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Original Article

Development and characterization of surface solid dispersion *Moringa oleifera* leaf extract-microcrystalline cellulose by co-grinding method

[Desarrollo y caracterización de una dispersión sólida superficial de extracto de hoja de *Moringa oleifera*-celulosa microcristalina mediante el método de co-molienda]

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Abstract

Context: *Moringa oleifera* leaf extract is rich in flavonoid compounds and potential antioxidant sources. However, the development of *M. oleifera* leaf extract into an herbal product is limited by challenges such as inconsistent quality, hygroscopicity, and poor solubility. Surface solid dispersion (SSD) is a versatile method to enhance both the physical properties and solubility of such extracts.

Aims: To enhance the physical and solubility properties of *M. oleifera* leaf extract using SSD with microcrystalline cellulose as a carrier.

Methods: SSD was prepared using a co-grinding method with extract-to-carrier ratios of 1:2 and 1:4.

Results: This study revealed that the SSD effectively absorbed the extract onto the surface of microcrystalline cellulose. Hence, the SSD powder exhibited improved moisture content and flowability. Thermal and crystallinity analyses revealed a reduction in crystal lattice regularity compared to the pure extract, as evidenced by decreased enthalpy and diminished peak intensity. The total flavonoid content of SSD 1:2 was 14.70 ± 0.35 mg QE/g, whereas SSD 1:4 was 7.00 ± 0.07 mg QE/g. Flavonoid solubility compound from SSD 1:2 was 62.26 ± 0.62 µg/mL, while the solubility of SSD 1:4 was 63.58 ± 0.62 µg/mL. The solubility of the flavonoid compound from SSD preparation increased about 1.16-fold compared to the physical mixture.

Conclusions: SSD preparation of *M. oleifera* leaf extract improves its physical and chemical properties, making it more suitable for formulation.

Keywords: co-grinding; microcrystalline cellulose; *Moringa oleifera*; surface solid dispersion.

Resumen

Contexto: El extracto de hoja de *Moringa oleifera* es rico en compuestos flavonoides y fuentes potenciales de antioxidantes. Sin embargo, el desarrollo del extracto de hoja de *M. oleifera* en un producto herbal está limitado por desafíos como la calidad inconsistente, la higroscopicidad y la baja solubilidad. La dispersión sólida superficial (SSD) es un método versátil para mejorar tanto las propiedades físicas como la solubilidad de dichos extractos.

Objetivos: Mejorar las propiedades físicas y de solubilidad del extracto de hoja de *M. oleifera* utilizando SSD con celulosa microcristalina como portador.

Métodos: La SSD se preparó utilizando un método de co-molienda con proporciones de extracto a portador de 1:2 y 1:4.

Resultados: Este estudio reveló que la SSD absorbió eficazmente el extracto sobre la superficie de la celulosa microcristalina. Por lo tanto, el polvo de SSD exhibió un contenido de humedad y una fluidez mejorados. Los análisis térmicos y de cristalinidad revelaron una reducción en la regularidad de la red cristalina en comparación con el extracto puro, como lo demuestra la disminución de la entalpía y la disminución de la intensidad de pico. El contenido total de flavonoides de SSD 1:2 fue de 14,70 ± 0,35 mg QE/g, mientras que SSD 1:4 fue de 7,00 ± 0,07 mg QE/g. La solubilidad del compuesto flavonoide de SSD 1:2 fue de 62,26 ± 0,62 µg/mL, mientras que la solubilidad de SSD 1:4 fue de 63,58 ± 0,62 µg/mL. La solubilidad del compuesto flavonoide de la preparación SSD aumentó aproximadamente 1,16 veces en comparación con la mezcla física.

Conclusiones: La preparación SSD del extracto de hoja de *M. oleifera* mejora sus propiedades físicas y químicas, haciéndolo más adecuado para la formulación.

Palabras Clave: celulosa microcristalina; co-molienda; dispersión sólida superficial; *Moringa oleifera*.

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INTRODUCTION

The utilization of *Moringa oleifera* leaf to maintain health status and nutritional application is increasing nowadays. *M. oleifera* leaf contains flavonoid compounds with phenolic hydroxyl groups such as quercetin, kaempferol, rutin, and myricetin. These compounds are beneficial as potent antioxidant properties to encounter reactive oxygen species (Gupta et al., 2018). Studies have demonstrated the antioxidant activity of *M. oleifera* leaf extract prepared with organic solvents such as ethanol. The IC_{50} value of the hydroalcoholic extract of *M. oleifera* leaf was 232.60 ± 7.61 µg/mL, determined by diphenyl-picrylhydrazine (DPPH). The IC_{50} values indicated moderate antioxidant activity of these extracts (Chanda et al., 2011). Additionally, the total phenol content of ethanolic extract was reported to be 47.70 ± 1.58 GAE/g (Gupta et al., 2018). The polyphenol content of these extracts is highly contributed to neutralizing free radicals. Hence, these extracts are useful to maintain our health status. *M. oleifera* leaf extracts are also reported to have the potential as antimicrobial, antihyperglycemic, antitumor, and anticancer compounds (Razis et al., 2014). *M. oleifera* leaf has also been widely used in dietary supplements and food fortification due to its rich composition, including protein, minerals, iron, magnesium, calcium, vitamins, and carotenoids (Gupta et al., 2018). *M. oleifera* leaves incorporation into traditional food recipes in India suggested the further application of these plants in the food industry. Their incorporation was successful in combating macro and micronutrient deficiencies (Nambiar and Parnami, 2008).

The development of medicinal products or dietary supplements from *M. oleifera* leaf extracts faces many major challenges. The preparation of standardized, stable, and ready-to-use extract in the production process should be conducted to ensure the quality of an herbal product. *M. oleifera* leaf extract has a thick consistency, aromatic odor, and bitter taste (Departemen Kesehatan Republik Indonesia, 2008). These characteristics became significant obstacles to develop this extract in medicinal products, especially in the solid dosage form. The previous study also explained that in the development of the *M. oleifera* leaf tablet, the poor flowability, high caking tendencies, and low tensile strength. The masking approach to reduce the bitterness of *M. oleifera* leaf powder and extract also must be conducted to enhance the acceptability of consumers (Ali et al., 2017). Moreover, the flavonoid compounds contained in the *M. oleifera* leaf extract exhibited low solubility and bioavailability. The solubilities of quercetin, kaempferol, and myricetin were reported 4.5 µg/mL, 0.36 µg/mL, and 1.5 µg/mL, respectively (Colombo et al., 2019; Franklin and Myrdal 2015; Kulkarni et al., 2019). The limited solubility of flavonoid compounds exhibited low bioavailability of these compounds after administration.

