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Sun Protection Factor Activity of Crude Extract, Nanoparticle Extract, and Lyophilised Powder of Arabian Bidara Leaves (*Ziziphus spina-christi* (L.) Desf.)

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ABSTRACT: Arabic Bidara Leaves (*Ziziphus spina-christi* (L.) Desf.) contain flavonoids, including quercetin, which has SPF properties. The goal of this research was to examine the properties and SPF activity of crude extract, nanoparticle extract, and lyophilized powder of Arabian Bidara Leaves. Arabian Bidara Leaves were extracted using the UAE technique with 70% ethanol solvent. The crude extract was converted into nanoparticle extract using the ionic glass process and then freeze dried to produce lyophilized powder. Then the properties and SPF value were investigated. The study found that the crude extract of Arabian Bidara Leaves has a brownish green color, 10.42%±0.36 water content, 4.68±0.01 pH, and 19.98±0.15 SPF. The nanoparticle extract has a clear yellow color, distinct odor, liquid form, and can be stored for 5 days without sediment formation. It has a particle size of 268.6 nm±3.75, polydispersity index of 0.547±0.06, zeta potential of -6.07 mV, adsorption efficiency of 89.60%, spherical morphology, and an SPF value of 29.96±0.69. The lyophilized powder is brown, flake-shaped, and has an SPF of 29.27±0.76. The results of this investigation show that the leaves of the Arabian bidara have SPF activity in the ultra protection category.

KEYWORDS: Arabic Bidara Leaves; Antioxidant; Ionic Gelation; Freeze Dried.

1. INTRODUCTION

Indonesia has a tropical climate and receives a lot of sunlight. Exposure to sunlight produces UV (Ultra Violet) rays, which are classified into three types: UV A (320-400 nm), UV B (290-320 nm), and UV C (200-290 nm). UV B rays can help the skin produce vitamin D. Meanwhile, excessive UV A and UV B rays induce collagen damage in the skin cell membrane, resulting in premature aging; this can be avoided by utilizing cosmetic items that act as sunscreen. SPF (Sun Protection Factor) is a worldwide metric used to quantify the effectiveness of protection provided by sunscreen. A sunscreen's SPF number (>2) indicates its level of skin protection [1].

Arabian Bidara leaves contain many secondary metabolite compounds, one of which is a flavonoid, Quercetin, which is useful as a sunscreen due to the presence of chromophore groups and single double bonds conjugated in Arabian Bidara leaf flavonoids, allowing them to absorb UV A and UV B radiation [2-4]. Arabian Bidara leaves' flavonoids are typically extracted using maceration and ethanol methods (3,5). The SPF value of Arabian Bidara leaves extracted with ethanol ranges from 9.4 (96% ethanol) (maximum protection) to 15.74 (96% ethanol) (ultra protection) and 23.01 (ultra protection) [4,6,7].

Because quercetin is less soluble in water, Arabian Bidara leaf extract has a high potential for being converted into nanoparticles via the ionic gelation process [8]. The resultant nanosuspension will next be dried using the freeze dry process to remove the water content in it and generate more stable nanoparticles.

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2. MATERIALS AND METHODS

2.1. Materials

The samples used in this study were Arabian Bidara leaves (*Ziziphus spina-christi* (L.) Desf.), Chitosan (CHIMULTIGUNA), NaTPP (ANHUI SUNTRAN CHEMICAL CO.), PVP K-30, maltodextrin (Ex Lihua DE 10-14), propylene glicol, and water (WILFARIN USP-997).

2.2. Equipment

Rotary Vacum evaporator (BUCHI R-205), Karl Fischer titration (870 KF Titrino Plus), HANNA Instruments HI 2211 pH/ORP Meter, Particle Size Analyzer (Malvem), Zeta sizer (Malvem), UV-Vis spectrophotometer 1900-UV (Shimadzu), freeze dryer, Ultrasonic (GT SONIC-P3).

2.3. Method

2.3.1. Provision of research materials

Arabian Bidara Leaves (*Ziziphus spina-christi* (L.) Desf.) obtained from BALITRO (Research Materials for Spices and Medicines), Bogor, West Java. The University of Indonesia's Faculty of Mathematics and Natural Sciences determined the Arabian Bidara (*Ziziphus spina-christi* (L.) Desf.) leaves to be used in study.

2.3.2. Preparing Arabian Bidara leaf extract (Ziziphus spina-christi (L.) Desf

Extraction was performed with 70% ethanol at a 1:5 ratio between the material and solvent. The extraction process was aided by ultrasonic waves at a frequency of 40 KHz for 25 minutes. Following that, the extract results were filtered using whatman paper no. 1, and the extract was concentrated using a rotary evaporator at a temperature of 40 °C, a pressure of 175 mbar, and a speed of 50 rpm [9].

