

Preparation and evaluation of surface solid dispersion of *Moringa oleifera* leaf extract using freeze-drying method

Karina Citra Rani ^{1*} , Roisah Nawatila ¹ , Agnes Nuniek Winantari ^{1*} , Aditya Trias Pradana , Nikmatul Ikhrom Eka Jayani ² , As-Syifa Dilut Tri Antopo ³ , Kezia Febriana ³ 

¹ Department of Pharmaceutics, Faculty of Pharmacy, University of Surabaya, Indonesia.

² Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, Indonesia

³ Faculty of Pharmacy, University of Surabaya

* Corresponding Author. E-mail: karinacitrarani@staff.ubaya.ac.id (K.C.R); Tel. +6281803042202.

Received: 04 May 2023 / Revised: 26 May 2023/ Accepted: 27 May 2023

ABSTRACT: Moringa leaf extract contains flavonoids, which are useful as a source of antioxidants. Its development into pharmaceutical dosage forms, however, has several problems, including thick consistency, low solubility in water, and heat-sensitive stability. Formation into surface solid dispersion (SSD) is one approach to increase the solubility of flavonoid compounds and improve the physical-mechanical characteristics of moringa leaf extract. This research aimed to develop SSD of moringa extract with microcrystalline cellulose as the carrier as well as to perform its physical and chemical characterization. The method used to prepare the SSD was freeze drying with two extract-to-microcrystalline cellulose ratios, namely 1:2 and 1:4. Results showed that the 1:2 ratio produced 6.09% moisture content and adequate powder flowability, while the 1:4 SSD system had 5.06% moisture content and poor flowability. In addition, crystallinity analysis and thermal characteristics indicated a reduction in the regularity of the crystal lattice, marked by a decrease in the specific peak intensity on the X-ray diffractogram, as well as a shift in the melting point and a decrease in the enthalpy of the SSD system in both ratios on the DSC thermogram. The total flavonoid contents of the SSD were 7.1 ± 0.0527 mg QE/g for the 1:2 ratio and 4.0 ± 0.0797 mg QE/g for the 1:4 ratio. Also, the solubility of flavonoid compounds of the 1:2 SSD system was 67.33 µg/ml, showing enhanced solubility compared to moringa leaf extract (64.11 µg/ml), physical mixture (54.60–58.81 µg/ml), and the 1:4 SSD system (48.09 µg/ml) ($p < 0.05$). Based on these results, it can be concluded that SSD of moringa leaf extract-microcrystalline cellulose (1:2) has the potential to be further developed into pharmaceutical dosage forms.

KEYWORDS: *Moringa oleifera*; surface solid dispersion; extract; freeze drying

1. INTRODUCTION

Moringa oleifera is a medicinal plant that rapidly grows in tropical areas such as Indonesia. *M. oleifera*, belonging to the family *Moringaceae*, has proved to be positive for human health. It is rich in nutrients, and the phytochemicals copiously present in the leaves, pods, and seeds are used as medicinal ingredients [1]. The leaves are a source of vitamin C, calcium, beta-carotene, and protein. In addition, they are natural antioxidants, a characteristic linked to the flavonoid, ascorbic acid, carotenoid, and phenolic contents [2], that also protect against oxidative stress, inflammation, hepatic fibrosis, and liver damage. Antibacterial properties, including against multiple drug-resistant gram-positive and gram-negative bacteria pathogens, have also been reported [3]. Moringa leaves are also a potential source of vitamin E, which is antioxidative and has an inhibitory effect on cell proliferation [4].

Moringa leaves have been widely known as a prominent source of polyphenol compounds such as flavonoids and phenolic acids [5]. The main flavonoid contents are myricetin, quercetin, and kaempferol [6]. Moringa leaves harvested in Ghana, Senegal, and Zambia have a total flavonoid content of 0.18% to 1.64% (g/dry weight). Phenolic acids are parts of phenolic compounds that produce antioxidant, anti-inflammatory, antimutagenic, and anticancer effects [5]. Gallic acid, chlorogenic acid, and caffeic acid are the most abundant phenolic compounds in the leaves. Moringa leaf extracts are preferably developed as active ingredients in dosage forms because of the concentrated active compounds and their applicability in small concentrations. The hydroalcoholic extract has potent antioxidant activity, as demonstrated by the IC₅₀ value of 232.6 ± 7.61 µg/ml from the DPPH (1,1-difenil-2-pikrilhidrazil) method [7]. Besides, it contains rutin (1.58 ± 0.06 w/w), quercetin ($0.26 \pm 0.01\%$), ellagic acid ($0.20 \pm 0.01\%$), chlorogenic acid ($0.23 \pm 0.01\%$), and ferulic acid ($0.16 \pm$

How to cite this article: Rani KC, Nawatila R, Winantari AN, Pradana AT, Jayani NIE, Antopo ASDT, Febriana K. Preparation and evaluation of surface solid dispersion of *Moringa oleifera* leaf extract using freeze-drying method. J Res Pharm. 2023; 27(6): 1-XX.

0.01%). In a different study, the ethanol extract produced the highest antioxidant activity ($1C_{50} = 103.98 \mu\text{g/ml}$) compared to the extracts separated using n-hexane ($448.17 \mu\text{g/ml}$) and ethyl acetate ($169.90 \mu\text{g/ml}$) [7]. Hence, ethanol extracts of *M. oleifera* leaves are promising ingredients for pharmaceutical dosage forms and nutraceutical products.

According to Indonesian Herbal Pharmacopeia, the moringa leaf extract is a thick extract with brownish-green color, distinctive odor, and bitter taste. The thick consistency, however, causes physico-mechanical problems when formulated into pharmaceutical dosage forms, especially the solid ones. Chemically, this thick extract has a total flavonoid content of not less than 6.30%, identified as quercetin [8]. Quercetin, the marker compound of moringa leaves, is categorized as class II (water-insoluble) in the Biopharmaceutical Classification System (BCS). In physicochemical and pharmacodynamic studies, Kulkarni et al. confirmed the solubility of quercetin in water at 60 mg/L, indicating insolubility [9]. The other flavonoids identified in the moringa leaf extract are myricetin and kaempferol, with low water solubility at 54.9 mg/L and 440 mg/L [10]. The characteristically low-solubility flavonoids of *M. oleifera* leaves in water limit the development of the extract into a dosage form. Solubility is an essential property that determines the dissolution, absorption, and bioavailability of a flavonoid in systemic circulation [11]. Therefore, techniques such as solid dispersion, inclusion complex, salt formation, and nanocrystals are needed to address the low solubility and dissolution problem.

Surface solid dispersion (SSD) is a mechanical engineering used to disperse an extract on the surface and in the pore of an insoluble-hydrophilic carrier [12]. This technique increases the surface area of the moringa leaf extract that is in contact with an aqueous medium while making it less prone to clumping. In this case, a higher contact surface area leads to a higher dissolution rate. SSD uses carriers that are hydrophilic, insoluble in water, and porous. It quickly disperses upon contact with water, generating rapid drug release into an aqueous medium [12]. Several hydrophilic materials with a high surface area are microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, and crospovidone. In this present study, microcrystalline cellulose was used as the carrier to produce the SSD of the *M. oleifera* leaf extract. Microcrystalline cellulose is a porous, hygroscopic, and water-insoluble powder that expands substantially when in contact with water and is characterized as having a high surface area of approximately $1.21\text{--}1.30 \text{ m}^2/\text{g}$ [13,14]. The hydrophilic group in the structure of this molecule strongly contributes to its hydrophilic character [15]. Hydrophilicity, high porosity, and wide surface allow for rapid drug release from SSD [16].

