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Phytochemical characterization, antioxidant activity, and physicochemical properties of *Peronema canescens*-based Granule

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Abstract. *Peronema canescens* Jack leaves are among the herbs with the highest antioxidant activity. Innovative preparations that can extend shelf life and improve the use of *P. canescens* leaves as herbal medicine or as an alternative to bolster functional foods are highly needed. This study assesses the phytochemical content, antioxidant activity, and physicochemical properties of 70% ethanol extract (EE70) and *P. canescens*-based granules at various concentrations (F0, F1, F2, and F3). Total phenolics and flavonoids were quantified, antioxidant activity was evaluated using the DPPH assay method, and physicochemical analysis was performed through FTIR (Fourier Transform Infrared) spectroscopy and morphology analysis. The total phenol and flavonoid contents were highest in the EE70 sample, followed by the F3, F1, and F2 granule samples, which showed significant differences from the F0 granule preparation. Linear trends were also observed in the assessment of antioxidant activity. The EE70 sample demonstrated very strong antioxidant activity, with an IC₅₀ of 32.67 ± 0.19 mg/mL, while the F1, F2, and F3 granules exhibited strong antioxidant activity with IC₅₀ values of 60.29 ± 0.42, 61.04 ± 2.83, and 59.59 ± 1.51 mg/mL, respectively. Physicochemical analysis of the extract samples and all *P. canescens*-based granules revealed the presence of phenolic compounds and similar microstructural features across all *P. canescens* granules. This study shows that the extract and the three *P. canescens*-based granules contain phenolic and flavonoid compounds, exhibiting strong antioxidant activity, and thus represent promising candidates for functional food fortification.



1. Introduction

Indonesia has identified 40,000 species of medicinal plants to date (1). *Peronema canescens* Jack, also referred to as Sungkai or Jati Sabrang, is a widely used medicinal plant, especially by people on the islands of Sumatra and Kalimantan. The community knows that *P. canescens* leaves can be used as medicine for toothaches, ringworm, anti-bloating, and fever (2). Additionally, the Dayak tribe in Kalimantan and the Musi tribe in South Sumatra use *P. canescens* leaves to treat hypertension and hypercholesterolemia. *P. canescens* leaves, as a medicinal plant, are traditionally prepared, often as tea or brewed (3). Because of this, storing the preparation for a long time reduces its effectiveness and quality. The bitter taste in tea or brewed leaves can be unpleasant when consumed. The bitterness of the sungkai leaf brew is caused by a complex reaction between tannins and proteins in the mouth (4).

Qualitative phytochemical analysis indicated that sungkai leaves possess various chemical compounds, including alkaloids, phenolics, flavonoids, tannins, saponins, steroids, and triterpenoids (5). The primary indicator of potential for plant antioxidant activity is the existence of phenolic and flavonoid compounds (6)(7). Assessment of antioxidant activity through the DPPH method revealed that the ethanol extract from sungkai leaves demonstrates a free radical scavenging ability classified as very strong to strong, with IC₅₀ values recorded at 10,489 ppm (8) and 116,786 ppm (9). Methanol extracts from Sungkai leaves demonstrated notable antioxidant properties, with IC₅₀ values varying from 8,667 to 60,263 ppm (10). The antioxidant activity testing results using the DPPH method regarding the extraction of ethyl acetate from Sungkai leaves showed IC₅₀ values that ranged relatively high, from 12,986 ppm (11) to 320 ppm (12). The powerful antioxidant properties of sungkai leaves are probably a result of their high levels of secondary metabolites, particularly phenolic and flavonoid substances.

Innovative preparations that can extend shelf life and improve the use of *P. canescens* leaves as herbal medicine or as an alternative to fortify functional foods are greatly needed. Granules are one of the innovative dosage forms that can be developed. Granules are dosage forms produced using the granulation method. The granulation method is a method of forming larger particles with a binding mechanism (13).

