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SURFACE SOLID DISPERSION OF *MORINGA OLEIFERA* LEAF EXTRACT-MICROCRYSTALLINE CELLULOSE PH 102-POLOXAMER 188: PREPARATION AND CHARACTERIZATION

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ABSTRACT

Objective: The development of pharmaceutical products containing *Moringa oleifera* leaf extract has not developed well due to the physical and chemical characteristics limitations. The development of Surface Solid Dispersion (SSD) of *Moringa oleifera* leaves extract-microcrystalline cellulose PH102-Poloxamer 188 with 1:2:0.5 and 1:4:0.5 was performed in this study to improve the physicochemical characteristics of this extract.

Methods: SSD were prepared by microwave irradiation method using 400 W of power and 3 min of time exposure. The prepared SSD were evaluated for flowability, compressibility, moisture content, thermal characteristics (Differential Scanning Calorimetry (DSC)), crystallinity (Powder X-ray Diffractometry (PXRD)), functional group interaction (Fourier Transform Infra-Red Spectroscopy (FT-IR)), morphology (Scanning Electron Microscopy (SEM)), total flavonoid content, solubility study, and antioxidant activity.

Results: SSD powder exhibited better flowability, compressibility, and moisture content compared to the physical mixture (PM). The results of thermal characteristics and crystallinity of SSD indicate partial transformation into an amorphous phase. The total flavonoid content of SSD 1:2:0.5 was 11.04±0.23 mg QE/g, whereas SSD 1:4:0.5 was 9.18±0.05 mg QE/g. The solubility of the flavonoid compound from SSD 1:2:0.5 (78.73±0.76 µg/ml) and SSD 1:4:0.5 (61.90±3.38 µg/ml) was higher than PM with the equal ratio. The antioxidant activity was expressed as IC₅₀ values of SSD, which are 276.72±24.18 ppm for a 1:2:0.5 ratio and 249.04±27.29 ppm for a 1:4:0.5 ratio.

Conclusion: SSD preparation successfully improved the physicochemical characteristics and solubility of *Moringa oleifera* leaf extract. SSD 1:2:0.5 was the optimized composition from this study.

Keywords: *Moringa oleifera*, Surface solid dispersion, Microwave irradiation

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INTRODUCTION

Moringa oleifera also called drumstick is a valuable plant, because all parts of this plant are beneficial in nutritional and medicinal properties. *Moringa oleifera* leaves extract contained high levels of phenolic content and flavonoids [1]. The flavonoid content in *Moringa oleifera* leaves mainly consists of quercetin and kaempferol derivatives [2]. The ethanolic extract of *Moringa oleifera* leaves extract contained a total phenolic content of 62.56±0.72 mg Gallic Acid Equivalent (GAE)/g extract and total flavonoid 10.47±0.22 mg Quercetin Equivalent (QE)/g extract [3]. The previous study also revealed that the ethanolic extract of *Moringa oleifera* leaves extract exhibited moderate antioxidant activity confirmed by IC₅₀ at the concentration of 46.77±0.13 µg/ml [1]. Hence, *Moringa oleifera* leaf extract is promising as a potential source of natural antioxidants. The antioxidant activity of this extract is beneficial as a pharmaceutical ingredient to prevent oxidative stress and degenerative disease [4]. This extract also showed antibacterial activity against several bacteria and fungi including *Vibrio alginolyticus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, and *Candida albicans* [1]. From the obtained results, *Moringa oleifera* leaf extract possessed potential value as an antibacterial and antifungal compound [5]. The activity and capacity of macrophage phagocytosis also increased after ethanolic extract of *Moringa oleifera* leaves administration. Based on this fact, *Moringa oleifera* leaves can also function as an immunomodulator [6]. The antioxidant, antibacterial, antifungal, and immunomodulator activity of *Moringa oleifera* leaves extract are beneficial to develop as an active pharmaceutical ingredient in a pharmaceutical dosage form.

The development of solid pharmaceutical dosage forms containing plant extract suffers several problems, such as hygroscopicity and thick consistency of the extract. The selection of a method and suitable excipient to improve the hygroscopicity of the extract is a

challenge for solid formulation [7]. The biological activity of flavonoids contained in *Moringa oleifera* leaf extract also needs to be preserved, due to their instability in high temperature, poor aqueous stability, sensitivity to oxidation, and bitter taste [8]. Several methods have been implemented to improve the physicochemical characteristics and stability of extract, including *Moringa oleifera* leaf extract. The previous study revealed that the application of solid dispersion, inclusion complex formation, encapsulation, and phospholipid complexation are successful in overcoming these problems [9-11]. Solid dispersion is a versatile method to resolve the hygroscopicity problems of the extract as well as to enhance the stability. Solid dispersion was conducted by mixing the extract with a suitable carrier and subsequently drying through the melting process, solvent evaporation, and solvent melting approach [7]. The preparation of solid dispersion with PVP K-30 as a water-soluble carrier method has proven to increase the solubility and dissolution of *Selaginella doederleinii* extract due to crystalline conversion into amorphous form [12]. Hence, the same approach is possible to apply in the development of other extracts. Nowadays, the application of water-insoluble, porous material, and hydrophilic characteristics in the preparation of solid dispersion is rapidly increasing compared to the water-soluble carrier [13]. Water-soluble carriers produce a soft and tacky mass of dispersion; hence this carrier are not suitable for producing starting material in solid dosage form preparation [14]. Furthermore, the incorporation of a high portion of a water-soluble carrier in the solid dispersion can decrease dissolution due to the high viscosity of the system in the stagnant layer [15].