To address these challenges, surface solid dispersion (SSD) offers a promising approach to simultaneously improve the physical, mechanical, and solubility characteristics of plant extracts. SSD is a technique that utilizes water-insoluble, porous, and hydrophilic carriers to enhance the physical properties and dissolution of active substances (Windriyati et al., 2019). The carrier characteristics, such as hydrophilicity, particle size, porosity, and surface area, play pivotal roles in the physical characteristics improvement and release profile of active ingredients (Patel et al., 2012). Various carriers, including microcrystalline cellulose, colloidal silicon dioxide, crospovidone, croscarmellose sodium, and sodium starch glycolate, were applied in the previous study to prepare SSD. Microcrystalline cellulose is a promising carrier for SSD preparation due to its large surface area, high porosity, good compressibility, and rapid wettability (Ali et al., 2009). The large surface area and porosity are beneficial for adsorbing extract and promote fast release of active ingredients (Patel et al., 2012). The preparation of solid dispersion based on plant extracts has also been shown to convert the active ingredients into an amorphous state, which significantly enhances their solubility and dissolution behavior (Fan et al., 2023).

In this study, SSD preparation of *M. oleifera* leaf extract was carried out using microcrystalline cellulose as the carrier to improve the physical and mechanical characteristics as well as the solubility of its active compounds. The co-grinding method was chosen to prepare the SSD of *M. oleifera* leaf extract due to its solvent-free nature and low thermal impact on the extract, which helps preserve the stability of the extract. The previous study revealed that the co-grinding method does not cause chemical interactions between the drug and the carrier (Yang et al., 2012). Additionally, the co-grinding method has been reported to enhance drug dissolution rates, likely due to particle size reduction and lower drug crystallinity following the grinding process (Nazem et al., 2023).

MATERIAL AND METHODS

Plant material

Moringa oleifera leaves were collected in Maret 2024 from Desa Bogo, Kapas, Bojonegoro, East Java, Indonesia (latitude 7.1995° S, longitude 7.1995°S 111.8926°E). Identification was confirmed by the Cen-

ter for Information and Development of Traditional Medicine (PIPOT), University of Surabaya, with determination letter number 1578/D.T/IV/2024, according to references. The *M. oleifera* leaves were dried and processed into powder with a moisture content of 10%-12%. The preparation of *M. oleifera* leaf powder was conducted by the woman empowerment group KWT Sri Rejeki, Bogo, before further processing into *M. oleifera* leaf extract.

Materials

Analytical-grade absolute ethanol (EMSURE®) was obtained from Supelco (Germany) for the extraction process. The carrier, microcrystalline cellulose (pharmaceutical grade), with the brand name VIVAPUR® 102, was procured from JRS Pharma (Germany). The reference standard quercetin was purchased from Sigma Aldrich (USA). The chemical reagents utilized in this study were analytical grade such as 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) (Sigma Aldrich USA), sodium hydroxide (Merck, Germany), ND aluminum chloride (Merck, Germany).

Preparation of *Moringa oleifera* leaf extract

M. oleifera leaf extract was prepared using a maceration method. A total of 500 grams of *M. oleifera* leaf powder was placed in a jar and macerated with 5 L of 70% ethanol (1:10 ratio) for 24 hours. After filtration in this sealed container, an additional 2.5 L of 70% ethanol (1:5 ratio) was added and re-maceration was performed for another 24 hours. In addition, the filtrate obtained has been removed and the first and second filtrate has been evaporated by a rotary evaporator until a thick extract is produced (Mahdi et al., 2016).

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

SSD containing *M. oleifera* leaf extract was prepared using the co-grinding method (Chaturvedi et al., 2017). Extract-to-carrier ratios of 1:2 and 1:4 ratio (w/w) were prepared. The amount of the extract and microcrystalline cellulose used in the SSD preparation are tabulated in Table 1. The PM was prepared by mixing the extract and microcrystalline cellulose as a carrier for 2 minutes in a mortar. SSD was prepared by grinding PM in a laboratory-size ball mill AR 403 (Erweka, Germany) using several porcelain balls (3-5 cm diameter). The grinding process of SSD was conducted in a 5 L milling chamber. The velocity of the ball mill was set at 120 rpm for 60 minutes (Nazem et al., 2023). After the uniform dispersion of the extract to the carrier was achieved, the SSD powder was sieved using standard sieve mesh No. 30. The SSD powder was stored in a desiccator until further processing.

Powder X-ray diffractometry (PXRD) analysis

The crystallinities of the samples were determined by analyzing the PXRD patterns (Fitriani et al., 2024). The PXRD patterns of *M. oleifera* leaf extract, microcrystalline cellulose, PM, and SSD powder were recorded using an X-ray diffractometer (PANalytical X'Pert Pro, UK) at 2θ 5-50°. For each analysis, 50 mg of the sample was weighed and placed in a glass holder, with the sample surface carefully flattened using a spatula to ensure uniformity. The scanning rate was set to 2°/minute.

Thermal analysis

The thermal analysis characteristics of *M. oleifera* leaf extract, microcrystalline cellulose, PM, and SSD powder were analyzed using differential scanning calorimetry (DSC) (Mettler Toledo, Switzerland). Approximately 4 mg of each sample was placed in a sealed aluminum pan. The scanning process was conducted at 10°C/minute from 30°C-200°C (Yang et al., 2012).

Fourier-Transform Infrared (FT-IR) analysis

The identities of extract and carrier, along with potential interaction between them, were evaluated using a Fourier transform infrared (FT-IR) spectrophotometer (Jasco FTIR-4200, USA). The infrared spectrum of each sample was scanned within the range 400 cm^{-1} – 4000 cm^{-1} with 1 cm^{-1} resolution (Chaturvedi et al., 2017).

Table 1. Physical mixture (PM) and surface solid dispersion (SSD) composition.

Material	Amount (g)			
	SSD 1:2	SSD 1:4	PM 1:2	PM 1:4
Moringa oleifera leaf extract	30	30	30	30
Microcrystalline cellulose	60	120	60	120

Morphology

Scanning electron microscopy (SEM) analysis was performed to examine the morphology and topography of microcrystalline cellulose, PM, and SSD powder. The analysis was conducted using JEOL JSM 5310 LV Scanning Electron Microscope (Japan). Previously, the sample was coated with a gold-aluminum layer and attached to an aluminum sample holder (Chen et al., 2020). The morphology of each sample was captured at a magnification of 500×.

Moisture content

The moisture content of PM and SSD powder of *M. oleifera* leaf extract-microcrystalline cellulose was determined using moisture content balance (Ohaus MB27 moisture balance, USA). A 5-gram sample of PM and SSD powder was placed in a sample pan of moisture balance, and the sample was heated at 105°C for 15 minutes. The constant weight of the samples after heating was recorded, and the weight difference before and after heating was calculated to obtain the moisture content of the sample (da Cunha-Filho et al., 2014).

Analytical method validation of total flavonoid content analysis

Analytical method validation was carried out to ensure the validity of the spectroscopic method during total flavonoid content determination in PM and SSD powder. The analysis of flavonoid content in PM and SSD powder was a crucial step, not only to evaluate the quality but also to validate the safety and efficacy (Da Silva et al., 2015). The flavonoid content was determined based on complex formation with aluminum chloride ($AlCl_3$) using quercetin as the reference standard. The absorbance of the complex formation was measured using a spectrophotometer (Wulandari et al., 2016). The analytical method validation in this study includes tests for specificity, linearity and range, accuracy, and precision.