Based on the literature study obtained, the evaluation of bioactivity and physicochemical properties related to granule preparations based on *P. canescens* leaves extract has never been reported. Two of the references collected conducted formulation studies utilising *P. canescens* leaf extract. In contrast to this investigation, neither study utilised granule preparations. Nurfauliyah et al. (14) quantified the total amount of phenolic content (TPC) and the total amount of flavonoid (TFC) in ethanol extracts and nanoemulsion formulations produced by *P. canescens* and conducted antioxidant activity assays. Tarigan et al. (15) conducted a physicochemical analysis and evaluated the antioxidant effectiveness of microencapsulated formulations derived from the ethanol extract of *P. canescens* leaves. Thus, this research intends to assess the phytochemical composition, antioxidant activity, and physicochemical characteristics of 70% ethanol extract (EE70) and *P. canescens*-based granules at various concentrations (F0, F1, F2, and F3). The total amounts of phenolics and flavonoids were measured quantitatively, antioxidant activity was assessed using the DPPH assay method, and the physicochemical properties were examined through FTIR spectroscopy and morphology analysis.

2. Materials and methods

2.1 Plant material and extraction

Peronema canescens leaves are dried and ground to prepare a simplicia sample for extraction. The collection site for the *P. canescens* leaf sample was located in Pino, Bengkulu Selatan Regency, Bengkulu Province. Identification of *P. canescens* leaf samples was carried out at the Plant Identification Laboratory, Directorate of Scientific Collection Management, National Research and Innovation Agency (BRIN) in Cibinong, West Java. The extraction is performed using ultrasound-assisted extraction (UAE) with 70% ethanol solvent at a 1:6 ratio (16). The results from the sonication process are purified and concentrated with the help of a rotary evaporator (BUCHI Rotavapor® R-300). The thick extract obtained from the rotary evaporator is then evaporated on a water bath at a temperature of 50°C for 1 hour (17), and frozen at -80°C for 24 hours before being lyophilised using a BUCHI L-200® freeze dryer. Subsequently, the percentage yield of the obtained extract is determined by utilizing the following formula (18) :

$$\text{Extract yield (\%)} = \frac{\text{weight of the extract after freeze drying (g)}}{\text{weight of the simplicia (g)}} \times 100$$

The water content of extract and *P. canescens*-based granule samples was measured by weighing 1 g of the sample, then placing it into a calibrated porcelain cup, and drying at 100 °c for 1 hour. The sample was placed in a desiccator for 30 minutes and reweighed until the weight remained constant. Water content was calculated using the formula (19):

$$\text{Water content (\%)} = \frac{A - B}{A} \times 100$$

Description:

A: Weight of the sample before heating

B: Weight of the sample after heating

2.2 Granulation and formulation

P. canescens-based granules were produced using the wet granulation method, employing 5 grams of extract per formulation. The ingredients were weighed according to the formula (Table 1). The extract is poured into a mortar, mixed, and stirred with other ingredients until evenly distributed. Other ingredients in question include stevia, maltodextrin, and polyvinylpyrrolidone (PVP), with the percentage amount according to the formula shown in Table 1. Specifically, PVP is first dissolved in 70% food-grade ethanol before being mixed into the granule formula; then, ethanol is added gradually until a mass that can be shaped into granules forms. The granule-shaped mass was then sieved through a mesh with a particle size of 1.41 mm, designated as mesh No. 14. The granule samples were dried in an oven at 50 °c for a duration of 2 hours. After drying, the granules were re-sieved using mesh 16 with a hole diameter of 1.18 mm, following the method by Rosaini et al (20) with minor adjustments. The water content for each granule formula was calculated using the method according to Marpaung et al. (19). The water content requirement for granules is <10% (21).

Table 1. Formulation of *P. canescens*-based granules

Ingredients	Function	Formula (%)			
		F0	F1	F2	F3
<i>P. canescens</i> leaves extract	Active compound	0	5	10	15
Polyvinylpyrrolidone	Binder	5	5	5	5
Stevia	Sweetener	10	10	10	10
Maltodextrin	Filler	Ad 100	Ad 100	Ad 100	Ad 100

2.3 Total phenolic content (TPC)

To quantify the total phenolic content of the extract and granules, this investigation implemented the *Folin-Ciocalteu* reaction method, which was applied with minimal modifications (16). 3.75 mL of distilled water was mixed with 250 mL of *Folin-Ciocalteu* solution to obtain homogeneity, and a 250 μ L portion of a 5 mg/5 mL sample was vortexed. The mixture was kept for 8 minutes at a temperature between 25°C and 28°C. Following incubation, 750 mL of a 20% Na₂CO₃ (w/v) solution in water was added, vortexed, and then incubated for an additional 2 hours in a dark place. Subsequently, 200 μ L of each mixture was transferred to a 96-well microplate. Absorbance was measured at a wavelength of 765 nm utilizing a microplate reader (Multiskan Go, Thermo Scientific™). Absorbance values were evaluated against a standard of gallic acid, encompassing a concentration range from 60 to 300 μ g/mL. The data collected from all experiments were recorded as milligrams of gallic acid equivalent (GAE) per gram of extract and were conducted in triplicate.