The preparation of solid dispersion using water-insoluble and porous characteristics of material is widely known as surface solid dispersion (SSD). Several excipients can be applied in the preparation of SSD, such as microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, sodium starch glycolate, pregelatinized starch, polyacrylic potassium, and lactose starch

compound [14]. Microcrystalline cellulose is a porous material with interparticle porosity of approximately 90-95%. This high porosity promotes rapid water penetration into the SSD structure. Microcrystalline cellulose median particle size is about 100 µm (D₅₀) measured by laser diffraction. The small particle size produces a high surface area of particles that are in contact with the dissolution medium; hence this characteristic is beneficial to promote faster dissolution of active ingredient [16]. Microcrystalline cellulose was successfully employed as a carrier for improving the dissolution of weakly acidic drugs like furosemide through high surface area. Microcrystalline cellulose also acts as an adsorbent which can convert the sticky behavior of sticky material into free-flow powder [17]. The addition of surfactant in the SSD preparation was also promising to enhance drug solubility and dissolution. Poloxamer 188 is a non-ionic surfactant, characterized by lower molecular weight compared to the other surfactant and hydrophilic properties. Poloxamer 188 can increase the solubility and dissolution of poorly soluble molecules by increasing the wettability and reducing surface tension [18]. Consequently, the development of SSD-contained *Moringa oleifera* leaf extract using microcrystalline cellulose and poloxamer 188 has the potential to be conducted to improve the physicochemical characteristics, solubility, and dissolution of this extract.

Microwave irradiation is a novel method that is suggested by previous research to produce SSD by hot-melt concept. The hot-melt method ensures the safety of the SSD obtained better than the solvent evaporation method due to the absence of residual solvent [19]. Microwave energy can penetrate the substance, allowing the production of heat at any point of the sample homogeneously. The dipolar moment in the molecule structure can absorb microwave energy and alter it into heat [20]. Therefore, the microwave irradiation method offers several predominance over conventional heating employed in the hot-melt method such as fast heating and preventing overheating at the surface [19]. Microwave energy also influences the conversion of the crystalline state of the active ingredient into amorphous forms, hence improving the solubility and dissolution [21]. The utilization of the microwave irradiation method possesses several advantages for preparing SSD based on plant extract due to shorter time application, non-contact heating, efficient cost, as well as a significant improvement of drug solubility and dissolution [21]. The development of surface solid dispersion (SSD) of *Moringa oleifera* leaves extract-microcrystalline cellulose PH 102-poloxamer 188 with 1:2:0.5 and 1:4:0.5 ratio was performed in this study to improve the physicochemical characteristics of this extract. The ratio of the extract and carrier determines the wetting properties, molecular dispersion, and dissolution profile of SSD prepared by microwave irradiation technique. Therefore, this study aimed to optimize the ratio of *Moringa oleifera* leaf extract, microcrystalline cellulose, and poloxamer 188 to obtain the desired characteristics of SSD.

Table 1: The composition of SSD containing *Moringa oleifera* leaf extract

Materials	Function	Quantity (g)	
		Ratio 1:2:0.5	Ratio 1:4:0.5
<i>Moringa oleifera</i> leaf extract	Active ingredient	5	5
Microcrystalline cellulose	Carrier	10	10
Poloxamer 188	Surfactant	2.5	2.5

The extract, microcrystalline cellulose, and poloxamer 188 were weighed accurately and then mixed in a mortar using a spatula. This mixture is transferred to the microwave for the heating process (Microwave LG Model, South Korea). The power was adjusted to 300 W and the time exposure of the energy was 4 min. After the heating process, the SSD powder was cooled for 5 min. The SSD powder was then sieved through a standard sieve with a 125-150 µm diameter and stored in a desiccator for further analysis [23].

Characterization of SSD

Fourier-transform infrared (FT-IR) spectroscopy

This characterization was conducted to analyze the functional group vibration and translation of the sample, to analyze the compatibility

MATERIALS AND METHODS

Material

Moringa oleifera leaf powders were acquired from Bogo Village, Bojonegoro, (East Java, Indonesia). Ethanol for the extraction process was pharmaceutical grade and supplied by CV. Mutiara Bersaudara (Surabaya, Indonesia). Microcrystalline cellulose used in this study was pharmaceutical grade with the brand name VIVAPUR® 102 (JRS Pharma, Germany). Poloxamer 188 was obtained from Merck, Germany and the grade was pharmaceutical grade. Quercetin as standard was analytical grade (Sigma Aldrich, USA). Absolute ethanol for analysis was analytical grade (Merck, Germany). The other materials were chemical reagents such as sodium hydroxide (Merck, Germany), aluminium chloride (Merck, Germany), 2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma Aldrich USA), ethyl acetate (Merck USA), methanol (Merck, Germany). All the chemical reagents were analytical grade. Membrane filters with 0.45 µm pore size (Merck Millipore, USA) were utilized in the solubility study. The preparation and analysis were conducted using distilled water during this study.

Methods

Preparation of *Moringa oleifera* leaf extract

Moringa oleifera leaf extract was prepared by maceration technique [22]. 500 gs of *Moringa* leaf powder was placed in the dry chamber, and then 5 l of ethanol 70% was added to the chamber. The ratio of *Moringa oleifera* leaf powder and solvent for maceration in the first cycle of maceration was 1:10. This mixture was macerated for 24 h. The mixture was then filtered using a funnel and filter paper with a pore diameter was 0.8 µm. The filtrate was collected in a sealed jar, while the residue was transferred to the new maceration chamber. The residue was added with 2.5 l of ethanol 70% and then re-macerated for 24 h to obtain the second cycle of filtrate. The filtrate from the first and second cycles of the maceration process was mixed, and then filtrates were evaporated using a rotary evaporator until the thick consistency of extract was produced.