Specificity

The specificity or selectivity parameter was determined to ensure that the spectrophotometric method can identify the analyte in a multi-component, matrix-containing sample (Abuzaid et al., 2020). The specificity of this method was evaluated through a comparison of the curves obtained from the SSD powder sample and quercetin standard solution using a UV-Vis spectrophotometer. For sample preparation, 10 mg of SSD powder was weighed and dispersed in distilled water. The solution was then transferred to a 10.0 mL volumetric flask. Hereafter, the solution was vortexed and centrifuged for 10 minutes. The solution was then analyzed using the UV-Vis spectrophotometer in the scanning mode from 400-600 nm. The obtained spectrum was compared with the spectrum of standard solution at concentrations of 5 ppm and 7 ppm (Shanko et al., 2024). Additionally, the spectrum of the matrix solution was also utilized in this study to determine the effect of microcrystalline cellulose on the absorbance of flavonoids.

Linearity and range

The linearity and range parameters were studied to evaluate the correlation between the independent variables (concentration of standard solution) and the dependent variables (absorbance) (Shanko et al., 2024). In this study, the linearity and range were determined by constructing three calibration curves using five different concentrations of quercetin standard solution (3, 5, 7, 9, and 10 ppm). The absorbance of the standard solution was analyzed at 435 nm, and the correlation between concentration and absorbance was plotted as a calibration curve. The linearity of the curve was evaluated by calculating the correlation coefficient (R^2) and Vxo value of the quercetin calibration curve. The obtained R^2 values greater than 0.995 and Vxo <5% were the acceptable range of the recommended linearity value (Oktriana et al., 2022; Shanko et al., 2024).

Precision

The precision of this method was evaluated through repeatability and intermediate precision. The repeatability was determined by performing six individual assays of the *M. oleifera* leaf extract, using a 100% concentration added to the PM and SSD powder. However, the intermediate precision was performed by two different analysts in the same laboratory and instrument (Abuzaid et al., 2020).

Total flavonoid content analysis

The total flavonoid content of PM and SSD powder was determined using a colorimetric assay and visible spectrophotometry (Da Silva et al., 2015). PM and SSD powder, serving as the samples, were weighed to provide an equivalent of 10 mg of *M. oleifera* leaf extract. The sample was dissolved in 5.0 mL of absolute ethanol until homogeneous and then transferred into a 10.0 mL volumetric flask. Additional absolute ethanol was added to reach a total volume of 10.0 mL. The solution was sonicated for 15 minutes and then centrifuged at 1500 rpm for 30 minutes to separate the sediment from the supernatant. From the supernatant, 1.0 mL was pipetted and transferred into a new 10.0 mL volumetric flask. The reagent, consisting of 0.1 mL of 10% $AlCL_3$ and 0.1 mL of NaOH, was added consecutively. The volume was adjusted to 10.0 mL with absolute ethanol (Sulaiman and Bala-

chandran, 2012). The mixture was allowed to react for 10 minutes until a yellow color was observed. The absorbance of the sample was then analyzed using a UV-visible spectrophotometer (Shimadzu UV 1900, Japan) at the maximum wavelength of 435 nm. The flavonoid concentration in the sample was calculated by extrapolating the absorbance onto a regression curve. The total flavonoid in PM and SSD powder was expressed as mg quercetin equivalent per gram of sample (mg QE/g).

Solubility study

The solubility study was conducted to determine the solubility of quercetin, a marker compound, in PM and SSD powders in an aqueous solution. A sample (PM and SSD powder) equivalent to 100 mg of *M. oleifera* leaf extract was weighed and placed in a 100 mL Erlenmeyer flask. Then, 50 mL of distilled water was added to the flask. The flask was placed on an incubation shaker set at 37°C and 120 rpm. After 20 minutes, approximately 5.0 mL of the sample was withdrawn using a disposable syringe. An equal volume of distilled water was added back to the Erlenmeyer flask to maintain sink conditions in the medium. The sample was then filtrated using a 0.45 µm filter membrane mounted in a 25 mm diameter of the filter holder. Next, 1.0 mL of filtrate was pipetted into a 10.0 mL volumetric flask. The reagent, consisting of 0.1 mL of 10% $AlCl_3$, 0.1 mL NaOH, and absolute ethanol, was added to the flask and shaken until a uniform solution was achieved. The sample was allowed to react for 10 minutes, after which the absorbance was measured at the maximum wavelength of 435 nm using a UV-visible spectrophotometer (Shimadzu UV 1900, Japan) (Saloko et al., 2020). The solubility of quercetin in the aqueous medium was calculated by interpolating the absorbance into the regression curve.

In vitro antioxidant capacity

The *in vitro* antioxidant capacity of PM and SSD powder against ABTS was determined using the spectrophotometry method (Stratil et al., 2006). The ABTS radical was prepared by reacting ABTS with potassium persulfate. Approximately 19.20 mg of ABTS powder and 5.50 mg of potassium persulfate were reacted in the distilled water to obtain the ABTS solution. The ABTS solution was incubated for 12 hours in a dark room. PM and SSD samples were prepared by dissolving and diluting them into six different sample concentrations (100, 250, 500, 750, and 1000 ppm). Five replicates were prepared for each sample concentration. The analysis involved pipetting 20 µL of ABTS solution and 160 µL of the sample solution into a microplate well. The absorbance was measured at the maximum wavelength of ABTS solution (734 nm) (Stratil et al., 2006). A blank solution, consisting solely of ABTS solution, was also prepared in a microplate well. The initial absorbance of the ABTS was recorded as 0.950. The decrease in ABTS absorbance with increasing sample concentration was tabulated and calculated as % inhibition. Using linear regression between sample concentration and % inhibition, the IC_{50} value of each sample was calculated to express the antioxidant activity of each sample.

Statistical analysis

The data were presented as mean ± standard deviation. The solubility and *in vitro* antioxidant capacity data for SSD powder (ratios 1:2 and 1:4) and PM powder (ratios 1:2 and 1:4) were analyzed using one-way ANOVA to identify significant differences between these data. When significant differences were detected, a post hoc analysis was conducted using Tukey's test. A $p<0.05$ was considered statistically significant. All statistical analyses were performed using IBM SPSS 23.

RESULTS AND DISCUSSION

Physical observation of PM and SSD powder

PM powder was prepared using the triturating method; hence, the SSD powder was prepared by the co-grinding method using a ball mill instrument. The PM powder in both ratios (1:2 and 1:4) appeared as light-yellow, aggregate, moist powder form with non-uniform particle sizes. In contrast, SSD powder was observed as a yellowish-brown powder with uniform particle sizes, good flowability, and better handling properties. SSD powder was neither moist nor lumpy, which contributed to its superior flowability and ease of handling compared to PM powder. A visual comparison of PM and SSD powders derived from *M. oleifera* leaf extract-microcrystalline cellulose is presented in Fig. 1.