2.4 Total flavonoid content (TFC)

The measurement of total flavonoids was conducted using a modified aluminium chloride colorimetric method (16), with minor adjustments to the procedure. A 96-well microplate was used to insert a 10 μ L sample (500 μ g/mL) along with a standard quercetin solution at different concentrations ranging from 25 to 600 μ g/mL. Additionally, 60 μ L of methanol, 10 mL of potassium acetate, 10 mL of aluminium chloride, and 120 mL of distilled water were administered. Following a 30-minute incubation of the resulting mixture, the absorbance was assessed at a wavelength of 415 nm. Quercetin was used as a reference standard, and the total quantity of flavonoids was measured in quercetin equivalents (QE) for every gram of extract.

2.5 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH test for the extract and granule samples was performed using the method outlined in prior studies (22). A total of 20 μ L of the sample or standard solution was combined with 80 μ L of the DPPH reagent in a 96-well microplate. The combination was allowed to rest for 30 minutes at room temperature, ensuring it was shielded from light. Following the incubation period, the absorbance at 517 nm was assessed utilising a microplate reader (Multiskan Go, Thermo Scientific™). Ascorbic acid served as a positive control for the preparation of a standard curve within a concentration range of 1 to 30 μ g/mL. The calculation of antioxidant activity percentage was determined by assessing the capture of DPPH radicals through the equation below:

$$\% \text{ DPPH radical scavenging effect} = \left[\frac{(\text{Abs. control} - \text{Abs. sample})}{\text{Abs. control}} \right] \times 100$$

Subsequently, the antioxidant activity was graphed by showing the percentage of DPPH scavenged against the concentration of standard antioxidants, and the half-maximal inhibitory concentration (IC₅₀) value of the extract was determined through linear regression analysis.

2.6 FTIR spectroscopy analysis

The analysis of FTIR spectra was conducted utilising an attenuated total reflectance (ATR)-FTIR spectrophotometer (Bruker, Vertex 10, Germany) to identify the functional groups present in the molecules derived from the extract and *P. canescens*-based granule sample (23). The spectra of the samples were obtained by positioning the sample on top of the ATR crystal and scanning

within the wavenumber range of 4000–500 cm^{-1} . The spectra were interpreted and analysed using OPUS 8.5 software (Bruker, Germany).

2.7 Morphology analysis

The morphology images of extract and *P. canescens*-based granule samples were analyzed using Scanning Electron Microscopy (SEM, Hitachi SU-3500, Japan) operating at 4.0 kV. The images captured for each sample were at a magnification of 1000x and 500x. For a period of 60 seconds, an Ion Sputter MC 1000 was applied to the samples at 20 mA. Selected images are provided as representative microstructural visuals.

3. Results and discussion

3.1 Yield and water content

Yield is the proportion of extract volume obtained after extraction by weight of the sample used. An extract is considered good if the yield exceeds 10% (24). According to Table 2, the *P. canescens* leaf extract obtained through the UEA method with a 70% ethanol solvent is considered satisfactory, as it has a yield value of greater than 10%. The yield percentage obtained in this study was 14.75%. This result shows a yield percentage value that closely resembles the research carried out by Sari et al. (25). Through the maceration technique with 70% ethanol solvent, the yield percentage of *P. canescens* leaf extract obtained by Sari et al (25) was 14%. Mahmudah et al. (26) also conducted research using the same samples and types of solvents but with a different method, which yielded a smaller percentage, namely 8%. Latief et al (27) reported a smaller percentage yield (13.7%) in their extraction of *P. canescens* leaves from Jambi province, despite using the same solvent as this study.

Differences in the percentage yield of an extraction process can be attributed to several factors. Several factors that influence the percentage yield value include the method used, sample type, solvent type, particle size, sample weight, and solvent. Additionally, the use of temperature and the length of extraction time also affects the percentage yield value (28). Another essential factor that impacts the percentage yield of the extraction process is the region of origin of the plant source. The origin of the plant affects the variety and quantity of metabolites it contains. Plants in regions with high ecological stress will produce more metabolic compounds than those with abundant nutrients (16).