Applying microcrystalline cellulose in SSD successfully improves cefuroxime dissolution rate and antibacterial activity [17]. Reduced particle size, increased contact surface area, and enhanced drug wettability have been predicted as the mechanisms responsible for better drug solubility and release from SSD [18]. Microcrystalline cellulose is also a potential adsorbent to convert active pharmaceutical ingredients that are sticky or strongly adhesive into free-flowing powders [19]. Its porous structure promotes the adsorption and release of a liquid extract upon contact with an aqueous medium—required characteristics as a suitable carrier for plant extracts in an SSD form [20]. Microcrystalline cellulose has proved to be an excellent carrier for the *Prunus padus* liquid extract due to its ability to protect from moisture-related interventions, prevent clumping, and improve flow properties [17]. Drug-to-carrier ratios also play a pivotal role in developing SSD to improve the dissolution rate [16]. In this study, the SSD of the moringa leaf extract was designed using microcrystalline cellulose as the carrier at 1:2 and 1:4 ratios.

The freeze-drying method was applied in this study to prepare the SSD. Freeze drying, widely known as lyophilization, exerts a positive influence on the development of pharmaceutical products. This process provides better stability during storage for thermolabile components such as protein drugs, plant-based constituents, and plant extracts [21]. A previous study revealed that the freeze-drying of thyme extracts into powders with higher bulk density and better flowability than the other drying methods. Moreover, these powders showed a lower reduction in total phenolic and flavonoid contents, indicative of stable antioxidant activity during storage [22]. Freeze-drying applies sublimation, thus creating powders with a porous structure and enhancing the wettability and solubility of the entrapped extract. The higher solubility and dissolution rate can be attributed to the rapid transformation into an amorphous phase during freeze-drying [23]. The SSD prepared in this study was further evaluated to determine the powder characteristics, physicochemical characteristics, total flavonoid contents, and solubility.

2. RESULTS AND DISCUSSION

2.1. Physical characteristics of surface solid dispersion (SSD) and physical mixture (PM)

The physical characterization revealed that the SSD powders formulated from the *Moringa oleifera* leaf extract and the carrier microcrystalline cellulose had a fine grain size, light yellow color, and no specific odor and taste. Meanwhile, the PM powders were in the form of greenish-yellow aggregates that were agglomerated and had the distinguished odor of the *M. oleifera* leaf extract. The observed physical characteristics of both powders are shown in Figure 1. From the observation results, it can be assumed that microcrystalline cellulose is an excellent carrier to formulate pharmaceutical preparations from active ingredients, i.e., flavonoids, with low water solubility. Further, the porous structure of microcrystalline cellulose allows the adsorption of a thick liquid extract like that of *M. oleifera* leaves, due to which the carrier covers the unfavorable physical properties of the extract [24].

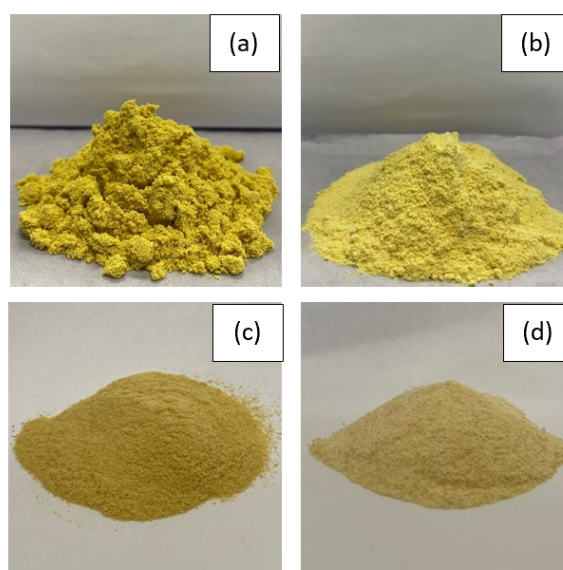


Figure 1. Powder appearances of the physical mixtures (PMs) of the moringa leaf extract and microcrystalline cellulose at (a) 1:2 ratio and (b) 1:4 ratio, and the surface solid dispersions (SSDs) of the moringa leaf extract and microcrystalline cellulose at (c) 1:2 ratio and (d) 1:4 ratio.

Both PM and SSD powders were made at different extract-to-carrier ratios, creating four different products: PM with a 1:2 ratio of the mixture (or PM 1:2), PM with a 1:4 ratio (PM 1:4), SSD 1:2, and SSD 1:4. The true, bulk, tapped densities, Hausner ratio, and compressibility index values are summarized in Table 1. SSD powders had a higher bulk density than PM powders, indicating that the same weight of powder occupies a smaller bulk volume in SSD. The same characteristics were also observed from the tapped densities. Further, The Hausner ratio and compressibility index were calculated to analyze the powder's flow and compressibility [25]. The Hausner ratio of all the prepared powders varied between 1.2995 and 1.3912, and the compressibility index was in the range of 23.03–27.91%. Based on both figures, it can be concluded that PM 1:2 and SSD 1:4 had poor flow characteristics. On the contrary, PM 1:4 and SSD 1:2 were free-flowing powders. Consequently, SSD 1:2 is considered the most reliable and promising system for the further development of solid dosage forms from *M. oleifera* leaf extracts. Powder flowability is contingent on the physical characteristics of the powder, such as particle size, shape, and density [26]. Moreover, SSD 1:2 showed only a slight difference between its bulk and tapped densities compared to the other tested systems, indicating enhanced flow properties. The homogeneous particle size observed in SSD 1:2 is also responsible for better flowability.

Moisture content is a measure of the ability of the powder to adsorb water vapor from the atmosphere, which may vary across the manufacturing and handling processes [27]. In addition to determining the powder's microbial activity [28], the moisture content is also an influencing factor of powder flowability; a decrease in powder flowability is most likely observed with an increased moisture level. Results showed that the moisture contents of the PM powders were 18.73% for the 1:2 ratio and 11.43% for the 1:4 ratio, or significantly higher than the SSD powders: 6.09% for the 1:2 ratio and 5.06% for the 1:4 ratio. A previous study revealed that the appropriate moisture content of *M. oleifera* leaf powder is approximately 5% [29]. Therefore, the SSD tested in the current study is the most suitable system for the development of moringa leaf extract

powders, especially because it is also less prone to instability during storage compared to the PM system. Moreover, based on the observed physical characteristics, SSD powders have better flowability and stability than PM powders.

Table 1. Physical characteristics of the physical mixture (PM) and surface solid dispersion (SSD) powders of the *Moringa oleifera* leaf extract formulated at different ratios of mixture to the carrier (microcrystalline cellulose).

Physical Characteristic	PM 1:2	PM 1:4	SSD 1:2	SSD 1:4
True density (g/ml)	1.45±0.28	1.89±0.19	1.48±0.10	1.67±0.06
Bulk density (g/ml)	0.26±0.02	0.25±0.01	0.34±0.01	0.31±0.00
Tapped density (g/ml)	0.36±0.08	0.33±0.01	0.45±0.01	0.43±0.03
Hausner ratio	1.39±0.01	1.31±0.01	1.30±0.03	1.36±0.08
Compressibility index (%)	27.88±5.11	23.71±0.34	23.03±1.71	26.55±4.13
Moisture content (%)	18.73±1.72	11.43±0.22	6.09±0.11	5.06±0.05

2.2. Solid-state characteristics of SSD and PM

2.2.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was conducted to identify any alterations in the thermal characteristics of *M. oleifera* leaf extract and microcrystalline cellulose after being formulated into SSD. The thermogram of PM powders was also analyzed for comparison. Also, DSC was used to determine physicochemical interactions between the extract and microcrystalline cellulose [30]. DSC thermograms of the extract, microcrystalline cellulose, PM powders, and SSD powders are shown in Figure 2. The *M. oleifera* leaf extract had an endothermic peak at 112.36°C, corresponding to the melting point of the extract, with a reaction enthalpy of -5733.61 J/g. Meanwhile, microcrystalline cellulose exhibited a semicrystalline character, with an endothermic peak at 79.92°C. The DSC thermograms of PM and SSD each had a lower endothermic peak than the extract: 74.74°C for PM 1:2, 100.86°C for PM 1:4, 76.17°C for SSD 1:2, and 53.13°C for SSD 1:4. These results indicated that the melting point of the extract shifts substantially when formulated into SSD. In addition, the DSC thermograms also show that the enthalpies of the SSD powders were -151.87 J/g for the 1:2 ratio and -34.59 J/g for the 1:4 ratio. The substantially lower melting point and enthalpy suggest that the extract experiences a decrease in the regularity of its crystal lattice and, hence, a change from a crystalline to an amorphous state when developed into SSD [12].