Powder X-ray diffractometry (PXRD) analysis

The diffractogram profiles of *M. oleifera* leaf extract, microcrystalline cellulose, physical mixture (PM), and surface solid dispersion (SSD) are presented in Fig. 2. The X-ray diffractogram of *M. oleifera* leaf extract confirmed its amorphous nature. The diffractogram of microcrystalline cellulose displayed specific peak intensity at 14.52°, 22.47°, and 34.53°, consistent with its semicrystalline nature. These results align with a previous study, which reported sharp peaks at 15.00°, 22.8°, and 34.50° (Terinte et al., 2017). The diffractogram of PM and SD exhibited patterns similar to that of microcrystalline cellulose. However, a reduction in peak intensity was observed in the SSD diffrac-

tion pattern for both 1:2 and 1:4 ratios. This reduction indicates the homogeneous dispersion of *M. oleifera* leaf extract into microcrystalline cellulose, serving as the carrier. The decrease in peak intensity in the SSD diffraction profile may be attributed to dilution with the carrier and reduced crystal phase regularity (Kiran et al., 2009).

Thermal analysis

Thermal analysis characterization was conducted to observe the absorption and emission of energy by each sample as a function of time and temperature. Changes in melting point and enthalpy were recorded using a differential scanning calorimetry (DSC) instrument (Fitriani et al., 2024). The DSC thermogram of *M. oleifera* leaf extract displayed an endothermic peak at 112.36°C, while microcrystalline cellulose exhibited a broad endothermic peak at 79.72°C. The thermogram of PM and SSD (ratios 1:2 and 1:4) showed an endothermic peak around 77-78°C, corresponding to the microcrystalline cellulose. A lower enthalpy value was observed from the thermogram of SSD 1:2 and SSD 1:4 compared to those of microcrystalline cellulose and PM. The disappearance of the extract's endothermic peak in the PM and SSD thermograms suggests the distribution of the extract within the microcrystalline structure as a carrier (Abd-El Bary et al., 2014). The reduction in peak intensity and enthalpy value may result from the strong adsorption of the extract onto the carrier during SSD preparation (Chaturvedi et al., 2017). The DSC thermogram of the extract, microcrystalline cellulose, PM, and SSD powder are presented in Fig. 3.

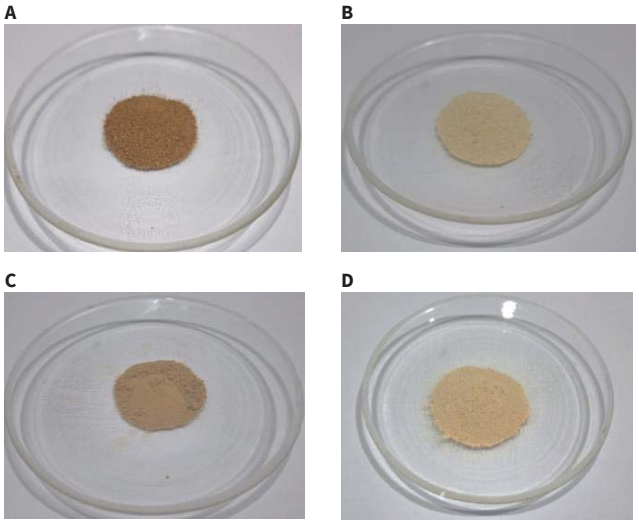

Figure 1. Physical characteristics of PM and SSD powder of *Moringa oleifera* leaf extract-microcrystalline cellulose **(A)** PM 1:2; **(B)** PM 1:4; **(C)** SSD 1:2; **(D)** SSD 1:4.

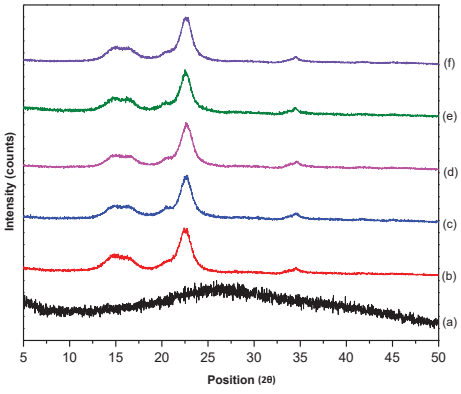

Figure 2. PXRD diffractogram of **(a)** *Moringa oleifera* leaf extract; **(b)** microcrystalline cellulose; **(c)** PM 1:2; **(d)** PM 1:4; **(e)** SSD 1:2; **(f)** SSD 1:4.

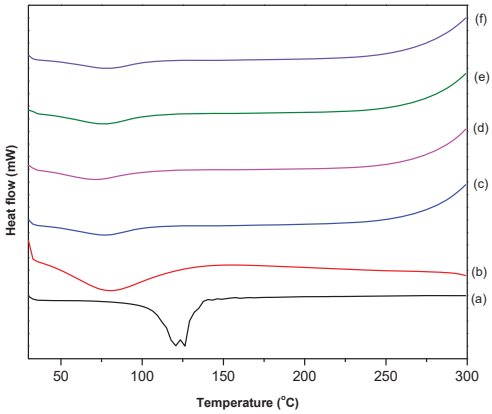

Figure 3. DSC thermogram of **(a)** *Moringa oleifera* leaf extract; **(b)** microcrystalline cellulose; **(c)** PM 1:2; **(d)** PM 1:4; **(e)** SSD 1:2; **(f)** SSD 1:4.

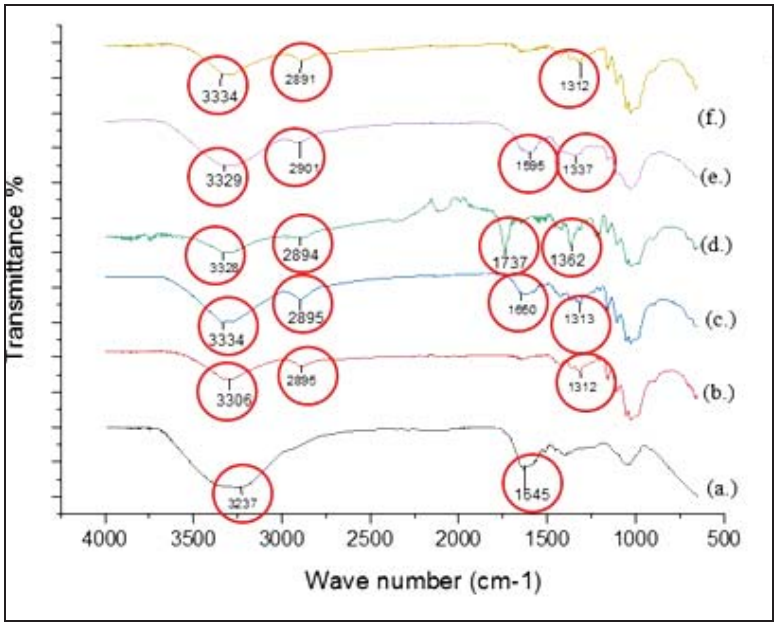

Figure 4. FT-IR spectrum of **(a)** *Moringa oleifera* leaf extract; **(b)** microcrystalline cellulose; **(c)** PM 1:2; **(d)** PM 1:4; **(e)** SSD 1:2; **(f)** SSD 1:4.