Preparation of surface solid dispersion (SSD) and physical mixture (PM) of *Moringa oleifera* leaf extract-microcrystalline cellulose-poloxamer 188

Surface solid dispersion (SSD) containing *Moringa oleifera* leaf extract was prepared by microwave irradiation method. There are two compositions of the extract and the carrier prepared in this study, which are 1:2:0.5 and 1:4:0.5 for *Moringa oleifera* leaf extract, microcrystalline cellulose, and poloxamer 188, respectively. The composition and quantity of each material for each preparation process are presented in table 1. The preparation of physical mixture (PM) was also performed in this study with the same ratio as SSD using the spatulation method.

between *Moringa oleifera* leaf extract and excipient. The sample consists of *Moringa oleifera* leaf extract, microcrystalline cellulose, poloxamer 188, PM powder, and SSD powder. The Attenuated Total Reflectance (ATR) sampling technique was performed in this section, and then the spectrum of these samples was analyzed by Jasco FT-IR-4200, USA spectrophotometer. The spectrum was recorded in the resolution interval 4000 to 400 cm⁻¹ [23].

Differential scanning calorimetry

Thermal characteristics of *Moringa oleifera* leaf extract, PM, and SSD during temperature exposure and time were determined using differential scanning calorimetry (DSC) (Mettler Toledo, Switzerland). 4 mg of samples were placed in a sealed aluminum

pan. The scanning was carried out at constant heating from 30 °C until 200 °C at a rate of 10 °C/min under air atmosphere [20].

Powder X-ray diffraction studies (PXRD)

PXRD studies were conducted to determine the amorphous and crystalline behavior of the extract, PM, and SSD preparation. The possibility of polymorphic transformation during the process was also can be identified from the diffractogram. X-ray diffractometer (PANalytical X'Pert Pro, UK) equipped CuK α radiation source, 40 KV, and 30 mA. The X-ray diffractogram was recorded at a scanning rate of 2°/minute and the angular range was from 5° to 50° [19].

Morphology analysis using scanning electron microscopy (SEM)

The surface morphology of PM and SSD powder was determined by using a Scanning Electron Microscope (SEM) (JEOL JSM 5310 IV). The samples were coated with a gold-aluminum layer to increase the conductivity of the electron beam. Afterward, the coated samples were placed in the SEM chamber [24]. The morphology of PM and SSD were examined at 500x magnification.

Moisture content

Moisture content is a critical parameter that influences the flow property and mechanical properties of powder [25]. The moisture content of PM and SSD powder was determined using a moisture content analyzer. PM and SSD powder were weighed 5 g and then placed in the plate. The heating process was set at 105 °C for 15 min until a constant weight was reached. The difference between the initial and dry weight of the sample was calculated and the results expressed as moisture content percentage.

True density, bulk density, tapped density

The evaluation of true density, bulk density, and tapped density was addressed to obtain physical characteristics and specific identification of PM and SSD powder. True density was determined by transferring 1 g of the powder to a pycnometer. The liquid used for this evaluation was liquid paraffin since the powder is insoluble in liquid paraffin. The amount of liquid penetrating the pore of the powder was calculated into true density [26]. The bulk density of PM and SSD powder was determined by pouring 25 g of these powders into 100 ml of cylindrical glass. The volume occupied by 25 g of powder was utilized to calculate the bulk density. The tapped density of the powder was determined by the sample of bulk density evaluation through 10, 500, and 1250 tapping intervals until a significant reduction in powder volume was achieved. The tapped density is calculated by dividing the weight of the powder (25 g) by the tapped volume [27].

Compressibility index and hausner ratio

The determination of bulk density, tapped density, compressibility index, and Hausner ratio were carried out to predict the flowability and compressibility characteristics of PM and SSD powder [28]. The compressibility index was calculated through the difference between tapped density and bulk density divided by tapped density. Moreover, the Hausner ratio is a ratio that reflects the ratio of tapped density and bulk density [27].

Total flavonoid content

Total flavonoid content in PM and SSD powder was determined by spectrophotometric method, based on flavonoid-aluminum chloride (AlCl $_3$) complexation. Total flavonoid content evaluation was carried out to analyze the amount of flavonoid in these samples which is correlated to its antioxidant activity. The result of total flavonoid content was expressed as mg QE/g powder [29]. The SSD and PM powder were dissolved in absolute ethanol; then, the solution was transferred into 10.0 ml of a volumetric flask. The absolute ethanol was added to 10.0 ml in a volumetric flask. This sample was then sonicated for 15 min and centrifuged at 1500 rpm for 30 min. The supernatant of the solution was pipetted 1.0 ml and transferred into 10.0 ml of volumetric flask. The supernatant continued to react with 0.1 ml of 10% AlCl $_3$ and 0.1 ml of NaOH. After the reagent was added, ethanol was added until a 10.0 ml mixture was obtained. The

mixture was shaken vigorously, and the reaction took about 10 min to get a yellow color of the solution. The absorbance of this solution has been analyzed with visible spectrophotometers at a maximum wavelength of 470 nm. To determine the concentration of total flavonoid, an absorbance was applied to the regression curve. The concentration has been calculated and the total flavonoid content has been expressed as mg Quercetin equivalent (QE) per g of powder.