2.3.4. Examining a crude extract of Arabian Bidara leaf extract (Ziziphus spina-christi (L.) Desf

a. The organoleptic test is performed visually by examining the shape, smell, and color.
b. The mixability test is performed by combining 1 gram of extract with a solvent mixture of 96% ethanol, propylene glycol, and clean water.
c. To evaluate water content, use Karl Fischer titration (870 KF Titrino Plus) and measure pH with a HANNA Instruments HI 2211 pH/ORP Meter.
ç. Ionic gelation is used to generate nanoparticles from Arabian Jujube leaf extract (*Ziziphus spina-christi* (L.) Desf.).

Table 1. Nanoparticle formula of Arabian Bidara leaf extract

Materials	Consentration (v/v%)
Thick extract of Arabian Bidara leaves (g)	0.05
Chitosan 0.2% (mL)	83.33
NaTPP 0.1% (mL)	16.67
Water (mL)	30
Ethanol 96% (mL)	15
Propylene glicol (mL)	5
PVP K-30 5% (mL)	3.33

2.3.5. Characterization of nanoparticles from Arabian Bidara leaf extract (Ziziphus spina-christi (L.) Desf.)

A characterization of Arabian Bidara leaf extract nanoparticles (*Ziziphus spina-christi* (L.) Desf.) is required, including examination of particle size distribution and polydispersity index using a Particle Size Analyzer (PSA), determination of zeta potential value, determination of adsorption efficiency, and examination of nanoparticle morphology using Transmission Electron Microscopy. The examination lasted 5 days at room temperature (25 °C), and the presence or absence of turbidity was determined. Observed by using a UV-Vis spectrophotometer to measure the percentage transmittance at a wavelength of 650 nm [6].

2.3.6. Preparation of lyophilized powder of Arabian Bidara leaf extract (Ziziphus spina-christi (L.) Desf.) using the freeze drying method.

Arabic Bidara leaf extract nanoparticles were mixed with 20% maltodextrin from the amount of extract used until dissolved, then frozen for 5 hours at 4 °C in a freezer, followed by 48 hours in a freeze dryer at -45 °C with a pressure of 1 atm [10]. The odor, form, and color of the lyophilized powder were next assessed.

2.3.7. Determination of SPF (Sun Protection Factor) value of extract, nanoparticle extract and lyophilized powder of Arabian Bidara leaf extract (*Ziziphus spina-christi* (L.) Desf.).

The SPF value was calculated using the Mansur equation [11].

1. 96% ethanol was utilized as a blank.
2. The absorption was measured at wavelengths of 290, 295, 300, 305, 310, 315, and 320 nm.
3. The absorption value was measured three times in replication.
4. The absorbance value obtained at each wavelength was multiplied by EE x I, and the results were summed together.
5. To calculate the SPF value, the addition result was multiplied by a correction factor of ten.
6. The SPF value was computed using the Mansur equation:

$$SPF = CF \times \sum (EE(\lambda) \times I(\lambda) \times Abs(\lambda))$$

CF = correction factor (10)
EE = spectrum of erythema effects
I = light intensity spectrum
Abs = sample absorbance

Table 2. The value of EE x I is constant

Wavelength	EE x I
290	0.0150
295	0.0817
300	0.2847
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

3. RESULTS AND DISCUSSION

3.1. Analysis of Arabic Bidara Leaf Extract

Table 2. Results of the examination of Arabic bidara leaf extract

Characteristics		Results
Organoleptic	Color	brownish green
	Dosage form	crude extract
	Smell	Typical of Arabic bidara
Solubility	Water : ethanol 96%: PPG (6:3:1)	Easily dissolved (1:10)
Water Content	10.41% 10.07% 10.78%	10.42%±0.36
pH	4.69 4.68 4.68	4.68±0.01

The water content of Arabian bidara leaves was 10.42%±0.36, meeting the quality requirements for crude extracts of 5%-30%. Lower water content reduces the possibility of microbial contamination. This is because the high concentration of water in thick extracts provides an ideal environment for microbial growth. The pH quality test of the crude extract of Arabian bidara leaves revealed a figure of 4.68±0.01, indicating that the extract is acidic. This is influenced by the phenolic content of the Arabian bidara leaf extract, which is acidic due to the presence of gallic acid compounds [6].

3.2. Characterization Results of Arabic Bidara Leaf Extract Nanoparticles

3.2.1. Results of the organoleptic examination of Arabian bidara leaf extract nanoparticles

According to the results of organoleptic examination of Arabian bidara leaf extract nanoparticles, a distinct odor was obtained but was not pungent to the five senses when compared to Arabian bidara leaf extract because the additional ingredients used to make Arabian bidara leaf extract nanoparticles do not have a pungent odor either. The nanoparticles of Arabian bidara leaf extract are liquid and translucent yellow, which is owing to the color of the extract itself.