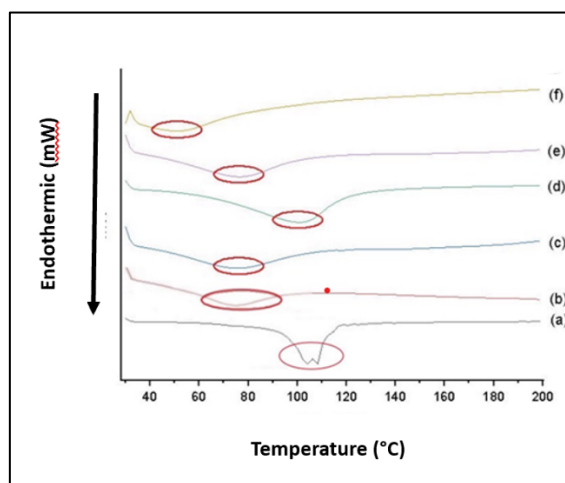


Figure 2. DSC thermograms of (a) *Moringa oleifera* leaf extract, (b) microcrystalline cellulose, (c) physical mixture (PM) 1:2, (d) PM 1:4, (e) surface solid dispersion (SSD) 1:2, and (f) SSD 1:4.

2.2.2. Powder X-ray diffraction (P-XRD)

A powder X-ray diffraction (P-XRD) study was conducted to analyze the crystallinity of the extract and the carrier at the initial and final stages, i.e., in SSD and PM powders [30]. The P-XRD patterns of the *M. oleifera* leaf extract, microcrystalline cellulose, PM, and SSD are presented in Figure 3. The diffractograms indicated an amorphous extract and a semi-crystalline carrier; the latter was determined from specific peaks at 14.52°, 22.46°, and 34.53° at the 2θ angle. An amorphous product creates a broad background pattern [23]. The major characteristics of the carrier's crystalline peaks were observed in the P-XRD patterns of the SSD and PM powders. Still, a reduction in the peak intensity was more significant in the SSD pattern. These results indicated that the carrier's crystallinity is reduced in the SSD system, possibly due to the partial conversion of the extract and the carrier into an amorphous state [31]. Moreover, with only the specific peaks of microcrystalline cellulose appearing in the SSD diffractograms, the moringa leaf extract's character is no longer present in SSD systems, suggesting that the extract is completely dispersed in the selected carrier [23].

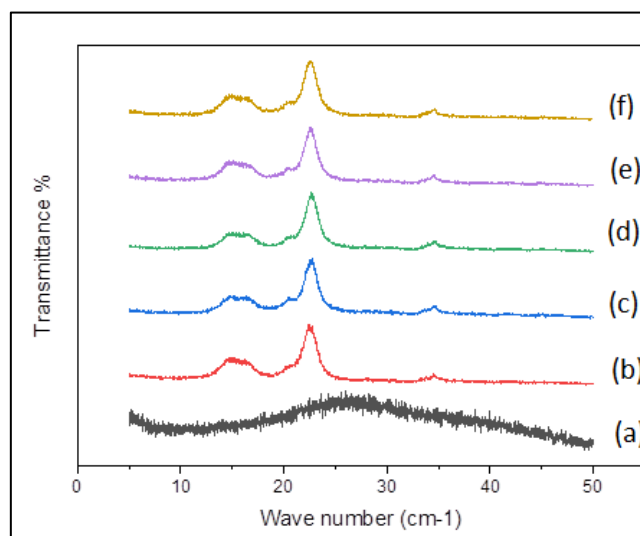


Figure 3. P-XRD diffractograms of (a) *Moringa oleifera* leaf extract, (b) microcrystalline cellulose, (c) physical mixture (PM) 1:2, (d) PM 1:4, (e) surface solid dispersion (SSD) 1:2, and (f) SSD 1:4

2.2.3. Scanning electron microscopy

Scanning electron microscopy (SEM) analysis was performed to evaluate the sample's morphology, surface roughness, fracture, cleavage, and crystal habit [32]. The micrographs of the PM and SSD formulated from the *M. oleifera* leaf extract and microcrystalline cellulose are shown in Figure 4. The PM micrograph shows that the extract was embedded at the surface of microcrystalline particles, which were columnar, fractured, and consolidated. Thus, it can be predicted that the particles form aggregates and agglomerate structures in PM. On the contrary, the SSD micrograph shows that the extract was dispersed on the surface of the carrier with columnar, porous particles that had hollow structures and smooth surfaces. Therefore, it can be concluded that the freeze-drying method produces SSD powders with a porous structure, as evident from the absence of agglomerates in the SSD micrograph. These results demonstrated that transforming the moringa leaf extract into an SSD system homogeneously disperses it into the carrier [33]. Besides, the porosity, as observed in the SSD micrograph, promotes water penetration into the powder and, as such, potentially enhances the extract dissolution from SSD [34].

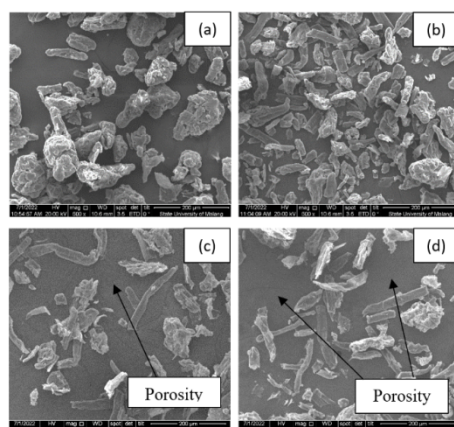


Figure 4. SEM micrographs of (a) physical mixture (PM) 1:2, (b) PM 1:4, (c) surface solid dispersion (SSD) 1:2, and (d) SSD 1:4 at 500x magnification.

2.2.4. Fourier-transform infrared (FT-IR) spectroscopy

FT-IR spectroscopy was employed to examine a possible interaction between the *M. oleifera* leaf extract and microcrystalline cellulose as a carrier [30]. The FT-IR spectra of the extract, microcrystalline cellulose, PM, and SSD are presented in Figure 5. The extract's spectrum shows characteristic bands at the wavenumbers 3329 cm⁻¹ (O-H stretch), 1580 cm⁻¹ (C=O stretch), 2924 cm⁻¹ (C-H stretch), and 1221 cm⁻¹ (C-O stretch). These bands are associated with polyphenol structures [23]. The microcrystalline cellulose's spectrum presents specific absorptions at wavenumbers 3324 cm⁻¹, 2892 cm⁻¹, 1616 cm⁻¹, and 1314 cm⁻¹, each corresponding to the O-H stretch, C-H stretch, C-O stretch, and C-H stretch. In addition, the specific adsorptions of some functional groups shifted in the PM and SSD spectra: the O-H stretch changed from 3329 cm⁻¹ in the extract to 3332 cm⁻¹ in SSD 1:2 and 3334 cm⁻¹ in SSD 1:4, the C=O stretch shifted from 1580 cm⁻¹ in the extract to 1605 cm⁻¹ in SSD 1:2 and 1618 cm⁻¹ in SSD 1:4, and the C-H stretch from the wavenumber 2924 cm⁻¹ in the extract to 2894 cm⁻¹ in both SSD systems. The extract-carrier interaction probably causes a slight shift in the specific adsorption of the functional group. It is assumed that O atoms in the C=O group of the moringa leaf extract interact with H atoms in the O-H group of microcrystalline cellulose through hydrogen bonding [23].