Antioxidant activity

The antioxidant activity of SSD powder was determined by a single electron transfer mechanism [30]. The antioxidant activity was analyzed by ABTS [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] free radical scavenging method [4]. Radical ABTS $^+$ was prepared through the oxidation of ABTS by potassium persulfate. ABTS powder weighed 19.20 mg; meanwhile, potassium persulfate weighed 5.5 mg. ABTS and potassium persulfate were mixed and dissolved in 5.0 ml of purified water until ABTS solution was obtained. The ABTS solution was incubated for 12 h and kept in the dark. ABTS solution was transferred into a 25.0 ml volumetric flask and purified water was added until 25.0 ml. The determination of antioxidant activity using ABTS in this study was conducted through sample preparation and the analysis of absorbance by a microplate reader (UVM 340-Biochrome). The sample was prepared in six concentrations, there are 5,25,75,100, and 150 ppm. Each concentration was replicated in five. Each concentration of ABTS and sample solution was pipetted 20 μ l and 160 μ l respectively (ratio 1:8). These solutions were transferred into a microplate well. The absorbance of the sample was measured at 726 nm wavelength. The blank solution was prepared by ABTS $^+$. The inhibition percentage of the sample was determined by the equation (1):

$$\text{Inhibition percentage} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\% \quad (1)$$

The IC $_{50}$ values were determined by the regression curve between sample concentration and inhibition percentage [31].

Solubility study

A solubility study was carried out in this study to evaluate the amount of quercetin from PM and SSD powder which are dissolved in the aqueous medium at saturated conditions [12]. An excess amount of PM and SSD powder (350 mg for 1:2:0.5 ratio and 550 mg for 1:4:0.5 ratio) was transferred into a 100 ml Erlenmeyer flask and added with 500 ml of purified water. The samples were placed in a thermostat oscillator and shaken at 120 rpm for 4 h at 37° C. The samples were taken out 6 ml from each Erlenmeyer flask; then the filtrate was filtered through a 0.45 μ m membrane filter [12]. The filtrate was pipetted 1.0 ml and transferred into a 10.0 ml volumetric flask. The reagent consisting of 0.1 ml of 10% AlCl $_3$, 0.1 ml of NaOH, and absolute ethanol was added to the volumetric flask and shaken until homogenized. The samples were incubated for 10 min, then the absorbance was measured at the maximum wavelength (435 nm) using a UV-Vis spectrophotometer. The concentration of quercetin dissolved in saturated conditions was calculated by subtracting the absorbance from the linear regression of the quercetin standard curve. The experiments were conducted in triplicate [32].

RESULTS AND DISCUSSION

Preparation of SSD and PM of *Moringa oleifera* leaf extract-microcrystalline cellulose-ploxamer 188

The extract of *Moringa oleifera* leaf was yellowish-green and thick consistency of liquid. SSD powder of *Moringa oleifera* leaf extract-microcrystalline cellulose-ploxamer 188 ratios 1:2:0.5 and 1:4:0.5 were prepared by microwave irradiation method. Meanwhile, the PM powder with the same ratio and composition was prepared by spatulation method. The physical observation of SSD powder revealed that the extract was dispersed homogenously at the carrier. SSD was a dry-light yellow powder with controlled particle size and aromatic odor. Whereas the PM powder was characterized as a rough moist agglomerate powder with a yellow color, and aromatic odor. The appearance of SSD and PM powder was described in fig. 1.



Fig. 1: Powder characteristics of (A) SSD 1:2:0.5 (B) SSD 1:4:0.5 (C) PM 1:2:0.5 (D) PM 1:4:0.5

Fourier-transform infrared (FT-IR) spectroscopy

The compatibility and interaction between *Moringa oleifera* leaf extract and carrier were studied using FT-IR spectroscopy [8]. The FT-IR spectrum of the extract, microcrystalline cellulose, poloxamer 188, PM, and SSD powder are presented in fig. 2.

For the FT-IR spectrum of *Moringa oleifera* leaf extract, the peak at 3332 cm^{-1} was associated with the O-H group stretching of flavonoid [33]. The specific peaks were also observed in the functional group region of *Moringa oleifera* leaf extract. C-H are stretching at 2924 cm^{-1} , C=O group at 1580 cm^{-1} , and C-O stretching at 1039 cm^{-1} . The spectrum of microcrystalline cellulose revealed specific peaks at 3324 cm^{-1} corresponding to the O-H group. The peak at 2892 cm^{-1}

and 1310 cm^{-1} were related to C-H stretching. The peak at 1616 cm^{-1} was assigned to C=O stretching. Poloxamer 188 spectrum revealed several specific peaks at 2874 cm^{-1} , 1345 cm^{-1} , and 1103 cm^{-1} . These peaks were assigned to the C-H stretching, -CH_3 stretching, and C-O stretching, respectively. The FT-IR spectrum of PM and SSD powder showed several peaks which are attributable to the individual spectrum of the extract, microcrystalline cellulose, and poloxamer 188 with a slightly reduced intensity or wavenumber shifting. The decrease in the intensity are related with the decrease amount of the component in PM and SSD powder [34]. This result indicated no interaction between *Moringa oleifera* leaf extract and carriers [24]. The extract possibly dispersed at the surface or inside of the carrier, however no chemical interaction has occurred [34].