Table 2. Yield and water content of the *P. canescens* leaves extract and *P. canescens*-based granules

Sample	Yield (%)	Water content (%)
EE70	14.75	3,96 ± 0,17
F0		1,26 ± 0,04
F1	-	1,69 ± 0,03
F2		1,43 ± 0,00
F3		1,32 ± 0,03

Note: The water content (%) presented is the average and standard deviation

The granules produced in this investigation exhibited an average water content of 1% (Table 2). This value indicates that the water content of the granule meets the criteria for optimal moisture levels, which is less than 10% (21). This study used the wet granulation process. The wet granulation method combines active compounds with supplementary components and

requires the addition of a liquid, making it suitable for active ingredients that remain stable at elevated temperatures. Wet granulation offers enhanced compressibility and excellent homogeneity, hence minimising the risk of component separation during the granulation process (29). The water content was measured based on the results of the collected granules. The Indonesian Pharmacopeia stipulates a water content requirement of 2-4%, which can influence the quality of the granule. This determination of water content aims to mitigate the risk of granule contamination by bacteria and fungi during storage, thereby ensuring granule stability(30).

3.2 TPC, TFC, and antioxidant activity

Table 3 displays the results for total phenolic content (TPC) and total flavonoid content (TFC). The measurements for both TPC and TFC in the extract and *P. canescens*-based granules derived from sungkai leaves showed a consistent, linear pattern. The greatest TPC results were shown in the *P. canescens* leaf extract with a value of 184.32 ± 0.16 mg GAE/g, and the greatest TFC levels were likewise obtained from the extract sample with a value of 73.49 ± 3.84 mg QE/g. For the four granule formulas, the highest TPC (168.91 ± 0.69 mg GAE/g) was shown in sample F3 with the highest *P. canescens* leaf extract content (15%), and the highest TFC levels from granule samples were also shown in sample F3 with a value of 44.80 ± 3.13 mg QE / g.

The present study employed the DPPH assay method to evaluate antioxidant activity, with results expressed as IC₅₀ values (Table 3). The IC₅₀ value indicates that the sample could inhibit 50% of the free radical compounds used. The greater the antioxidant activity, the lower the IC₅₀ value (31). The IC₅₀ values of the *P. canescens* leaf extract sample utilized in this research were found to be 32.67 ± 0.19 mg/mL using the antioxidant activity test. Furthermore, the granule samples that had been formulated produced antioxidant activity from the weakest to the strongest, which were expressed in the IC₅₀ values, respectively, namely sample F0, 153.50 ± 22.74 mg/mL; F2, 61.04 ± 2.83 mg/mL; F1, 60.29 ± 0.42 mg/mL; and F3, 59.59 ± 1.51 mg/mL.

Table 3. TPC, TFC, and antioxidant activity (IC₅₀) of the *P. canescens* leaves extract and *P. canescens*-based granules

Sample	TPC (mg GAE/g)	TFC (mg QE/g)	IC ₅₀ (mg/mL)
EE70	184.32 ± 0.16^a	73.49 ± 3.84^a	$32,67 \pm 0,19^c$
F0	17.39 ± 0.08^e	1.58 ± 0.15^c	$253,50 \pm 22,74^a$
F1	153.36 ± 0.86^c	42.00 ± 2.97^b	$60,29 \pm 0,42^b$
F2	147.67 ± 1.11^d	41.93 ± 0.63^b	$61,04 \pm 2,83^b$
F3	168.91 ± 0.69^b	44.80 ± 3.13^b	$59,59 \pm 1,51^b$
Standard Ascorbic acid	-	-	$10,61 \pm 0,16^e$

Note: Each value represents the mean of triplicate measurements, and different superscripted letters in the same column signify notable variations.