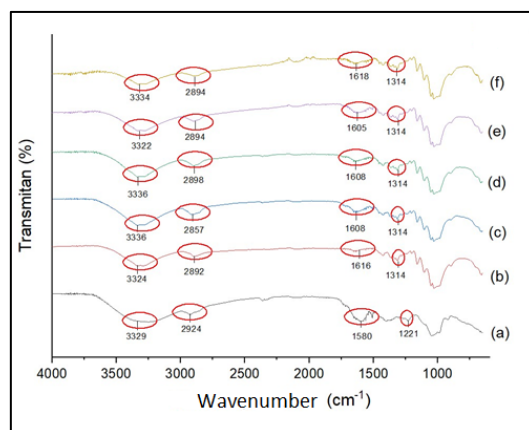


Figure 5. FT-IR spectra of (a) *Moringa oleifera* leaf extract, (b) microcrystalline cellulose, (c) physical mixture (PM) 1:2, (d) PM 1:4, (e) surface solid dispersion (SSD) 1:2, and (f) SSD 1:4.

2.3. Total flavonoid contents

The total flavonoid contents of the PM and SSD powders were assessed using visible spectrophotometry [35]. Quercetin was used as the marker compound (i.e., standard solution) in this study. The regression equation of the standard solution curve was $y = 0.0861x - 0.0665$, with the determination coefficient (r^2) of 0.9991 and the regression function coefficient (V_{x0}) of 1.45%. These figures indicated a linear correlation between the standard solution's concentration (ppm) and absorbance. The regression equation was used to calculate the total flavonoid content, expressed in mg of quercetin equivalent per g of the sample (mg QE/g sample) [36]. As shown in Table 2, the total flavonoid contents were 7.00 ± 0.19 mg QE/g for PM 1:2, $4.00 \pm$

0.08 mg QE/g for PM 1:4, 7.10 ± 0.05 mg QE/g for SSD 1:2, and 4.00 ± 0.08 mg QE/g for SSD 1:4. Further analysis revealed that the total flavonoid contents of the 1:4 systems are significantly lower ($p < 0.05$) than their 1:2 counterparts. One possible reason is that, in the system with more carrier composition, the carrier structure entraps the extract more strongly. This interaction influences the bioavailability of flavonoids in the sample [37].

Table 2. Total flavonoid content of the physical mixture (PM) and surface solid dispersion (SSD) powders of the *Moringa oleifera* leaf extract formulated at 1:2 and 1:4 ratios to the carrier (microcrystalline cellulose).

Total flavonoid content (mg QE/g powder)			
PM 1:2	PM 1:4	SSD 1:2	SSD 1:4
7.00 ± 0.19	4.00 ± 0.08	7.10 ± 0.05	4.00 ± 0.08

2.4. Solubility

The aqueous solubility of quercetin (a standard marker of the *M. oleifera* leaf extract) in the SSD powder was determined and compared to that of the pure extract and PM powder. As shown in Figure 6, the solubility of quercetin from the pure extract was 64.11 ± 1.95 µg/ml, while lower solubility values were identified in the PM powders: 54.60 ± 1.73 µg/ml (1:2 ratio) and 58.81 ± 0.37 µg/ml (1:4 ratio). In SSD 1:2, the quercetin had a higher solubility of 67.33 ± 1.00 µg/ml, which is about 1.05 times higher than the pure extract and 1.14 times higher than the PM powder. The improved solubility can be explained by better wettability, increased porosity and water penetration, reduced particle size, and partial conversion from a crystalline into an amorphous structure [38]. Moreover, the higher solubility of the SSD 1:2 system is potentially correlated with the reduced crystallinity, as evidenced by the DSC and P-XRD results.

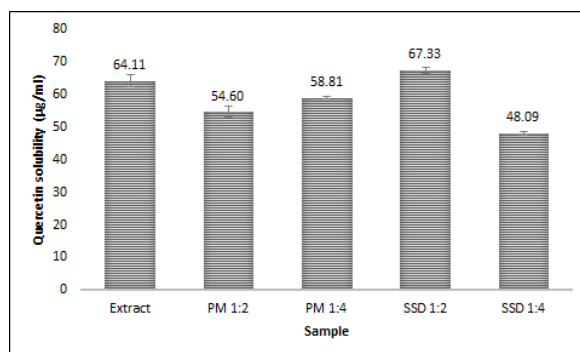


Figure 6. Aqueous solubility of quercetin from the *Moringa oleifera* leaf extract, physical mixture (PM) powder, and surface solid dispersion (SSD) powder (mean \pm SD, n=3)

3. CONCLUSION

Formulating the *Moringa oleifera* leaf extract with microcrystalline cellulose as the carrier into surface solid dispersion (SSD) proves effective in improving the extract's physical-mechanical characteristics. SSD powders have better flowability, compressibility, and moisture content than the extract and PM powders. Moreover, when developed into SSD with a 1:2 extract: carrier ratio, the solubility of quercetin (a marker compound of the moringa leaf extract) increases. The mechanisms involved in this solubility improvement result from better wettability, higher water penetration into the porous structure, particle size reduction, and partial conversion of a crystalline into an amorphous state.

4. MATERIALS AND METHODS

4.1. Materials

Herbal materials used in this study were *Moringa oleifera* leaf powders obtained from KWT (Kelompok Wanita Tani) Sri Rejeki, Bogo-Bojonegoro (East Java, Indonesia). Microcrystalline cellulose was of pharmaceutical grade from the brand VIVAPUR® 102 (JRS Pharma, Germany). Liquid paraffin (Bumi Agung Group, Indonesia) was used to determine true density. Other materials were the USP reference standard of quercetin as standard (Sigma Aldrich, USA), absolute ethanol (Merck, USA), and analytical-grade reagents, including sodium hydroxide (Merck, Germany), aluminum chloride (Merck, Germany), ethyl acetate (Merck USA), and methanol (Merck, Germany). Membrane filters with a 0.45 µm pore size (Merck Millipore, USA)

were used in the solubility study. Distilled water was used for all the total flavonoid assessments and solubility studies.

4.2. Preparation of *Moringa oleifera* leaf extract

The *Moringa oleifera* leaf extract was prepared by maceration [7]. Five hundred grams of the moringa leaf powder were weighed, put in a jar, and then added with 5 L of 70% ethanol (1:10). This jar was closed, and the mixture was macerated for 24 h and then filtered. Next, the filtrate was set aside, while the residue was added with 2.5 L of 70% ethanol (1:5) and then re-macerated for 24 h to produce another filtrate. Finally, the first and second filtrates were evaporated using a rotary evaporator until a thick extract was obtained.

4.3. Preparation of surface solid dispersion (SSD) and physical mixture (PM)

The surface solid dispersion (SSD) of the *Moringa oleifera* leaf extract was prepared by freeze-drying with two extract-to-microcrystalline cellulose ratios, 1:2 and 1:4. First, 5 g of the extract was weighed, and 10 g of microcrystalline cellulose was used for the 1:2 ratio and 20 g for the 1:4 ratio. Then, the extract and microcrystalline cellulose were dispersed in 96% ethanol in a beaker glass and stirred using a magnetic stirrer at 500 rpm for 30 minutes until a homogenous mixture was obtained. For the freezing process, this mixture was placed in an ultra-low temperature freezer at -70°C. Afterward, the frozen mixture was dried using a freeze-dryer at -50°C for 24 h. The final product was triturated and passed through a 30-mesh sieve. Then, the resulting SSD was stored in a desiccator (airtight container) protected from light until further use.