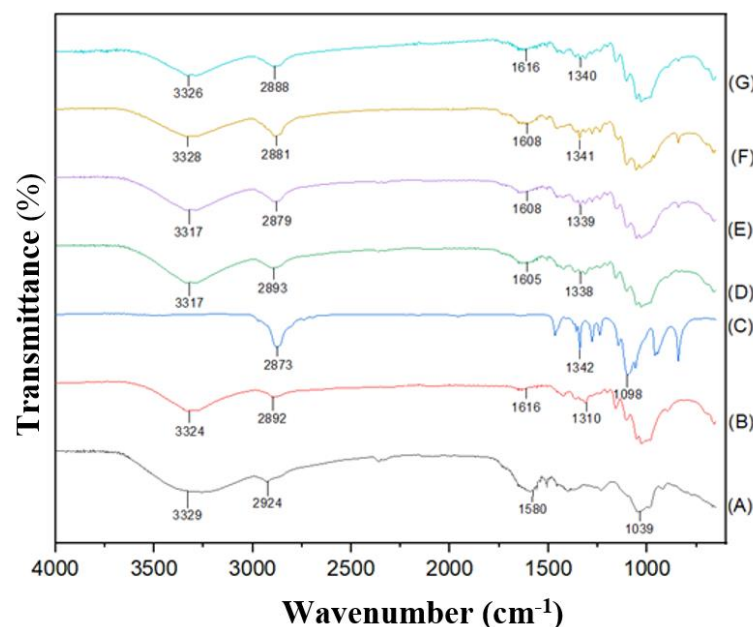


Fig. 2: FT-IR spectrum of (A) *Moringa oleifera* leaf extract (B) Microcrystalline cellulose PH 102 (C) Poloxamer 188 (D) PM 1:2:0.5 (E) PM 1:4:0.5 (F) SSD 1:2:0.5 and (G) SSD 1:4:0.5

Differential scanning calorimetry (DSC)

Thermal characteristics analysis using DSC was conducted to analyze the thermal properties and solid phase transformation of samples [35]. This characterization was also able to capture the exothermic and endothermic changes of the samples due to an increase in temperature [9]. The melting point and enthalpy of the samples were compared to analyze the crystallinity state of each sample and the possibility of interaction between components. DSC thermogram of *Moringa oleifera* leaf extract, microcrystalline

cellulose, poloxamer 188, PM, and SSD powder is presented in fig. 3. *Moringa oleifera* leaf extract exhibited an endothermic peak at $112.36\text{ }^{\circ}\text{C}$ and the enthalpy was -5733.61 J/g . Moreover, both microcrystalline cellulose and poloxamer 188 also showed an endothermic peak. The melting points of microcrystalline cellulose and poloxamer 188 were $79.72\text{ }^{\circ}\text{C}$ and $54.35\text{ }^{\circ}\text{C}$, respectively. The thermogram of PM and SSD powder did not present the endothermic peak of the extract. The endothermic peak of microcrystalline cellulose and poloxamer 188 were slightly shift into lower melting points at PM and SSD thermogram. The reduction of enthalpy values

was also observed in the PM and SSD thermograms compared to the pure extract and excipient thermograms. The higher reduction of enthalpy and the lower melting point shift was exhibited by SSD

1:2:0.5. This phenomenon could be attributed to the dispersion of *Moringa oleifera* leaf extract into the carrier and partially transformation from crystalline into amorphous state [24].

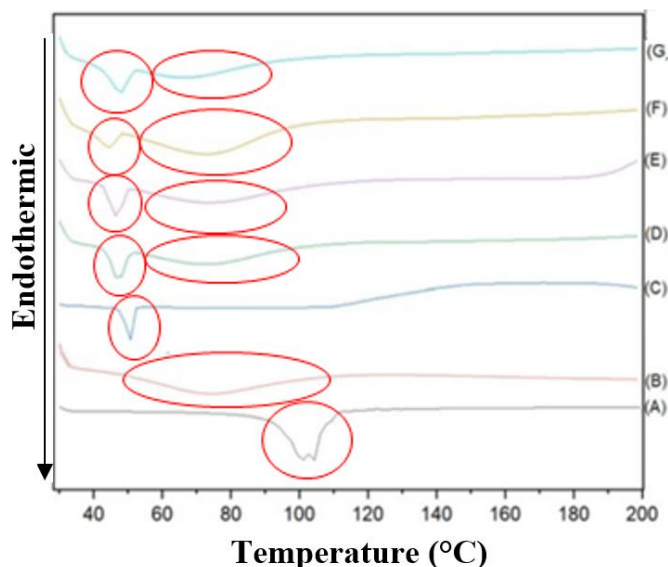


Fig. 3: DSC thermogram of (A) *Moringa oleifera* leaf extract (B) Microcrystalline cellulose PH 102 (C) Poloxamer 188 (D) PM 1:2:0.5 (E) PM 1:4:0.5 (F) SSD 1:2:0.5 and (G) SSD 1:4:0.5