The extract of *P. canescens* leaves contains a number of secondary metabolites, such as flavonoids and phenolic components. These substances are the main secondary metabolites found in plants. Both the antioxidant and free radical-scavenging capabilities of these classes are well-known (14). The possible effects of flavonoid chemicals, which are secondary metabolites with antioxidant properties, depend on the quantity and location of free hydroxyl (OH) groups. By giving hydrogen atoms to free radicals, flavonoid molecules act as important antioxidant components that neutralize them. Furthermore, their molecular structures are characterized by features that are particularly conducive to free radical scavenging, thereby implying potential therapeutic benefits against diseases mediated by free radicals (32). Quercetin, catechins, and

other flavonoid chemicals are well-known for their antioxidant properties and ability to neutralize free radicals (33). Suryani et al. (16), demonstrated the presence of the quercetin 3,7-dimethyl ether compound in the ethanol extract of *P. canescens* leaves through secondary metabolite identification techniques using UHPLC-HRMS instruments. The presence of this quercetin compound can be one of the factors causing strong antioxidant activity from *P. canescens* leaf extract, as well as from *P. canescens*-based granules.

3.3 FTIR spectroscopy

In this study, *P. canescens* leaf extract and four *P. canescens*-based granule formulas with different concentrations were analysed using FTIR to identify functional groups related to the contained substances. Figure 1 shows the FTIR analysis results of *P. canescens* leaf extract (EE70) and *P. canescens*-based granules at various concentrations (F0, F1, F2, and F3). Overall, the FTIR spectra of *P. canescens* leaf extract and the granules containing it displayed similar patterns. The only notable variation was observed in sample F0, which was a granule formulation without the addition of *P. canescens* extract. However, in general, several peaks observed in the formula containing *P. canescens* extract were also present in sample F0. This is likely due to the addition of stevia in the granule formula. The identification of functional groups in the extract of *Stevia rebaudiana* revealed the presence of alcohol/phenol, carboxylic acids, alkanes, and aldehydes (34). The similarity of the same peaks in sample E70 with samples F1, F2, and F3 demonstrates that the FTIR analysis method can effectively identify key compound groups in natural materials or plants. Additionally, FTIR is recognized as a fingerprinting technique, implying that distinct samples will produce unique FTIR spectra. This characteristic was examined using a highly sensitive infrared spectroscopy technique in the wavenumber range of 4000 to 500 cm^{-1} .

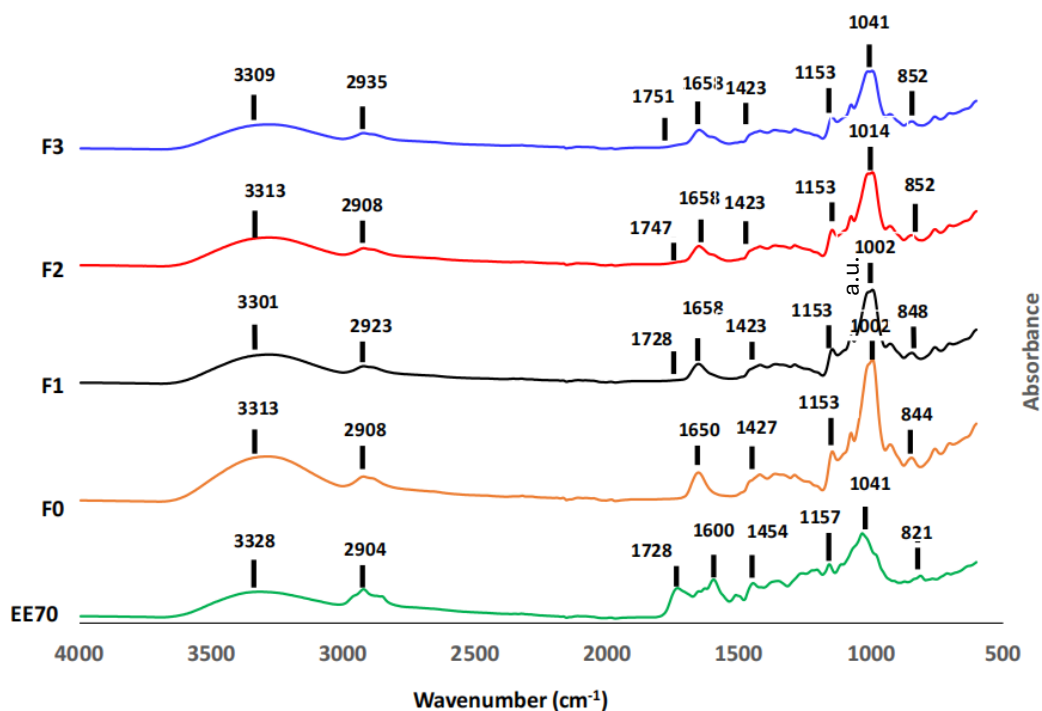


Figure 1. FTIR spectra of *P. canescens* leaf extract (EE70) and *P. canescens*-based granule (F0 = 0 % of *P. canescens* leaf extract; F1 = 5 % of *P. canescens* leaf extract; F2 = 10 % of *P. canescens* leaf extract; F3 = 15 % of *P. canescens* leaf extract).