A physical mixture of the moringa leaf extract and microcrystalline cellulose was prepared by trituration. The extract and microcrystalline cellulose were mixed in a mortar without applying pressure until a homogenous mixture was obtained. The mixed powder was then passed through a 30-mesh sieve and stored in a desiccator before further evaluation.

4.4 Physical characterization of SSD and PM

4.4.1 Powder density

The solid products, i.e., SSD and PM powders, were tested to characterize the true, bulk, and tapped densities. For the true density, 1 g of the powder was dispersed in liquid paraffin using a pycnometer. The powder and liquid paraffin weights were calculated to determine the amount of liquid inside the powder structure and then used to calculate the true density [39]. The bulk density was obtained by dividing the weight of the powder occupying the cylinder glass (20 g) by its volume. Then, the tapped density was determined by tapping the powder that occupied the cylinder glass until a significant reduction in volume was observed. It was calculated by dividing the weight of the powder by the tapped volume [40].

4.4.2 Hausner ratio and compressibility index

The Hausner ratio is an indirect index used to predict the powder's flow, and the compressibility index measures the relative interaction between powder particulates, which represents the tendency to consolidate [41,42]. In this study, the Hausner ratio was calculated by dividing the tapped density by the bulk density of the SSD and PM powders. The compressibility index was calculated using the equation below:

$$\text{Compressibility index} = \frac{\text{Tapped density} - \text{bulk density}}{\text{Tapped density}} \times 100\%$$

4.4.3 Moisture content

The moisture content was evaluated to estimate the amount of water in the SSD and PM powders, which indicates their physical, chemical, and microbiological stabilities as well as flowability [43]. First, 5 g of the powder (SSD or PM) was put into a moisture analyzer and then heated at 105°C for 15 minutes until a constant dry weight was obtained. The difference between initial and dry weights was calculated to obtain the powder's moisture content.

4.5. Solid-state characterization of SSD and PM

SSDs made with the 1:2 and 1:4 extract-to-microcrystalline cellulose ratios were evaluated to determine the interaction between the extract and the carrier. Solid-state characterization aimed to analyze any alterations to the characteristics of the *Moringa oleifera* leaf extract after being formulated into SSD. Physical mixtures with the same ratios were also characterized for comparison.

4.5.1 Differential scanning calorimetry (DSC)

The DSC curves of the extract, microcrystalline cellulose, SSD, and PM were recorded using a differential scanning calorimeter (DSC-2) from Mettler Toledo (Switzerland). A 4 mg sample was weighed and sealed in the aluminum pan. Then, scanning was performed in a temperature range of 30–200°C at a constant heating rate of 10°C/minute; the temperature was measured using a thermocouple [23].

4.5.2 Powder X-ray diffraction (XRD) studies

The powder XRD patterns of the extract, microcrystalline cellulose, SSD, and PM were obtained using an X-ray diffractometer (PANalytical X'Pert Pro, UK). The sample was scanned at 2θ from 5° to 50° at a speed of 2°/minute. The operating voltage and current were 40 Kv and 30 mA, respectively.

4.5.3 Scanning electron microscopy

Morphological studies of the extract, microcrystalline cellulose, SSD, and PM were conducted using a JEOL JSM 5310 LV Scanning electron microscope (Japan) with an accelerating voltage of 4 kV. The sample was sprinkled on the adhesive tape attached to a thin gold-coated aluminum plate under an argon atmosphere to make it conductive [44]. The sample surface was observed at various magnifications ranging from 500x to 2500x.

4.5.4 Fourier-transform infrared spectroscopy

The sample's Fourier-transform infrared (FT-IR) spectrum was recorded with an FT-IR spectrophotometer (Jasco FTIR-4200, USA) using the attenuated total reflectance technique. The extract, microcrystalline cellulose, SSD, or PM sample was scanned at different frequencies from 4000 to 400 cm^{-1} . The interaction between the extract and the carrier was observed from the absorption bands of a specific functional group and band shifts in the SSD spectrum.

4.6 Total flavonoid content analysis

Total flavonoid content analysis was conducted to estimate the amount of the functional ingredient, namely flavonoids, in the extract, PM, and SSD. The flavonoid content was analyzed using the UV-Vis spectrophotometry method (Shimadzu UV-Vis spectrophotometer, Japan). Colorimetry was also used to obtain the absorption of flavonoids in the sample. First, the sample was dissolved in absolute ethanol, transferred into a 10.0 ml volumetric flask, and added with ethanol up to the 10.0 ml mark. The solution was sonicated for 15 minutes and centrifuged at 1500 rpm for 30 minutes. Afterward, the supernatant was collected. A sample (1.0 ml) of this supernatant was transferred into a 10.0 ml volumetric flask, reacted with 0.1 ml of 10% AlCl_3 and 0.1 ml of NaOH, and then added with ethanol until a 10.0 ml mixture was obtained. Next, this sample was shaken vigorously and left for 10 minutes for an optimal reaction. When it turned yellow, the absorbance value was quantitatively determined using visible spectrophotometry at the maximum wavelength (435 nm). It was then inputted into the regression curve equation to obtain the total flavonoid content, expressed in mg of quercetin equivalents per gram of sample (mg QE/g). The mg QE/g is equivalent to the amount (mg) of the total flavonoid content of the sample [45].

4.7 Solubility study

The solubility study was performed to analyze the amount of quercetin (a standard marker of the moringa leaf extract) dissolved in an aqueous medium at saturated conditions. In the test, the samples were 300 mg of SSD or PM powder made with a 1:2 extract-to-carrier ratio and 500 mg of the 1:4 system. Each sample was placed in a 50 ml Erlenmeyer flask and added with 50 ml of distilled water. Then, it was put into an incubator shaker at 30°C and agitated at 120 rpm. During the process, 6 mL of the filtrate was taken using an injection syringe at 1 h, 2 h, 3 h, and 4 h. For each removed filtrate, the same volume of distilled water (equivalent to the aliquot withdrawn) was immediately added. The collected filtrate was passed through a 0.45 μm membrane filter. Then, 1.0 mL of the resulting filtrate was put into a 10.0 mL volumetric flask, added with 0.1 mL of 10% AlCl_3 , 0.1 mL of NaOH, and absolute ethanol up to the mark, and then shaken until homogeneous. Next, the sample was left for 10 minutes, and the absorbance was measured using a UV-Vis spectrophotometer (Shimadzu, Japan) at the maximum wavelength (435 nm). The amount of quercetin dissolved in an aqueous medium was then calculated for each sampling time. These data were then used to create a curve depicting the correlation between time and the amount of the dissolved quercetin. Finally, the solubility value of quercetin was determined in saturated conditions from the curve.

Acknowledgments: This study was supported by the Research and Community Services Institute of the University of Surabaya, Indonesia, based on the assignment letter No. 015/ST-Lit/LPPM-01/FF/IV/2023. The authors would also like to express gratitude to Bogo Village, Bojonegoro, East Java, Indonesia, for providing the moringa leaves as active ingredients in this study.

Author contributions: Concept – K.C.R., R.N., N.I.E; Design – K.C.R., N.I.E., A.N.W., A.T.P.; Supervision – A.N.W., A.T.P.; Resources – K.C.R., A.D.T.A., K.F.; Materials – A.D.T.A., K.F.; Data Collection and/or Processing – K.C.R., N.I.E., A.D.T.A., K.F.; Analysis and/or Interpretation – K.C.R., N.I.E, R.N., A.N.W., A.T.P.; Literature Search – A.D.T.A., K.F., R.N.; Writing – K.C.R.; Critical Reviews – N.I.E, A.N.W, A.T.P.

Conflict of interest statement: The authors declare no conflict of interest.