3.2.2. Analysis of the particle size and polydispersity index of Arabian bidara leaf extract nanoparticles

The particle size and polydispersity of Arabian bidara leaf extract nanoparticles were investigated using the Particle Size Analyzer (PSA) tool and the light scattering method. This approach works by scattering light at various angles created by a light beam or laser hitting particles in the nanosuspension. The particle size identified is proportional to Brownian motion; the faster the Brownian motion, the smaller the particle size of the nanosuspension [12]. The evaluation of the nanosuspension's particle size occurs concurrently with the examination of the nanosuspension's polydispersity index, which is beneficial for determining the size and distribution of nanoparticles in the suspension.

Table 4. Particle size test and polydispersity index of Arabian Bidara leaf extract nanoparticles

Characteristics	Result	Average
Particle size	263.7 nm	268.6 nm±3.75
	271.2 nm	
	270.9 nm	
Polydispersity Index	0.605	0.547±0.06
	0.487	
	0.549	

According to the results of the examination of particle size and polydispersity index of nanoparticles of bidara arab leaf extract with three replications, the average particle size was 286.6 nm, with an average polydispersity index value of 0.547. The particle size and polydispersity index were created by nanoparticles of bidara arab leaf extract prepared with a magnetic stirrer (IKA C-MAG HS 7) at a speed of 250 rpm. The nanosuspension of bidara arab leaf extract met the requirements with particle sizes ranging from 10-1000 nm and a polydispersity index of <0.7, indicating that the particles were monodisperse [6].

3.2.3. The zeta potential of nanoparticles derived from Arabian Bidara leaf extract

Examining the zeta potential value of nanoparticles is useful for determining their stability. The zeta potential value of a nanoparticle is determined by its electrostatic interaction, which determines whether the particles repel or aggregate. The zeta potential of the Arabian bidara leaf extract nanoparticles produced good results (see attachment 15). This is because the obtained value fits the nanoparticle's zeta potential requirements, which are more than or less than +30 mV (20). The negative charge of Arabian bidara leaf extract nanoparticles comes from the NaTPP molecule, which has a $P_3O_{10}^{5-}$ group. As a result, the amount of NaTPP used in nanoparticle production increases the negative charge of the nanoparticles themselves. The extract is not absorbed in nanoparticles because of the negative charge of the quercetin component (OH^-).

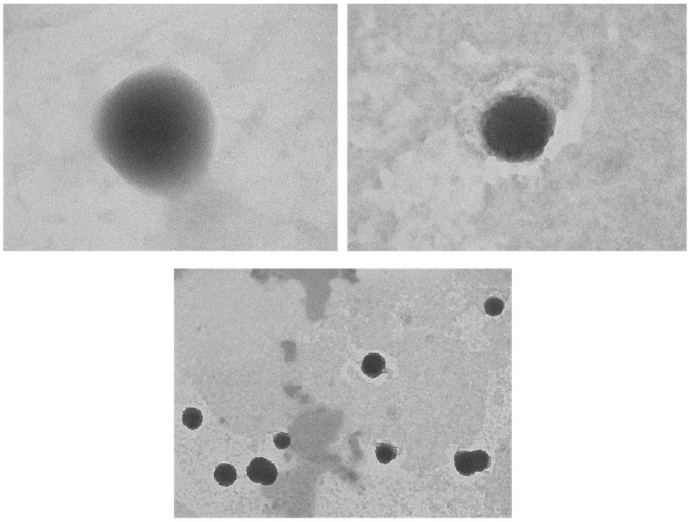
3.2.4. Results of the investigation of the efficiency of nanoparticle adsorption of bidara arab leaf extract

The adsorption effectiveness of nanoparticles is evaluated by the amount of bidara arab leaf extract adsorbed. According to research, this adsorption efficiency is determined indirectly by measuring the absorption of the supernatant containing free active compounds and precipitated nanoparticles containing active compounds that are adsorbed at a wavelength of 440.5 nm (Quercetin Standard) over a 30-35-minute period.

Based on the results of the nanoparticle adsorption efficiency, an average result of 89.06% was obtained, indicating that the adsorption efficiency of bidara arab leaf extract nanoparticles is good because it is greater than 60% and approaching 100%. The negative charge of quercetin's hydroxyl group (OH^-) combined with the positive charge of chitosan's NH^{3+} group results in a high adsorption capability.