Previous studies by Windarsih et al. (35) guided the interpretation of FTIR vibrations. The absorption of the –OH hydroxyl (phenol/alcohol group), which may come from carbohydrates or other substances, such as ketones and carboxylic acids, is linked to the vibration in the range of 3200-3550 cm^{-1} . Stretching vibrations of –CH, –CH₂, and –CH₃ were seen in the peaks ranging from 2850 to 2990 cm^{-1} . The C=C (alkene) bond can be detected in the range of 1600-1680 cm^{-1} . This vibration may also originate from the amide vibration, primarily linked to protein molecules. Furthermore, absorption at around 1000–1157 cm^{-1} was associated with the stretching vibration of the C–O bond. The bending vibrations of –CH, CH₂, and CH₃ were observed at wavenumbers of 1423-1454 cm^{-1} , 900-1000 cm^{-1} , and 800-900 cm^{-1} , respectively. According to the above results, the FTIR spectra support the presence of phenolic compounds in the *P. canescens* leaf extract and four *P. canescens*-based granule formulas with varying concentrations.

3.4 Morphology analysis

In this study, morphological evaluations were conducted on *P. canescens* leaf extract samples and four *P. canescens*-based granule formulations. All granule formulations were examined using a scanning electron microscope. The extract concentrations used in the granule formulations were 0% (F0), 5% (F1), 10% (F2), and 15% (F3). Figure 2a displays a photograph of granules derived from *P. canescens* extract. The image shows an increase in the green color associated with increasing extract percentages used in the granule formulations. Figure 2b illustrates the morphological characteristics of *P. canescens* leaves and four different granule formulations prepared with varying concentrations of *P. canescens* extract.