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2630-6344

Publisher

Marmara University

Frequency

Bimonthly (Six issues / year)

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J.Res.Pharm.

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Hatice Kübra ELÇİOĞLU

Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye

kubra.elcioglu@marmara.edu.tr (mailto:kubra.elcioglu@marmara.edu.tr)

Vice Editors

Levent KABASAKAL

Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye

lkabasakal@marmara.edu.tr (mailto:lkabasakal@marmara.edu.tr)

Esra TATAR

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
etatar@marmara.edu.tr (mailto:etatar@marmara.edu.tr)

*Analytical Chemistry & Therapeutic Drug Monitoring***Anil Kumar DWIVEDI**

Central Drug Research Institute, Lucknow, India
anilcdri@gmail.com (mailto:anilcdri@gmail.com)

Anisa ELHAMILI

Department of Medicinal & Pharmaceutical Chemistry, Faculty of Pharmacy, University of Tripoli, Tripoli, Libya
aaelhamili2000@gmail.com (mailto:aaelhamili2000@gmail.com)

Emirhan NEMUTLU

Department of Analytical Chemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye
enemutlu@hacettepe.edu.tr (mailto:enemutlu@hacettepe.edu.tr)

Lorena MEMUSHAJ

Department of Pharmacy, Faculty of Medical Sciences, Aldent University, Tirana, Albania
lorena.memushaj@ual.edu.al (mailto:lorena.memushaj@ual.edu.al)

Mehmet GÜMÜŞTAŞ

Department of Forensic Toxicology, Institute of Forensic Sciences, Ankara University, Ankara, Türkiye
mgumustas@hotmail.com (mailto:mgumustas@hotmail.com)

Mohd Younis RATHER

Multidisciplinary Research Unit, Government Medical College Srinagar, Srinagar, India
younis.rather78@gmail.com (mailto:younis.rather78@gmail.com)

Pablo MIRALLES IBARRA

Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (FISABIO-Public Health), Valencia, Spain
pablo.miralles@fisabio.es (mailto:pablo.miralles@fisabio.es)

Pınar TALAY PINAR

Department of Analytical Chemistry, Faculty of Pharmacy, Yüzüncü Yıl University, Van, Türkiye
ptalay@gmail.com (mailto:ptalay@gmail.com)

*Biochemistry & Cancer Research***Beyza Ecem ÖZ BEDİR**

Department of Medical Biology, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Türkiye
beoz@ybu.edu.tr (mailto:beoz@ybu.edu.tr)

Débora DUMMER MEIRA

Department of Biological Sciences, Nucleus of Human and Molecular Genetics, Federal University of Espirito Santo, Vitória- Espirito Santo, Brazil
debora.dummer.meira@gmail.com (mailto:debora.dummer.meira@gmail.com)

Derya ÖZSAVCI

Department of Biochemistry, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
derya.ozsavci@marmara.edu.tr (mailto:derya.ozsavci@marmara.edu.tr)

Emine TERZİ

Department of Medical Biology, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Türkiye
emineterzi1990@hotmail.com (mailto:emineterzi1990@hotmail.com)

Gülberk UÇAR

Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye
gulberk@hacettepe.edu.tr (mailto:gulberk@hacettepe.edu.tr)

Haidar A ABDULAMIR

College of Pharmacy, Al-Maaql University, Basra, Iraq
h_al_attar@yahoo.com (mailto:h_al_attar@yahoo.com)

Hamide Sena ÖZBAY

Department of Biochemistry, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye
senaozbay@hacettepe.edu.tr (mailto:senaozbay@hacettepe.edu.tr)

İşıl YILDIRIM

Pharmacy Services Program, Beykent University, Istanbul, Türkiye
assistant.professor.isil.yildirim@gmail.com (mailto:assistant.professor.isil.yildirim@gmail.com)

Lokman AYAZ

Department of Biochemistry, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
lokmanayaz@yahoo.com (mailto:lokmanayaz@yahoo.com)

Sahar AL-OKBI

Nutrition and Food Sciences Department, National Research Centre, Cairo, Egypt
s_y_alokbi@hotmail.com (mailto:s_y_alokbi@hotmail.com)

Lynda BOUREBABA

Department of Experimental Biology, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, Wrocław, Poland
lynda.bourebaba@upwr.edu.pl (mailto:lynda.bourebaba@upwr.edu.pl)

Selma HOUCHI

Laboratory of Applied Biochemistry, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif, Algeria
houchi.selma@univ-setif.dz (mailto:houchi.selma@univ-setif.dz)

*Biotechnology***Ali Demir SEZER**

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
adsezer@marmara.edu.tr (mailto:adsezer@marmara.edu.tr)

Ammad Ahmad FAROOQI

Department of Molecular Oncology, Institute of Biomedical and Genetic Engineering (IBGE), Islamabad, Pakistan
farooqiamdahmad@gmail.com (mailto:farooqiamdahmad@gmail.com)

Ceyda EKENTOK ATICI

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
ceyda.ekentok@marmara.edu.tr (mailto:ceyda.ekentok@marmara.edu.tr)

Murat DOĞAN

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Cumhuriyet University, Sivas, Türkiye
mdogan@cumhuriyet.edu.tr (mailto:mdogan@cumhuriyet.edu.tr)

Uğur KARAGÖZ

Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
ugurkaragoz@trakya.edu.tr (mailto:ugurkaragoz@trakya.edu.tr)

*Clinical Pharmacy & Social Pharmacy & Pharmacoeconomy***Abdikarim Mohammed ABDI**

Department of Clinical Pharmacy, Faculty of Pharmacy, Yeditepe University, Istanbul, Türkiye
abdikarim.abdi@yeditepe.edu.tr (mailto:abdikarim.abdi@yeditepe.edu.tr)

Ahmed Hamza AL-SHAMMARI

Department of Pharmacy, Kut University College, Alkut, Wasit, Iraq
Ahmedhamzamezaal@gmail.com (mailto:Ahmedhamzamezaal@gmail.com)

Betül OKUYAN

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
betul.okuyan@marmara.edu.tr (mailto:betul.okuyan@marmara.edu.tr)

Emre KARA

Department of Clinical Pharmacy, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye
emrekara@hacettepe.edu.tr (mailto:emrekara@hacettepe.edu.tr)

Maja ORTNER HADŽIABDIĆ

Centre for Applied Pharmacy, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia
mortner@pharma.hr (mailto:mortner@pharma.hr)

Mesut SANCAR

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
mesut.sancar@marmara.edu.tr (mailto:mesut.sancar@marmara.edu.tr)

Mirela MIRAÇI

Faculty of Pharmacy, University of Medicine, Tirana, Albania
mirela.miraci@umed.edu.al (mailto:mirela.miraci@umed.edu.al)

Nasir IDKAIDEK

Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy and Medical Sciences, Petra University, Amman, Jordan
nidkaidek@uop.edu.jo (mailto:nidkaidek@uop.edu.jo)

Tarik CATIĆ

Department of Pharmacy, Sarajevo School of Science and Technology, Sarajevo, Bosnia and Herzegovina
tarik.catic@ssst.edu.ba (mailto:tarik.catic@ssst.edu.ba)

*General Chemistry***Sinem GÖKTÜRK**

Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Marmara University, İstanbul, Türkiye
sgokturk@marmara.edu.tr (mailto:sgokturk@marmara.edu.tr)

*In Silico Studies***Berna DOĞAN**

Department of Chemistry, Faculty of Science and Letters, Istanbul Technical University, Istanbul, Türkiye
bernadogan@itu.edu.tr (mailto:bernadogan@itu.edu.tr)

Gizem TATAR YILMAZ

Department of Biostatistics and Medical Informatics, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye
gizemtatar@gmail.com (mailto:gizemtatar@gmail.com)