3.2.5. Analysis of nanoparticle morphology in bidara arab leaf extract

The Transmission Electron Microscopy (TEM) method was used to examine the morphology of nanoparticles, which works by interacting the sample with a high energy electron beam. The sample must be in a very thin layer on a carbon grid, such that electrons cannot penetrate it. The electron beam will enter the soft part and be held back by the harder part, which is the particle to be detected, resulting in a shadow of the particle caught by the detector [13].



Picture 1. Results of nanoparticle morphology of Arabian Bidara leaf extract

The morphology of bidara leaf extract nanoparticles was observed using a TEM HTT700 equipment with a magnification of 80,000x, and the results showed that the nanoparticles were relatively spherical with a reasonably smooth surface. Nanoparticles with this form will be more stable because the shape of the nanoparticles will be less spherical and smooth, allowing for faster agglomeration and aggregation of nanoparticles due to increased particle interaction.

3.2.6. Results of the stability of the Arabian bidara leaf extract nanoparticles

Based on measurements over 5 days at room temperature (25° C), no sediment was discovered, and there was no turbidity in the nanoparticles from the first to the last day. As a result, these Arabian bidara leaf extract nanoparticles appear to be stable or have undergone no physical changes, as evidenced by the fact that they retain their original physical form. PVP K-30 can prevent or inhibit the agglomeration and aggregation process, making it a helpful stabilizer for reducing particle size [14].

3.3. Organoleptic Evaluation of Lyophilization Effects of Arabian Bidara Leaf Extract Nanoparticles

Because phenolic chemicals degrade at high temperatures, Arabic bidara leaf extract nanoparticles with a concentration of 0.03% (0.05 grams/153.33 mL) were freeze dried. Freeze drying has a sublimation concept, which is to eliminate the water mass contained in the nanosuspension in a solid state by evaporating it in a frozen condition [15]. According to the results of organoleptic examination of the results of lyophilization of Arabian bidara leaf extract nanoparticles carried out visually using the five senses, the lyophilization results produced a distinct odor that was identical to the odor of Arabian bidara leaf extract nanoparticles. Furthermore, the outcomes of nanoparticle lyophilization are formed like brown plates or flakes.

3.4. Results of Determınatıon of SPF Values of Crude Extract, Extract Nanopartıcles, And Lyophilized Powder of Arabic Bidara Leaf Extract

The SPF (Sun Protection Factor) value test performed on the extract and nanoparticles of Arabic bidara leaf extract is beneficial for assessing if the extract and nanoparticles exhibit SPF activity and hence may be used as sunscreen. Another goal is to determine whether there is a difference in SPF value between the crude

extract and extract nanoparticles of Arabic bidara leaf extract used as sunscreen. The SPF test results are presented in the table below:

Table 5. SPF results for Arabian Bidara leaf (crude extract, extract nanoparticles, and lyophilized powder)

Formula	Result	Average	Category
Arabian Bidara Leaf Crude Extract	20.00 19.90 20.02	19.98±0.15	ultra protection
Arabian Bidara Leaf Extract Nanoparticles	29.62 31.00 29.27	29.96±0.69	ultra protection
Lyophilized powder of Arabian Bidara Leaf extract	29.81 29.70 28.29	29.27±0.76	ultra protection

The nanoparticles of the Arabian bidara leaf extract had a higher SPF test result (29.96) than the Arabian bidara leaf crude extract (19.96). This can be attributed to the huge surface area created by the small particle size, which improves the solubility of the active chemical, resulting in increased interaction between particles and UV B radiation. Particles with a size closer to the wavelength of UV B rays will absorb UV B photons more easily. The SPF value of the lyophilized nanoparticles of Arabian bidara leaf extract was 29.27 (ultra protection). When compared to the SPF value of the nanoparticles from the Arabian bidara leaf extract (which did not undergo freeze drying), these results are marginally lower (29.96).

4. CONCLUSION

Crude extract has color brownish green with the water content 10.42%±0.36, lower water content reduces the possibility of microbial contamination and the resulting pH is 4.68±0.01, indicating that the extract is acidic. Nanoparticles of 0.03% bidara leaf extract have particle size characterization results of 268.6 nm±3.75; polydispersity index of 0.547±0.06; zeta potential of -6.07 mV; adsorption efficiency of 89.60%±2.72; and spherical nanoparticle morphology, so it can be said to meet quality requirements. Nanoparticles of 0.03% bidara leaf extract that meet quality criteria generate a lyophilized product containing 0.1 grams of bidara leaf extract nanoparticle. The extract, nanoparticles, and lyophilized results of bidara leaf extract nanoparticles that have passed quality standards have activity as sunscreen with SPF values of 19.98, 29.96, and 29.27, which have the extreme protection class for all three.

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