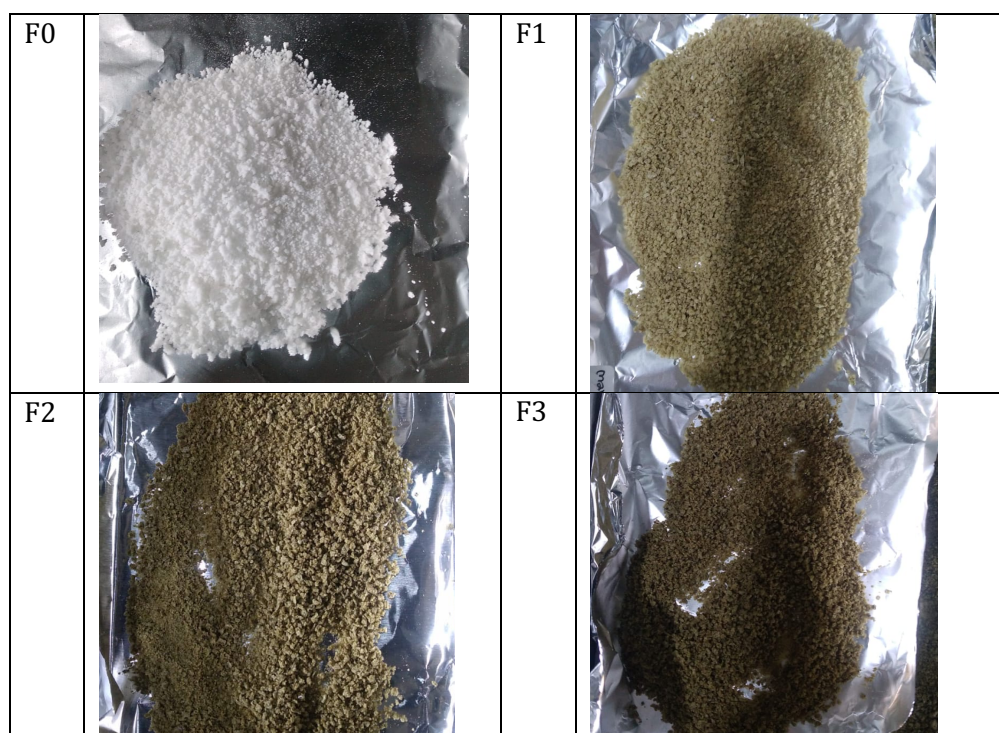
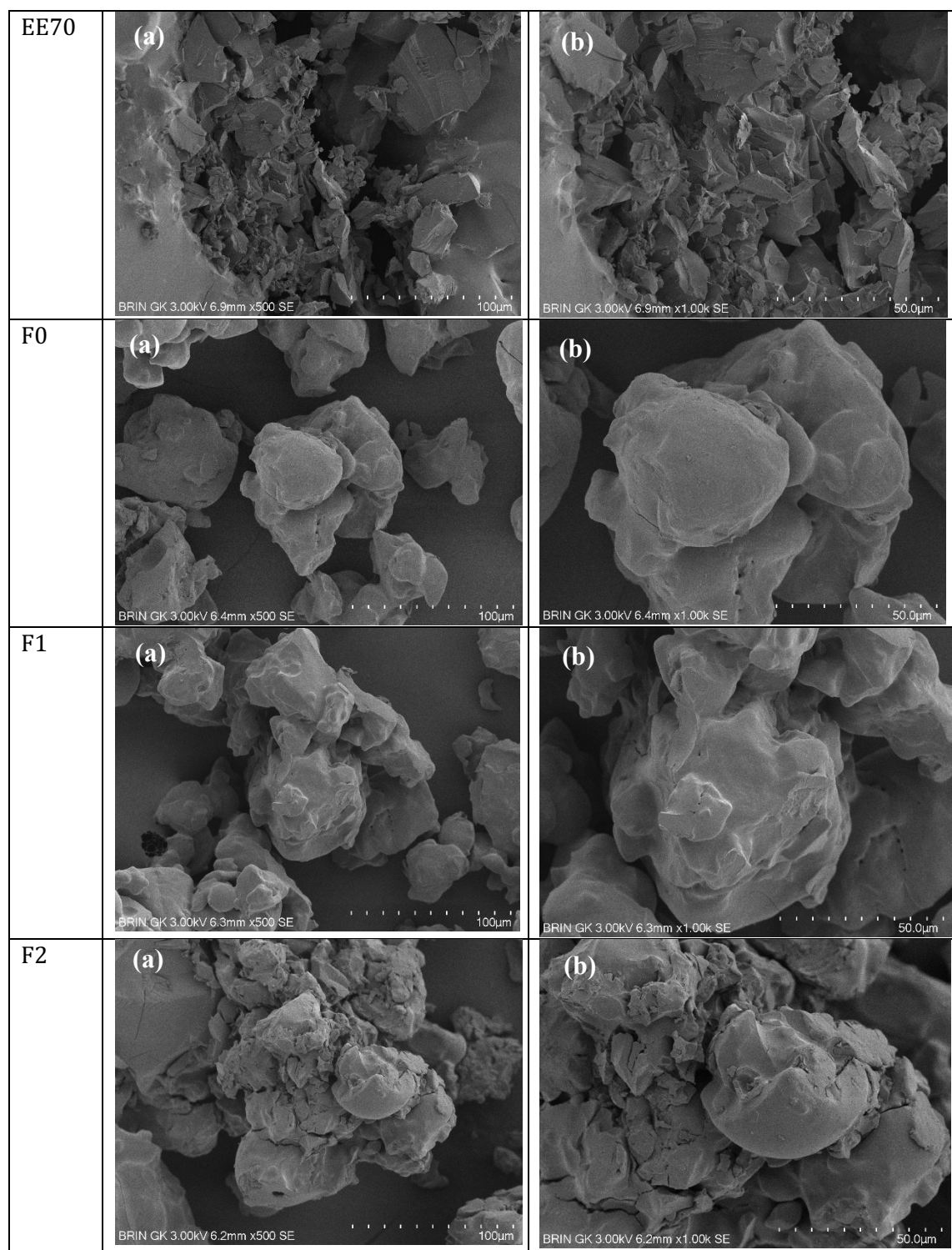


Figure. 2a. The photograph of *P. canescens*-based granule (F0 = 0 % of *P. canescens* leaf extract; F1 = 5 % of *P. canescens* leaf extract; F2 =10 % of *P. canescens* leaf extract; F3=15 % of *P. canescens* leaf extract).