Powder X-ray diffraction (PXRD) study

The PXRD diffractogram was recorded to observe the crystalline and amorphous characteristics of PM and SSD powder [21]. The PXRD diffractogram of *Moringa oleifera* leaf extract explained that this extract was amorphous, hence, microcrystalline cellulose and poloxamer 188 were characterized as semicrystalline substances [36]. The PXRD diffractogram of *Moringa oleifera* leaf extract, microcrystalline cellulose, poloxamer 188, PM, and SSD powder was presented in fig. 4. Microcrystalline cellulose presented sharp peaks at 2θ 16.71°, 22.46°, and 34.53°. Meanwhile, poloxamer 188 exhibited specific peaks at 15.15°, 19.10°, and 23.51°. The PXRD pattern of PM and SSD showed that the peaks observed in this spectrum were a combination of the specific peaks of

microcrystalline cellulose and poloxamer 188. The sharp peak indicates the presence of crystalline nature in the sample [37]. However, the peak height was reduced in the PM and SSD powder due to the dilution effect of the carriers [38]. Reduction in the peak height was observed higher in SSD 1:2:0.5 compared to the other ratio and PM powder. These findings indicated a decrease in crystallinity in the SSD preparation [39]. The reduction of crystallinity occurred due to the interaction of *Moringa oleifera* leaf extract, microcrystalline cellulose, and poloxamer 188 during exposure to microwave energy [40]. The microwave irradiation method also influenced the alteration of crystal lattice regularity. Microwave irradiation method is able to break down molecules in low temperature; hence it can maintain the stability of *Moringa oleifera* leaf extract against exposure to high temperatures [37].

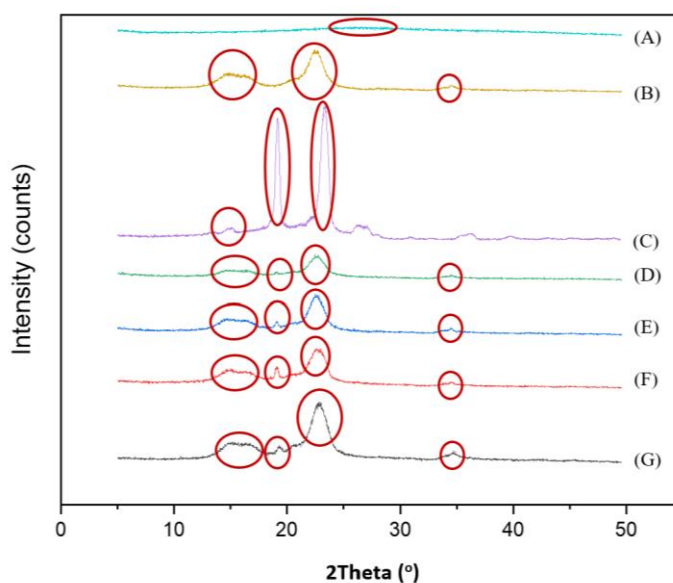


Fig. 4: PXRD diffractogram of (A) *Moringa oleifera* leaf extract (B) Microcrystalline cellulose PH 102 (C) Poloxamer 188 (D) PM 1:2:0.5 (E) PM 1:4:0.5 (F) SSD 1:2:0.5 and (G) SSD 1:4:0.5

Morphology analysis using scanning electron microscopy (SEM)

The micrograph of the extract, microcrystalline cellulose, poloxamer 188, PM, and SSD are tabulated in fig. 5. The SEM micrograph of microcrystalline cellulose was exhibited as columnar, wide, and thin particles. Meanwhile, poloxamer 188 was characterized as a spherulite particle. The PM and SSD powder micrographs revealed that the extract was attached to the surface of microcrystalline cellulose. The PM powder was observed as an aggregate and agglomerate structure. The SSD powder is constructed as a columnar particle similar to

microcrystalline cellulose particle; nevertheless, the particles are scattered and there is a gap between columnar particles. The presence of microcrystalline cellulose as a carrier influenced the particle size of the resultant SSD particles [41]. The SEM micrograph of SSD powder constructs a rough surface, contrary to the amorphous solid dispersion, which exhibits a smooth and porous surface. It indicates the extract is dispersed in the microcrystalline cellulose as a carrier. Poloxamer 188 might be also attached to the surface of the extract and microcrystalline cellulose, producing the surface modification into hydrophilic characteristic [42].

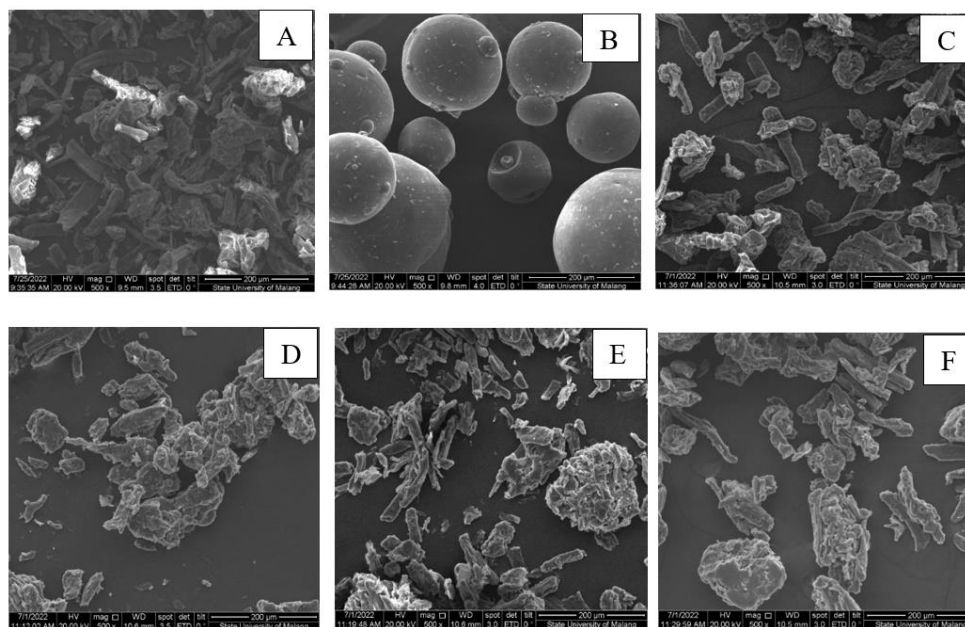


Fig. 5: SEM micrographs of: (A) Microcrystalline cellulose PH 102 (B) Poloxamer 188 (C) PM 1:2:0.5 (D) PM 1:4:0.5 (E) SSD 1:2:0.5 and (F) SSD 1:4:0.5