Onur SERÇİNOĞLU

Department of Bioengineering, Faculty of Engineering, Gebze Technical University, Kocaeli, Türkiye
osercinoglu@gtu.edu.tr (mailto:osercinoglu@gtu.edu.tr)

Mehmet ÖZBİL

Department of Bioengineering, Faculty of Engineering, Gebze Technical University, Kocaeli, Türkiye
mozbil@gtu.edu.tr (mailto:mozbil@gtu.edu.tr)

*Medicinal Chemistry***Bahadır BÜLBÜL**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Düzce University, Düzce, Türkiye
bahadir.bulbul@yahoo.com.tr (mailto:bahadir.bulbul@yahoo.com.tr)

Efe Doğukan DİNCEL

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul, Türkiye
efe.dincel@istanbul.edu.tr (mailto:efe.dincel@istanbul.edu.tr)

Entela HALOCI

Faculty of Pharmacy, University of Medicine, Tirana, Albania
entela.haloci@umed.edu.al (mailto:entela.haloci@umed.edu.al)

Hasan Erdinç SELLİTEPE

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye
esellitepe@ktu.edu.tr (mailto:esellitepe@ktu.edu.tr)

Kerem BURAN

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Health Sciences, Istanbul, Türkiye
Kerem.buran@sbu.edu.tr (mailto:Kerem.buran@sbu.edu.tr)

Simone CARRADORI

Department of Pharmacy, "G. d'Annunzio" University of Chieti-Pescara, Chieti, Italy
simone.carradori@unich.it (mailto:simone.carradori@unich.it)

Somaieh SOLTANI

Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
soltanis@tbzmed.ac.ir (mailto:soltanis@tbzmed.ac.ir)

*Microbiology & Immunology***Shahram KHADEM VATAN**

Department of Medical Parasitology and Mycology, Urmia University of Medical Sciences, Urmia, Iran
Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran
Khademvatan@yahoo.com (mailto:Khademvatan@yahoo.com)

Erkan RAYAMAN

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
erayaman@marmara.edu.tr (mailto:erayaman@marmara.edu.tr)

Gülgün TINAZ

Department of Basic Pharmaceutical Sciences, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
gulgun.tinaz@marmara.edu.tr (mailto:gulgun.tinaz@marmara.edu.tr)

Zahraa AMER HASHIM

Department of Microbiology and Immunology, College of Pharmacy, Mosul University, Mosul, Iraq
hashimz@uomosul.edu.iq (mailto:hashimz@uomosul.edu.iq)

*Pharmaceutical Botany & Pharmacognosy & Chemistry of Natural Products***Ahmet EMİR**

Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir, Türkiye
ahmet.emir@ege.edu.tr (mailto:ahmet.emir@ege.edu.tr)

Annalisa CHIAVAROLI

Department of Pharmacology, Faculty of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
annalisa.chiavaroli@unich.it (mailto:annalisa.chiavaroli@unich.it)

Antoaneta TREDAFILOVA

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
antoaneta.trendafilova@orgchm.bas.bg (mailto:antoaneta.trendafilova@orgchm.bas.bg)

Ayşe Esra KARADAĞ

Department of Pharmacognosy, Faculty of Pharmacy, Istanbul Medipol University, Istanbul, Türkiye
ayseesraguler@gmail.com (mailto:ayseesraguler@gmail.com)

Ceren EMİR

Department of Pharmacognosy, Faculty of Pharmacy, Ege University, Izmir, Türkiye
ceren.acir@ege.edu.tr (mailto:ceren.acir@ege.edu.tr)

Claudio FERRANTE

Department of Pharmacology, Faculty of Pharmacy, G. d'Annunzio University of Chieti-Pescara, Chieti, Italy
claudio.ferrante@unich.it (mailto:claudio.ferrante@unich.it)

İ. İrem TATLI ÇANKAYA

Department of Pharmaceutical Botany, Faculty of Pharmacy, Hacettepe University, Ankara, Türkiye
iremchankaya@gmail.com (mailto:iremchankaya@gmail.com)

Laleh KHODAIE

Department of Pharmacognosy, Faculty of Traditional Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
khodaiel@gmail.com (mailto:khodaiel@gmail.com)

Lejla KLEPO

Department of Chemistry, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
klepolejla@gmail.com (mailto:klepolejla@gmail.com)

Mirjana MARČETIĆ

Department of Pharmacognosy, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
mirjana.marcetic@pharmacy.bg.ac.rs (mailto:mirjana.marcetic@pharmacy.bg.ac.rs)

Nurettin YAYLI

Department of Pharmacognosy, Faculty of Pharmacy, Karadeniz Technical University, Trabzon, Türkiye
yayli@ktu.edu.tr (mailto:yayli@ktu.edu.tr)

Patrícia RIJO

Research Center for Biosciences & Health Technologies, Lusofona University, Lisbon, Portugal
p1609@ulusofona.pt (mailto:p1609@ulusofona.pt)

*Pharmacognosy***Sneha AGRAWAL**

Department of Pharmacognosy, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, Maharashtra, India
sneha.agrawal@bvcop.in (mailto:sneha.agrawal@bvcop.in)

Turgut TAŞKIN

Department of Pharmacognosy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
turguttaskin@marmara.edu.tr (mailto:turguttaskin@marmara.edu.tr)

Viktorija MAKSIMOVA

Department of Applied Sciences, Faculty of Medical Sciences, Goce Delcev University, Shtip, Republic of N. Macedonia
viktorija.maksimova@ugd.edu.mk (mailto:viktorija.maksimova@ugd.edu.mk)

Vildan ÇELİKSOY

School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK
celiksoyv92@gmail.com (mailto:celiksoyv92@gmail.com)

Vilma TOSKA PAPAJANI

Department of Pharmacy, University of Medicine, Tirana, Albania
toskavilma@gmail.com (mailto:toskavilma@gmail.com)

Zoran ZEKOVIĆ

Faculty of Technology, University of Novi Sad, Novi Sad, Serbia
zzekovic@tf.uns.ac.rs (mailto:zzekovic@tf.uns.ac.rs)

*Pharmaceutics***Monika DWIVEDI**

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, India
monika.nbri@gmail.com (mailto:monika.nbri@gmail.com)

Afife Büşra UĞUR KAPLAN

Department of Pharmaceutical Technology, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye
afife.busra.ugur@gmail.com (mailto:afife.busra.ugur@gmail.com)

Rajanikant PATEL

Granules Pharmaceuticals Inc., Chantilly, VA - 20151, USA
rajnipharmacy@gmail.com (mailto:rajnipharmacy@gmail.com)

Burcu ÜNER

Pharmaceutical and Administrative Sciences, The University of Health Science and Pharmacy in St. Louis, USA
uner.burcu@yahoo.com (mailto:uner.burcu@yahoo.com)

Dhanashree P. SANAP

Department of Pharmaceutics, Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai, India
dhanashree.sanap@bvcop.in (mailto:dhanashree.sanap@bvcop.in)

Dinesh KUMAR

Department of Pharmaceutical Engineering & Technology, Indian Institute of Technology (BHU), Varanasi, India
dinesh.phe@itbhu.ac.in (mailto:dinesh.phe@itbhu.ac.in)

Ebru ALTUNTAŞ

Department of Pharmaceutical Technology, Faculty of Pharmacy, Istanbul University, Istanbul, Türkiye
ebru.altuntas@istanbul.edu.tr (mailto:ebru.altuntas@istanbul.edu.tr)

Ela HOTI

Faculty of Pharmacy, University of Medicine, Tirana, Albania
ela.hoti@umed.edu.al (mailto:ela.hoti@umed.edu.al)

Emrah ÖZAKAR

Department of Pharmaceutical Technology, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye
emrahozakar@atauni.edu.tr (mailto:emrahozakar@atauni.edu.tr)