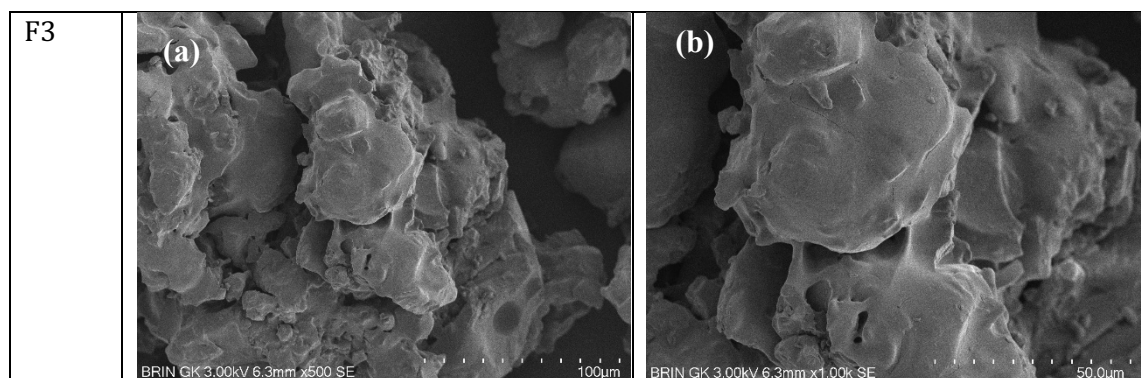


Figure. 2b. The SEM image of *P. canescens* leaf extract (EE70) and *P. canescens*-based granule (F0 = 0 % of *P. canescens* leaf extract; F1 = 5 % of *P. canescens* leaf extract; F2 = 10 % of *P. canescens* leaf extract; F3 = 15% of *P. canescens* leaf extract).

The results of this study indicate that all four variations of *P. canescens*-based granule formulations have uniform granule surface morphology with irregular granule shapes and varying sizes under SEM. Liu et al. (36) stated that the characteristics of particles formed from the granulation process of herbal powders are coarse and irregular due to gelatinization during the granulation process. The morphological similarity of these four formulations may be due to the use of the same binder (PVP), sweetener (stevia), and filler in each granule formulation at similar percentages. Binders play a crucial role in binding small particles in the powder mixture into granules and facilitating the granule formation process from inhomogeneous particles to larger, homogeneous ones (37). Al Hakim et al (38) stated that the use of a 5% PVP concentration as a binder can improve the physical properties of stevia granule preparations to be better than concentrations of 1% and 3%.

Nurhadi et al. (39) indicated that the binder formulation of the encapsulant, which includes the extract powder, influences the shapes and quality of the produced powder. The dense, compact, and uniform morphology of the powder ensures its durability and results in stable granules that shield the bioactive substances from evaporation or harm during being stored (40). In this study, *P. canescens* leaf extract was added to the granule formulation at a percentage ranging from 5% to 15%. The concentration variation did not significantly affect the morphological features of the granules obtained. A study conducted by Liu et al. (41) indicated that granule formulations with different amounts of mushroom extracts did not result in notable differences in the morphological structure as observed through SEM. The compact and dense morphological arrangement, featuring a fairly consistent particle size in the four formulations that include *P. canescens* leaf extract, enhanced the effectiveness of its bioactive substances, which include phenolic compounds, flavonoids, and terpenoids (42).

4. Conclusion

According to the findings shown in this research, it is clear that the *P. canescens*-based granule formula with an extract percentage of 15% (F3) exhibits the greatest total phenolic content, total flavonoids, and antioxidant activity compared to other granule formulas. Physicochemical analysis of all *P. canescens*-based granule formulas showed the existence of phenolic compounds and similar microstructural features in all *P. canescens*-based granules. Therefore, the granule formula with a *P. canescens* extract content of 15% can be used as one of the herbal preparations or as an alternative for fortification of functional foods that are beneficial for health. Further

research, measuring the tensile strength or mechanical integrity of the granules, is necessary. Additionally, toxicity assays are also needed to determine its safety level. In summary, there is considerable potential for the *P. canescens* leaf to be employed as a supplement in food and pharmaceutical products.

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