Fourier Transform Infra-Red (FT-IR) spectroscopy

FT-IR spectroscopy was used to investigate the interaction between active ingredients and carriers (Patel et al., 2012). This analysis focused on identifying functional groups and potential intermolecular interactions between *M. oleifera* leaf extract and microcrystalline cellulose. Vibrational and translational motions of the functional group in the sample were observed as an intensive peak absorption spectrum. Intermolecular interactions in the surface solid dispersion were suggested by reductions in peak intensity and shifts in absorption, as shown in the FT-IR spectrum (Wicaksono et al., 2022). The FT-IR spectrum of *M. oleifera* leaf extract, microcrystalline cellulose, PM, and SSD powder is presented in Fig. 4.

The FT-IR spectrum of *M. oleifera* leaf extract showed a broad absorption peak at wavenumber 3237 cm^{-1}, corresponding to O-H stretching. Additionally, the spectrum exhibited specific peaks at 1645 cm^{-1} (C=O stretching) and 1221 cm^{-1} (C-H stretching). Several distinct absorption peaks were also observed in the FT-IR spectrum of microcrystalline cellulose, indicating O-H stretching at 3306 cm^{-1}, C-H stretching at 2895 cm^{-1}, and C-H bending observed in wavenumber 1312 cm^{-1}. The FT-IR spectrum of PM displayed the specific absorption peaks of both materials, the extract, and microcrystalline cellulose as carriers. A reduction in peak intensity and a shift in specific absorption peaks were observed in the FT-IR spectrum of SSD. Notably, the absorption peak at 1645-1650 cm^{-1}, corresponding to C=O stretching, was absent in the FT-IR spectrum of SSD 1:4. The absence of this peak, along with the reduction in peak intensity, indicates the interaction between *M. oleifera* leaf extract and microcrystalline cellulose as carriers. The interaction likely involves hydrogen bonding, which contributes to the miscibility of the extract upon contact with water (Fitriani et al., 2024). Consequently, this interaction may enhance the solubility and dissolution of phytochemical compounds from the extract.

Morphology

The morphological characteristics of PM and SSD powders were examined using scanning electron microscopy (SEM), as shown in Fig. 5. The morphology of PM powder was observed as columnar and cracked particles, with aggregate formation visible in the micrograph. However, while the SSD powder displayed similar columnar and cracked particle morphology, aggregate formation was notably absent. This distinction is presumably attributed to the microwave irradiation process, which reduces the system's moisture content and promotes a more uniform deposition of the extract onto the microcrystalline cellulose compared to the PM powder. The SEM micrograph also revealed that the porous surface of microcrystalline cellulose facilitated the deep penetration of the extract into the carrier's internal structure and influenced extract deposition during SSD preparation (Singh et al., 2014).

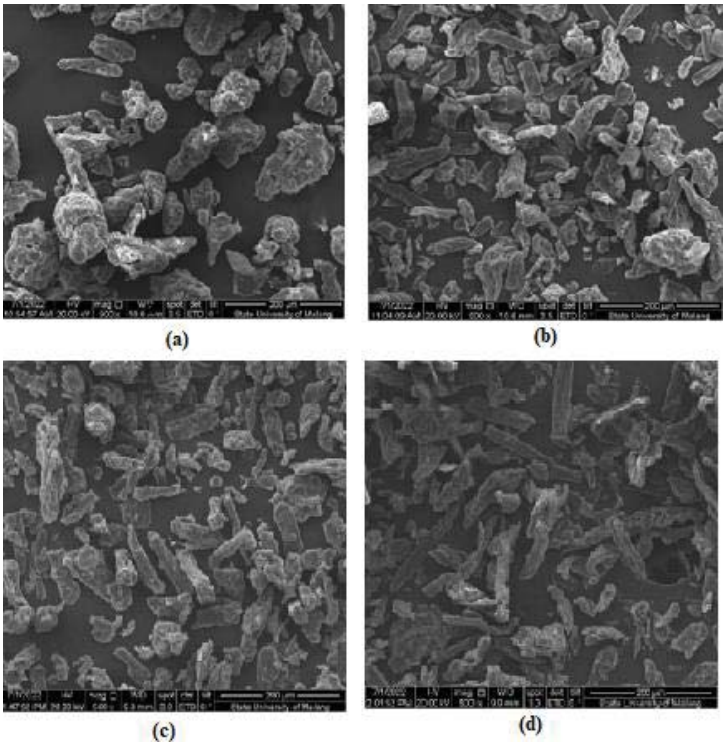

Figure 5. SEM micrograph of **(a)** PM 1:2; **(b)** PM 1:4; **(c)** SSD 1:2; **(d)** SSD 1:4. Magnification 5000×.

Moisture content

Moisture content analysis was conducted to evaluate the moisture percentage in the prepared PM and SSD powders. Moisture content influences powder flowability and compaction properties (Sun, 2008). Changes in the moisture content of active ingredients and excipients have been shown to affect interparticle lubrication and reduce density, leading to compaction products with lower mechanical strength (Crouter and Briens, 2013). The analysis revealed a significant difference in moisture content between PM and SSD powders ($p<0.05$). SSD powder exhibited lower moisture content than PM powder. This condition might be due to the dispersion process facilitated by grinding. The co-grinding process of the extract and microcrystalline cellulose induced direct collisions between the extract and the carrier. This process also reduced the particle size of microcrystalline cellulose, enhancing the adsorption of the extract onto the carrier's surface and pores (Siahi-Shadbad et al., 2014). These simultaneous processes significantly reduced the moisture content of SSD compared to PM. In addition, the increased microcrystalline ratio in SSD preparation contributed to the reduced moisture content (Cegledi et al., 2022). Among the tested samples, SSD 1:4 exhibited the lowest moisture content. The results of the moisture content of PM and SSD powders are presented in Table 2.

Table 2. The moisture content of PM and SSD of *Moringa oleifera* leaf extract-microcrystalline cellulose.

Sample	Moisture content (%)
PM 1:2	18.73 ± 1.72^{a}
PM 1:4	11.43 ± 0.22^{b}
SSD 1:2	8.99 ± 0.23^{c}
SSD 1:4	4.69 ± 0.14^{d}

Data are expressed as mean ± SEM or SD (n = 3). Treatments not sharing the same letters in the same column are significantly different by ANOVA followed by a Tukey's test ($p<0.05$). PM: physical mixture; SSD: surface solid dispersion.

