate more efficient traversal of the stratum corneum [2,3].

Supporting data from Table 18 show that NbSS had slightly higher flux (1.7931 µg/cm²·min), permeability constant (2.899×10^{-5} cm/min), and diffusion coefficient (3.479×10^{-5} cm²/min) compared to EbSS. These differences, though modest, suggest that NbSS offers more effective skin penetration due to optimized physicochemical properties.

Scientifically, the improved flux and Kp values reflect better membrane permeability, likely influenced by formulation and excipient interactions. The elevated diffusion coefficient indicates faster molecular movement, possibly due to enhanced solubility or carrier efficiency.

Practically, NbSS may be preferred for cosmeceutical or pharmaceutical applications requiring rapid and efficient delivery. Nonetheless, EbSS remains a viable option where other factors—such as cost, stability, or sensory attributes—are prioritized.

CONCLUSION

The 70% ethanol extract of Syzygium myrtifolium leaves demonstrates remarkable antioxidant efficacy, evidenced by an impressive IC50 value of 15.96 ppm. Then, the extract can be synthesized into gelatin nanoparticles with an IC50 of 32.59 ppm through a precipitation method that meets stringent physical and chemical parameters. The serum spray formulations, which incorporates both the extract and nanoprecipitates, achieved IC50 values of 32.29 ppm (indicative of very potent antioxidant activity) and 59.98 ppm (indicating potent antioxidant activity), respectively. These compelling results suggest that while the spray serum formulation is effective, the antioxidant activity of the formulation with only the 70% ethanol extract of Syzygium myrtifolium leaves remains superior.

The Franz cell penetration test demonstrated that NbSS formulations exhibit superior transdermal delivery characteristics compared to EbSS. This is evidenced by consistently higher values across key parameters: Flux (J): NbSS showed a higher flux than EbSS, indicating a faster rate of flavonoid penetration. Permeability constant (Kp): The increased Kp in NbSS suggests improved membrane permeability. Diffusion coefficient (D): A higher D value in NbSS reflects more efficient molecular diffusion through the membrane. These enhancements are likely due to the reduced particle size in the nanoparticle formulation, which facilitates better interaction with the stratum corneum and promotes deeper skin penetration. Additionally, the consistent stirring and controlled temperature (37 ± 0.5°C) ensured optimal diffusion conditions, minimizing variability.

To further enrich the conclusion, 1. Implications for formulation design: The data support the use of nanoparticle technology to improve bioavailability and skin delivery of flavonoids, making NbSS a promising candidate for topical antioxidant therapies. 2. Potential for controlled release: Nanoparticles may offer sustained release profiles, which could extend therapeutic effects and reduce dosing frequency. 3. Recommendations for future studies: Investigating long-term stability, skin retention, and in vivo efficacy would provide a more comprehensive understanding of NbSS performance.

Author Contributions

Conceptualization: K.K. and L.S.; methodology: K.K. and A.W.Z; investigation: K.K.; formal analysis: K.K. and L.S.; software: K.K., A.W.Z. and L.S.; data curation: K.K., A.W.Z. and L.S.; writing—original draft: A.W.Z.; writing—review and editing: K.K.; project administration: A.W.Z.; funding acquisition: K.K. and A.W.Z. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest
The authors declare no conflicts of interest

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