Moisture content

Moisture content was a crucial parameter examined from the SSD powder based on plant extract due to its influence on the stability and activity parameter of *Moringa oleifera* leaves extract. The moisture content of SSD powder was extremely lower compared to the PM powder. The moisture content of SSD powder was $7.50 \pm 0.24\%$ and $6.11 \pm 0.14\%$ respectively for ratios 1:2:0.5 and 1:4:0.5. Meanwhile, the moisture content of PM powder was $17.77 \pm 0.97\%$ for ratio 1:2:0.5 and $11.48 \pm 0.83\%$ for ratio 1:4:0.5. This result confirmed that the preparation of SSD powder was successful for preparing sticky materials into dry powder. Microcrystalline cellulose as a carrier was also

promising to reduce the hygroscopicity of the dried products [43].

True density, bulk density, tapped density, compressibility index, and Hausner ratio

The determination of powder density, compressibility index, and Hausner ratio was performed to predict the compression behavior and flowability of PM and SSD powder. The compressibility index was able to measure powder bridge strength and stability, hence the Hausner ratio was a tool to measure interparticle friction [44]. The results of powder density measurement, compressibility index, and Hausner ratio are presented in table 2.

Table 2: Powder density, compressibility index, and Hausner ratio of SSD and PM powder

Physical characteristic	SSD 1:2:0.5	SSD 1:4:0.5	PM 1:2:0.5	PM 1:4:0.5
True density (g/ml)	1.4089 ± 0.089	1.4417 ± 0.096	2.0162 ± 0.053	1.3374 ± 0.084
Bulk density (g/ml)	0.3577 ± 0.065	0.3266 ± 0.038	0.3381 ± 0.038	0.3433 ± 0.088
Tapped density (g/ml)	0.4303 ± 0.034	0.4440 ± 0.077	0.4453 ± 0.097	0.4082 ± 0.000
Hausner ratio	1.24 ± 0.03	1.25 ± 0.02	1.28 ± 0.02	1.30 ± 0.03
Compressibility index (%)	19.65 ± 1.64	19.99 ± 0.86	21.06 ± 1.23	22.69 ± 0.71

Data are expressed as mean \pm SD, n=3

All the powders, not only SSD but also PM presented as passable powder regarding these data, as described in United States Pharmacopeia. The microcrystalline cellulose successfully entraps the extract into its structure, reduces the sticky consistency of the extract, and promotes better flowability as a dry product. High interparticle porosity, approximately 90-95% and wide surface were responsible for adsorbing the *Moringa oleifera* leaves extract [16]. Microcrystalline cellulose was effectively used in the previous study

to reduce the moisture content and enhance the flowability of powdered ginger due to the capacity of adsorption and fibrous morphology of this particle [16].

Total flavonoid content

The total flavonoid content of PM and SSD powder was evaluated using the visible spectrophotometry method. The principle of total

flavonoid content determination was colorimetric assay [45]. Quercetin was utilized as a marker compound in this study. The total flavonoid content of SSD powder was 11.04 ± 0.23 mg QE/g for SSD 1:2:0.5 and 9.18 ± 0.05 mg QE/g for SSD 1:4:0.5. Meanwhile, the total flavonoid content of PM powder was 6.78 ± 0.44 mg QE/g and 4.86 ± 0.36 mg QE/g for 1:2:0.5 and 1:4:0.5 ratio respectively. The results of total flavonoid content are presented in fig. 6. The total

flavonoid content of SSD powder was significantly different compared to the PM powder ($p < 0.05$). The total flavonoid content of SSD powder was higher than PM powder. This condition was influenced by the dispersion and encapsulation process of extract, which are better in the SSD preparation [11]. The homogenous physical characteristics of SSD powder compared to the PM powder promoted these findings.

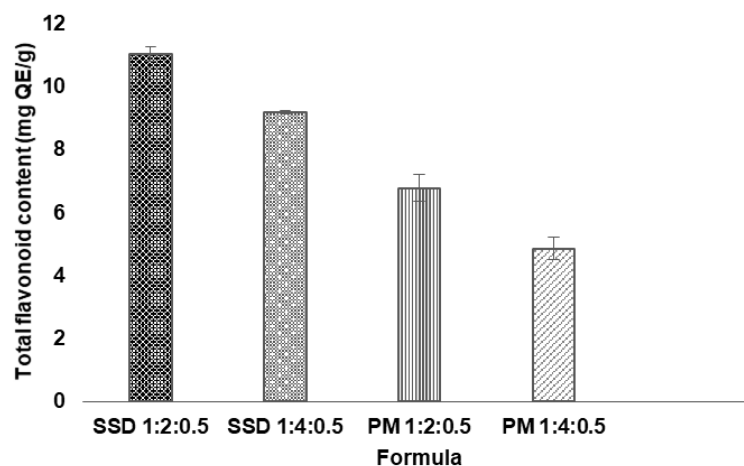


Fig. 6: Total flavonoid content of SSD and PM of *Moringa oleifera* leaves extract microcrystalline cellulose PH 102-poloxamer 188 (Data are expressed as mean ± SD, n=3; error bars indicate SD values)