Analytical method validation of total flavonoid content analysis

Specificity

The specificity parameter was evaluated to verify the ability of a colorimetric-spectrophotometric method to identify the analyte (flavonoid compound) within the sample, which consisted of several components (Shanko et al., 2024). The UV-Vis spectrophotometer was used to analyze the spectrum of a quercetin standard solution (5 ppm and 7 ppm), *M. oleifera* leaf extract, SSD powder, and matrix solution. The scanning range was 400-600 nm. The results indicated that the maximum wavelength of quercetin as a marker of

flavonoid compound was observed at 435 nm in both the extract and standard solution. These findings confirm the method's capability to detect the primary chemical compound in the sample (Abuzaid et al., 2020). Furthermore, the matrix solution's spectrum exhibited no absorption within the 400-600 nm range, indicating no interference with analyte determination at the maximum wavelength of 435 nm. The spectrum of the quercetin standard solution, *M. oleifera* leaf extract, and matrix solution are presented in Fig. 6.

Linearity and range

The calibration curve of quercetin as a standard solution was prepared in triplicate. The absorption of a yellow complex formed between quercetin and $AlCl_3$ was analyzed using a visible spectrophotometer at 435 nm. Linearity of the calibration curve was observed within the range of 3.06-10.20 ppm. The correlation coefficients (R^2) for the three replicates were 0.9991, 0.9970, and 0.9960, respectively, indicating excellent linearity, as R^2 values exceeded 0.99 (Abuzaid et al., 2020). The Vxo values for the calibration curve ranged from 1.45%-4.95%. The Vxo value < 5.0% possessed linearity of the regression curve (Oktriana et al., 2022). The regression equation was y=0.0861x-0.0665 and the correlation coefficient was 0.9991. The calibration curve of the standard quercetin-$AlCl_3$ complex, which was determined spectrophotometrically at 435 nm, is presented in Fig. 7.

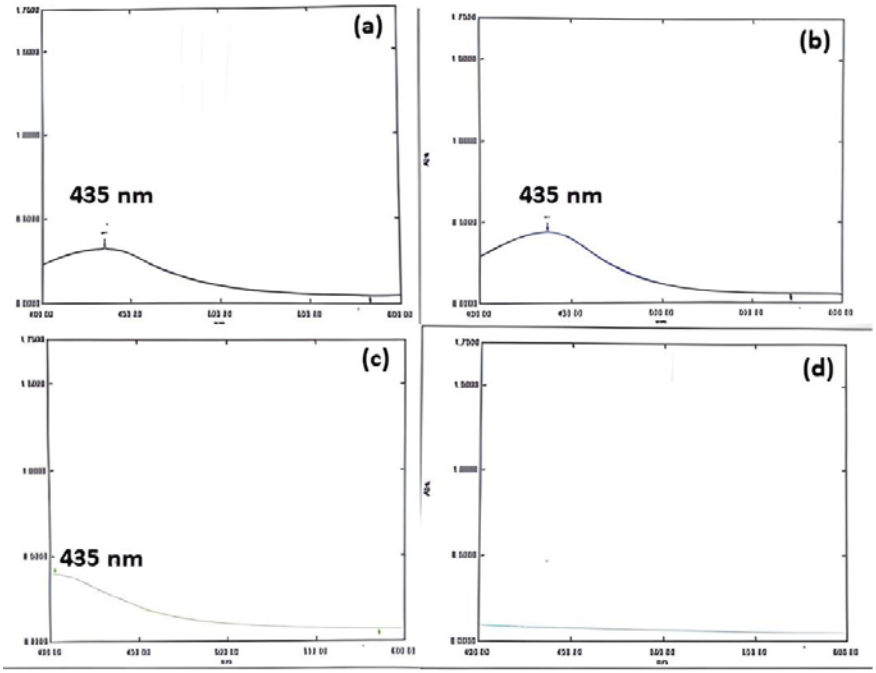

Figure 6. UV/Vis spectrum of **(a)** standard solution of quercetin 5 ppm; **(b)** standard solution of quercetin 7 ppm; **(c)** SSD 1:2, and **(d)** matrix solution (microcrystalline cellulose).

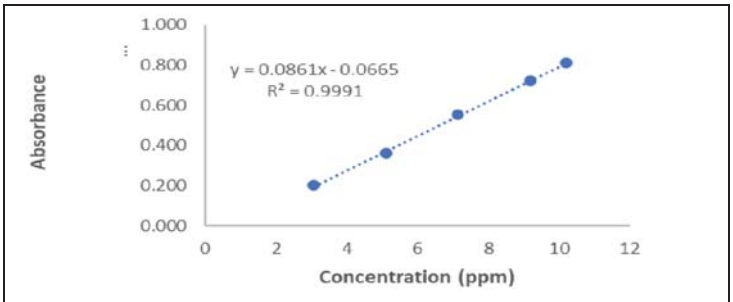

Figure 7. Calibration curve of standard quercetin-aluminum chloride complex assayed by visible spectrophotometry at 435 nm.

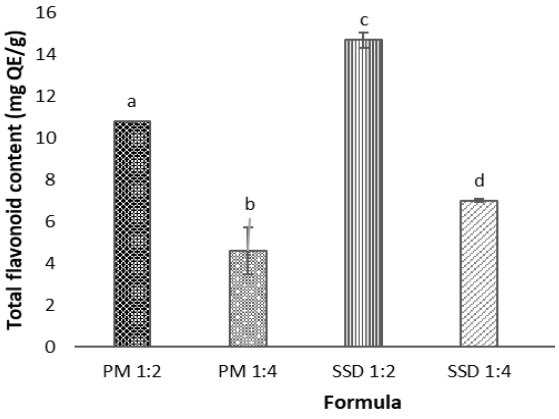

Figure 8. Total flavonoid content of the physical mixture (PM) and surface solid dispersion (SSD) of *Moringa oleifera* leaf extract – microcrystalline cellulose in 1:2 and 1:4 ratio.

Precision

The precision parameter described the closeness of the results from a series of measurements of the same sample. It was analyzed through the standard deviation or relative standard deviation of the samples, which are determined in the same analysis parameter condition (Oktriana et al., 2022). In this study, repeatability and intermediate precision showed RSD% values of 1.88% and 3.86%, respectively. The complex and multicomponent nature of the extract contributed to the higher inherent variation in total flavonoid content. Additionally, variations in the extraction process and complexation preparation further influenced the precision results (Da Silva et al., 2015). Despite these factors, the RSD% values obtained from this study were below 5%, indicating acceptable precision and reproducibility of the method (Abuzaid et al., 2020). The results for repeatability and intermediate precision are presented in Table 3.

Table 3. The results of repeatability and intermediate precision.

Parameter	Repeatability	Intermediate precision
Mean (mg QE/g)	31.00	32.61
SD	0.58	1.26
RSD%	1.88	3.86

Total flavonoid content analysis

The total flavonoid content analysis was conducted to quantify the total flavonoid group compound in the samples. The flavonoid content was expressed as quercetin equivalent (%QE). A colorimetric method was employed in this study to calculate the flavonoid compound in the PM and SSD samples. The reaction between the flavonoid compound and $AlCl_3$ formed a yellow complex. The flavonoid compound and $AlCl_3$ form an acid-resistant complex between the ketone group at the C-4 atom and the hydroxy (-OH) group at the C-3 or C-5 atom (Martono et al., 2019). The intensity of the yellow complex was measured using a visible spectrophotometer at 435 nm wavelength.