Enkelejda GOCI

Pharmacotherapeutic Research Center, Aldent University, Tirana, Albania
enkelejda.goci@ual.edu.al (mailto:enkelejda.goci@ual.edu.al)

Kleva SHPATI

Department of Pharmacy, Albanian University, Tirana, Albania
k.shpati@albanianuniversity.edu.al (mailto:k.shpati@albanianuniversity.edu.al)

Sakine TUNCAY TANRIVERDİ

Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University, İzmir, Türkiye
sakine.tuncay@ege.edu.tr (mailto:sakine.tuncay@ege.edu.tr)

Gülşah GEDİK

Department of Pharmaceutical Technology, Faculty of Pharmacy, Trakya University, Edirne, Türkiye
gulsahgedik@trakya.edu.tr (mailto:gulsahgedik@trakya.edu.tr)

Ongun Mehmet SAKA

Department of Pharmaceutical Technology and Biotechnology, Faculty of Pharmacy, Ankara University, Ankara, Türkiye
omsaka@gmail.com (mailto:omsaka@gmail.com)

Oya KERİMOĞLU

Department of Pharmaceutical Technology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
osipahigil@marmara.edu.tr (mailto:osipahigil@marmara.edu.tr)

Pankaj DWIVEDI

Pharmaceutical and Administrative Sciences, The University of Health Science and Pharmacy in St. Louis, USA
dwivedipank@gmail.com (mailto:dwivedipank@gmail.com)

Rezarta SHKRELI

Department of Pharmacy, Faculty of Medical Sciences, Aldent University, Tirana, Albania
rezarta.shkreli@ual.edu.al (mailto:rezarta.shkreli@ual.edu.al)

Renuka KHATIK

Washington University in St. Louis, USA
renukadops@gmail.com (mailto:renukadops@gmail.com)

Rukiye SEVİNÇ ÖZAKAR

Department of Pharmaceutical Technology, Faculty of Pharmacy, Atatürk University, Erzurum, Türkiye
rukiyeso@atauni.edu.tr (mailto:rukiyeso@atauni.edu.tr)

Saeideh SOLTANI

Novel Drug Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
soltanisa@pharm.mui.ac.ir (mailto:soltanisa@pharm.mui.ac.ir)

*Pharmacology & Toxicology***Ana V. PEJČIĆ**

Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
anapejic201502@yahoo.com (mailto:anapejic201502@yahoo.com)

Ayfer BECEREN

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
ayfer.tozan@marmara.edu.tr (mailto:ayfer.tozan@marmara.edu.tr)

Ayşenur GÜNAYDIN AKYILDIZ

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Bezmialem Vakıf University, Istanbul, Türkiye
gunaydinaysenur@gmail.com (mailto:gunaydinaysenur@gmail.com)

Ayça TOPRAK SEMİZ

Vocational School of Health Services, Giresun University, Giresun, Türkiye
ayca.toprak@giresun.edu.tr (mailto:ayca.toprak@giresun.edu.tr)

Büşra ERTAŞ

Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
busra.ertas@marmara.edu.tr (mailto:busra.ertas@marmara.edu.tr)

Vasudevan MANI

Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Al Qassim, Kingdom of Saudi Arabia
v.samy@qu.edu.sa (mailto:v.samy@qu.edu.sa)

Fatiha MISSOUN

Laboratory of Pharmacognosy and Api-Phytotherapy, University of Mostaganem, Mostaganem, Algeria
fatiha.missoun@univ-mosta.dz (mailto:fatiha.missoun@univ-mosta.dz)

Klodiola DHAMO

Faculty of Technical Medical Sciences, Aldent University, Tirana, Albania
klodiola.dhamo@ual.edu.al (mailto:klodiola.dhamo@ual.edu.al)

Long Chiau MING

School of Medical and Life Sciences, Sunway University, Sunway City, Malaysia
longchiauming@gmail.com (mailto:longchiauming@gmail.com)

Merve KABASAKAL

Department of Medical Pharmacology, Faculty of Medicine, University of Health Sciences, Istanbul, Türkiye
merve.kabasakal@sbu.edu.tr (mailto:merve.kabasakal@sbu.edu.tr)

Miloš N. MILOSAVLJEVIĆ

Department of Pharmacology and Toxicology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia
milosavljevicmilos91@gmail.com (mailto:milosavljevicmilos91@gmail.com)

Mohammed Jabbar MANNA

Department of Pharmacology, College of Dentistry, Al-Mustansiriya University, Baghdad, Iraq
mohammedalmanna@uomustansiriyah.edu.iq (mailto:mohammedalmanna@uomustansiriyah.edu.iq)

Nurdan TEKİN

Department of Medical Pharmacology, Faculty of Medicine, University of Health Sciences, Istanbul, Türkiye
nurdan.tekin@sbu.edu.tr (mailto:nurdan.tekin@sbu.edu.tr)

Rümeysa KELEŞ KAYA

Department of Medical Pharmacology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
rumeysakeles@sakarya.edu.tr (mailto:rumeysakeles@sakarya.edu.tr)

Sana REHMAN

Department of Pharmacology, HIMSR & HAHC Hospital, Jamia Hamdard, New Delhi, INDIA
drsanarehman2012@gmail.com (mailto:drsanarehman2012@gmail.com)

Ünzile YAMAN

Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Katip Çelebi University, İzmir, Türkiye
unzileyaman@gmail.com (mailto:unzileyaman@gmail.com)

Zarife Nigar ÖZDEMİR KUMRAL

Department of Physiology, Faculty of Medicine, Marmara University, Istanbul, Türkiye
znozdemir@marmara.edu.tr (mailto:znozdemir@marmara.edu.tr)

Zeina ALTHANOON

Department of Pharmacology and Toxicology, College of Pharmacy, Mosul University, Mosul, Iraq
dr.zeina@uomosul.edu.iq (mailto:dr.zeina@uomosul.edu.iq)

*Copy Editor***Ayşe Nur HAZAR YAVUZ**

Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
ayse.hazar@marmara.edu.tr (mailto:ayse.hazar@marmara.edu.tr)

Büşra ERGEN

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
busra.ergen@marmara.edu.tr (mailto:busra.ergen@marmara.edu.tr)

Elif Beyzanur POLAT

Department of Pharmacology, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
elif.beyzanur@marmara.edu.tr (mailto:elif.beyzanur@marmara.edu.tr)

Fatih Taha ÇİFTÇİ

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
fatih.ciftci@marmara.edu.tr (mailto:fatih.ciftci@marmara.edu.tr)

Müzeyyen AKSOY

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
muzeyyen.aksoy@marmara.edu.tr (mailto:muzeyyen.aksoy@marmara.edu.tr)

Ömer Faruk ÖZKANLI

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
oozkanli@marmara.edu.tr (mailto:oozkanli@marmara.edu.tr)

Sinan SERMET

Istinye University Faculty of Medicine, Department of Clinical Sciences and Department of Pharmacology and Clinical Pharmacology, Istanbul, Türkiye
sinan.sermet@istinye.edu.tr (mailto:sinan.sermet@istinye.edu.tr)

Şeyma GÖZELİZMİR

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
seyma.gozelizmir@marmara.edu.tr (mailto:seyma.gozelizmir@marmara.edu.tr)

Yeliz ŞAHİN

Department of Clinical Pharmacy, Faculty of Pharmacy, Marmara University, Istanbul, Türkiye
yeliz.sahin@marmara.edu.tr (mailto:yeliz.sahin@marmara.edu.tr)

*Language Editor***Khadija ALJESRI**

Department of Pharmacology, Institute of Health Sciences, Marmara University, Istanbul, Türkiye

*Biostatistics Editor***Gülnaz NURAL BEKİROĞLU**

Department of Biostatistics, Faculty of Medicine, Marmara University, Istanbul, Türkiye
nural@marmara.edu.tr (mailto:nural@marmara.edu.tr)

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