Solubility study

The solubility of quercetin in the aqueous medium from PM and SSD powder was determined by the spectrophotometry method. The solubility of quercetin from PM and SSD powder was presented in fig. 7. The solubility of quercetin was 78.73 ± 0.76 µg/ml for SSD 1:2:0.5 and

61.90 ± 3.38 µg/ml for SSD 1:4:0.5. The PM powder exhibited solubility of quercetin in 68.20 ± 0.40 µg/ml for PM 1:2:0.5 and 62.41 ± 0.08 µg/ml for PM 1:4:0.5. SSD 1:2:0.5 presented highest solubility among all preparations ($p < 0.05$). The higher solubility of SSD 1:2:0.5 compared to the PM powder was attributed to a reduction of crystal lattice regularity by microwave energy and improved wettability due to poloxamer 188.

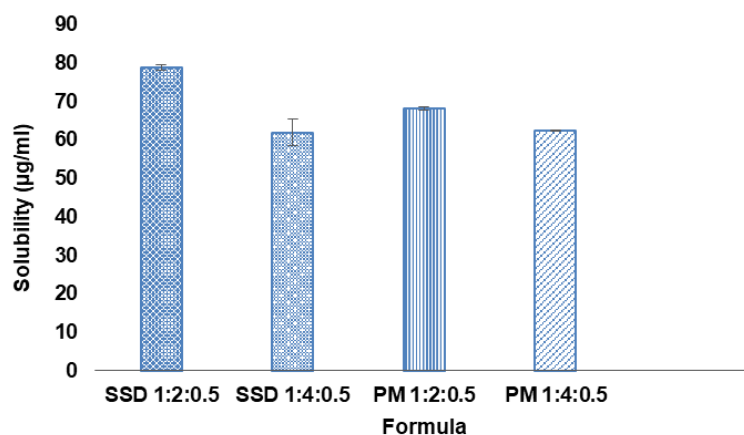


Fig. 7: Saturation solubility of PM and SSD powder containing *Moringa oleifera* leaf extract in water (Data are expressed as mean ± SD, n=3; error bars indicate SD values)

Antioxidant activity

The improved solubility of flavonoids observed in the solubility study promoted the observation of the antioxidant activity of SSD powder. 2,2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid (ABTS) is a chemical compound frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods and pharmaceutical products [46]. The antioxidant activity of SSD powder in this study was determined by ABTS scavenging assays. The IC_{50} values were calculated to express the antioxidant activity of SSD powder. The IC_{50} of SSD powder 1:2:0.5 and 1:4:0.5

are tabulated in table 3. The antioxidant activity of SSD powder was lower compared to the pure extract and vitamin C, which is 125.56 ± 10 ppm for pure extract and 9.02 ± 0.56 for vitamin C. Vitamin C as positive control exhibited very strong antioxidant activity ($IC_{50} < 50$ ppm) due to the radical scavenging mechanism of this substance. Nevertheless, the *Moringa oleifera* leaf extract and SSD powder exhibited weak antioxidant activity. This is due to the flavonoid content, which are contributed as an antioxidant substance in extract was not equally strong as vitamin C to stabilized the free radical but still potential to be developed as an antioxidant sources. The antioxidant activity of SSD powder was lower compare

to the pure *Moringa oleifera* leaf extract. These results implied that the microwave irradiation process led to an increase in the solubility of flavonoid compound in the aqueous medium; however the reduction of antioxidant activity may occur [11]. The optimization of power energy and time exposure should be conducted in further study to prevent the reduction of antioxidant activity.

Table 3: The results of antioxidant activity evaluation of PM and SSD powder expressed as IC₅₀ value

Samples	IC ₅₀ (ppm)
Vitamin C	9.02±0.56
<i>Moringa oleifera</i> leaf extract	125.56±10
SSD 1:2:0.5	276.72±24.18
SSD 1:4:0.5	249.04±27.29

Data are expressed as mean±SD, n=3

CONCLUSION

The development of surface solid dispersion (SSD) of *Moringa oleifera* leaves extract-Microcrystalline cellulose PH102-Poloxamer 188 with 1:2:0.5 and 1:4:0.5 successfully improved the physicochemical characteristics of the extract. The microwave irradiation method was able to incorporate the extract uniformly into the carrier structure and reduce crystal lattice regularity in the prepared SSD. This mechanism caused improvement in quercetin solubility from SSD powder in the aqueous medium. However, the optimization of power energy and time exposure of the microwave irradiation method should be optimized in further study to preserve the antioxidant activity of *Moringa oleifera* leaf extract.

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AUTHORS CONTRIBUTIONS

Concept and design of the ideas, literature search and concept of the intellectual content was performed by Karina C Rani, Roisah Nawatila, and Nikmatul IE Jayani. Formulation and characterization of SSD, data analysis, and statistical analysis was performed by Karina C Rani, Roisah Nawatila, Zulviata PD Natasya, and Veronika G Angela. *In vitro* antioxidant activity was determined by Winda M Wanti and Nikmatul IE Jayani. Manuscript preparation, manuscript editing, and review was conducted by Karina C Rani, Roisah Nawatila, and Nikmatul IE Jayani.

CONFLICT OF INTERESTS

Declared none

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