The total flavonoid content of PM and SSD powders is presented in Fig. 8. The results showed that the total flavonoid content for PM and SSD of *M. oleifera* leaf extract-microcrystalline cellulose with 1:2 and 1:4 ratios were 10.9 ± 0.73, 4.6 ± 1.12, 14.70 ± 0.35, and 7.00 ± 0.07 µg/mL, respectively. The total flavonoid content of SSD powder with 1:2 and 1:4 ratios showed a significant difference compared to the PM powder ($p<0.05$). The SSD preparation method effectively enhanced the solubility of flavonoid compounds in *M. oleifera*. This improvement is likely attributed to the deposition of the *M. oleifera* leaf extract on the surface of the hydrophilic carrier, leading to particle size reduction and increased wettability. These factors collectively contribute to improved solubility and dissolution of flavonoid compounds (Sapkal et al., 2020).

On the contrary, the increased proportion of microcrystalline cellulose in PM and SSD preparations significantly impacted the total flavonoid content in these samples. Microcrystalline cellulose was a carrier with a strong ability to entrap the plant ethanolic extracts due to the high surface area and porosity (Widyaningsih et al., 2021). Additionally, microcrystalline cellulose is insoluble in water. This character emphasizes the ability of microcrystalline cellulose to adsorb the *M. oleifera* leaf extract within its structure. When exposed to water, the high porosity of microcrystalline cellulose facilitates water penetration into the powder's structure. The water penetration process into an insoluble carrier produces a high-viscosity film layer

on the surface of SSD and PM powders. The high viscosity and thick layer produced by this mechanism can hinder the release of bound substances, such as *M. oleifera* extract (Bajracharya et al., 2022). This phenomenon correlated with the lower total flavonoid content observed in PM and SSD powder with 1:4 ratio compared to 1:2 ratio.

Solubility study

The equilibrium solubility of total flavonoid compounds from PM and SSD powders is presented in Fig. 9. The results show that the preparation of PM and SSD powders of *M. oleifera* leaf extract in 1:2 and 1:4 ratios yielded solubility values of 54.60 ± 1.73, 58.80 ± 0.37, 62.26 ± 0.62, and 63.58 ± 0.62 µg/mL, respectively. The SSD preparation using 1:2 and 1:4 ratios significantly increased total flavonoid solubility by 1.14 and 1.08-fold compared to the physical mixture at the same ratio ($p<0.05$). The improved solubility from SSD powder was related to the high interparticle porosity of microcrystalline cellulose, which provides an internal surface area of approximately 90-95%. This high porosity promoted water penetration through capillary action into the SSD powder structure. The penetration of water into this structure easily disrupted hydrogen bonding between the active ingredient and carrier, enhancing particle wettability and solubility (Thoorens et al., 2014). The significant reduction of particle size during SSD formation by the milling process also contributed to the increase of total flavonoid solubility from SSD powder. The reduction of particle size induced the higher surface area exposed to the dissolution medium and improved wettability of the extract, hence the solubility was also increased (Patel et al., 2012). Furthermore, the improvement in drug solubility could be attributed to the reduction of crystal lattice regularity of the extract in the SSD-loaded *M. oleifera* leaf extract (Siahi-Shadbad et al., 2014). From this result, it can be concluded that the SSD preparation containing *M. oleifera* leaf extract is a promising approach for improving not only the physicochemical properties of the extract but also the solubility profile.

In vitro antioxidant activity

Antioxidant activity was evaluated using the free radical ABTS. Flavonoids (quercetin) contain a catechol group in ring B that serves as a target for free radicals due to its excellent electron-donating properties, which enables flavonoids to stabilize free radicals (Chaves et al., 2020). The principle of the ABTS test is to remove the color of the ABTS cation to measure the antioxidant capacity, which directly reacts with ABTS cation radicals. If reduced by antioxidants, it will turn colorless (Nguyen et al., 2015). The parameter used to determine antioxidant activity is the inhibitory concentration 50 (IC_{50}) value. IC_{50} value was utilized to interpret the results of free radical scavenging activity testing. The IC_{50} value is defined as the concentration of the test compound that can reduce free radicals by 50%. The smaller the IC_{50} value, the higher the free radical scavenging activity (Fattahi et al. 2014). The IC_{50} values of PM and SSD powders are presented in Fig. 10.

The antioxidant activity of SSD and PM powders of *M. oleifera* leaf extract-microcrystalline cellulose at ratios of 1:2 and 1:4 was evaluated using a microplate reader at a wavelength of 726 nm. The results of the IC_{50} values for surface solid dispersion powder and physical mixture powder with a ratio of 1:2 and ratio of 1:4 were 396.65 ± 42.33, 524.44 ± 7.6, 499.9 ± 9.62 and 539.38 ± 12.18, respectively. The IC_{50} value results show that SSD powder has better antioxidant activity compared to PM powder. The IC_{50} value of a 1:2 ratio SSD has better antioxidant activity than a 1:4 ratio.

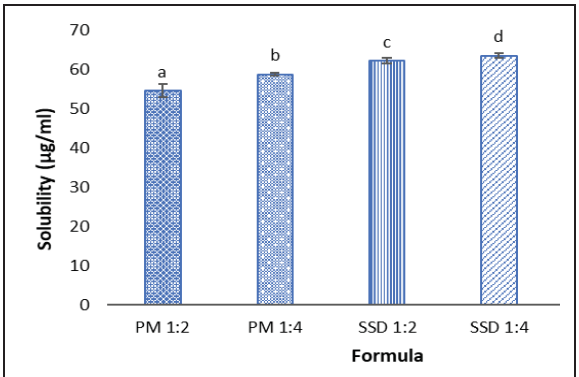

Figure 9. Solubility of physical mixture (PM) and surface solid dispersion (SSD) of *Moringa oleifera* leaf extract – microcrystalline cellulose in 1:2 and 1:4 ratio.

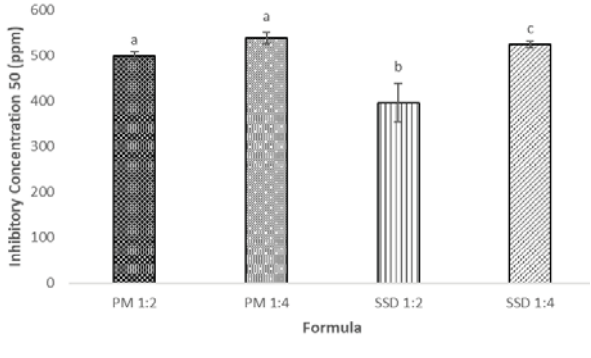

Figure 10. Inhibitory concentration 50 of the physical mixture (PM) and surface solid dispersion (SSD) of *Moringa oleifera* leaf extract – microcrystalline cellulose in 1:2 and 1:4 ratio.

Research conducted by Sankhalkar and Vernekar (2016) showed a correlation between increased antioxidant activity and increased phenolic and flavonoid content. The increase in antioxidant activity is greatly influenced by the content of flavonoid compounds contained in the extract. This can happen because more flavonoid compounds can donate hydrogen ions (H^+) to free radicals. Thus, free radicals become more stable (Chaves et al., 2020). This correlation suggests that an increase in total flavonoid content enhances antioxidant activity.

Limitations of the study

This study has several limitations related to the analytical method validation parameters. Due to the complex composition of the extract and the limited data on the concentration of flavonoid compounds, the validation parameters assessed were restricted to specificity, linearity, range, and precision. Additionally, antioxidant activity was evaluated solely using the ABTS method. Another antioxidant assay could not be performed due to constraints in sample availability and extract quantity.

CONCLUSION

The preparation of surface solid dispersion (SSD) containing *Moringa oleifera* leaf extract was successfully achieved using microcrystalline cellulose as a carrier. The co-grinding method significantly improved the characteristics of SSD powder, including reduced moisture content and particle size. Furthermore, surface solid dispersion also contributed to the thermal characteristics of the extract and the reduction of crystal lattice regularity due to the interaction between the extract and microcrystalline cellulose. The solubility of the total flavonoid content increased by approximately 1.16-fold in the SSD compared to the PM. These findings suggest that SSD preparation using the co-grinding method is a promising approach to enhance the physicochemical characteristics of *Moringa oleifera* leaf extract, paving the way for its formulation into a solid dosage form.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Rani KC	Prameswari P	Matulatan C	Putri APH	Setiawan F	Jayani NIE
Concepts or ideas	x				x	x
Design	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x
Literature search	x	x	x	x		
Experimental studies	x	x	x	x	x	x
Data acquisition	x	x	x	x	x	x
Data analysis	x	x	x	x	x	x
Statistical analysis	x	x	x	x		
Manuscript preparation	x	x	x	x	x	x
Manuscript editing	x					x
Manuscript review	x	x	x	x	x	x

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5.- Original Article

Andri Prasetiyo, Esti Mumpuni, Dewi Luthfiana, Rina Herowati, Galih Satrio Putra (2025) ***In silico* discovery of potential sodium-glucose cotransporter-2 (SGLT-2) inhibitors from *Smallanthus sonchifolius* (Poepp.) H.Rob. via molecular docking and molecular dynamics simulation approach.** | [Descubrimiento *in silico* de potenciales inhibidores del cotransportador de sodio-glucosa-2 (SGLT-2) de *Smallanthus sonchifolius* Poepp.) H.Rob. mediante un enfoque de acoplamiento molecular y simulación de dinámica molecular]. J Pharm Pharmacogn Res 13(3): 716-728. https://doi.org/10.56499/jppres24.2104_13.3.716 [1.09 Mb] [ABSTRACT]

6.- Original Article

Athiyah Layla, Sri Widyarti, Sutiman Bambang Sumitro (2025) ***In silico* study of α-synuclein pathology in Parkinson's disease and the potential of H_2O_2 as complementary therapy.** | [Estudio *in silico* de la patología de la α-sinucleína en la enfermedad de Parkinson y el potencial del H_2O_2 como terapia complementaria]. J Pharm Pharmacogn Res 13(3): 729-743. https://doi.org/10.56499/jppres24.2122_13.3.729 [1.28 Mb] [ABSTRACT]

7.- Original Article

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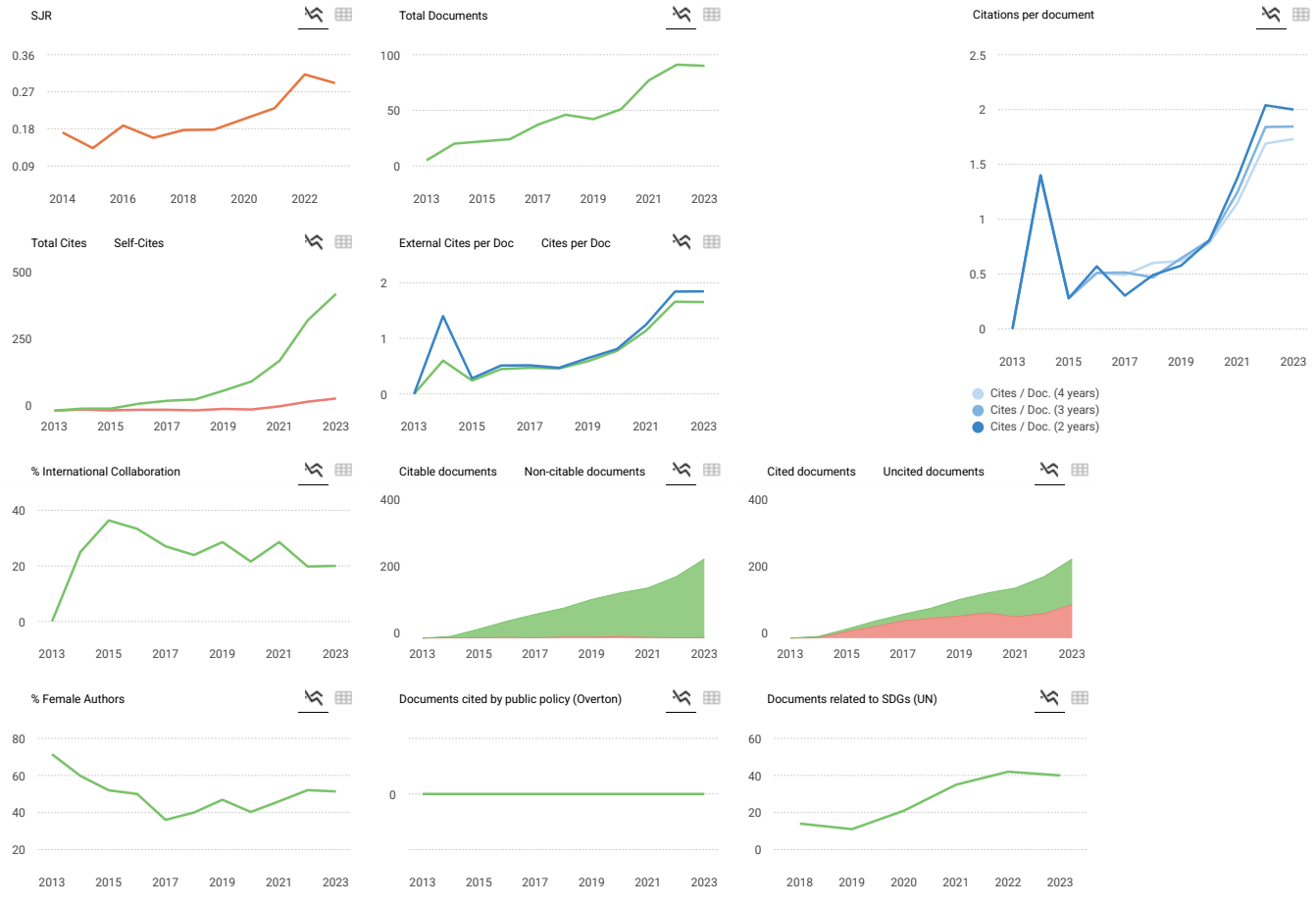

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