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Formulatıon And Stabılity Test Of Red Pıdada (Sonneratia Alba (L) Engl) Ethyl Acetate Nano Fractıon As Sunscreen

Supomo[1*], Siti Jubaidah[2], Eka Siswanto Syamsul[3]

Departement of Pharmaceutical Biology, Sekolah Tinggi Ilmu Kesehatan Samarinda, East Kalimantan, Indonesia[1]
Departement of Pharmacology, Sekolah Tinggi Ilmu Kesehatan Samarinda, East Kalimantan, Indonesia[2]
Departement of Pharmaceutical Chemistry, Sekolah Tinggi Ilmu Kesehatan Samarinda, East Kalimantan, Indonesia[3]

Corresponding author: 1*

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ABSTRACT

Red Pidada leaves (Sonneratia alba (L) Engl) have sun-protecting properties. Meanwhile, nanotechnology is widely used to extracted serum formula which are stable, safe, effective, and comfortable nanoparticle-sized. Therefore, this study targeted to formulate serum nanoparticles based on extracted Red Pidada leaves into a sunscreen serum preparation. It is detailed, nano serum was built in six formulas completed by different HPMC gelling agent concentrations, and evaluated stability in relation to the particle size, organoleptic, pH, viscosity, homogeneity, spraying pattern, irritation, and preference test. It showed the phytochemical screening carried out the extract contains alkaloids, flavonoids, saponins, tannins, and steroids. The detail finding explained the particle size was 308 nm, the color of the preparation was transparent white supported by distinctive smell. The completion of result study showed, the formulas had a semisolid gel dosage form, homogeneous, stable pH around 7.6-5.7, viscosity ranging from 401-751 cps, and all fulfilled the physicochemical stability test criteria.

1. Introduction

The Red Pidada (*Sonneratia alba* (L) Engl) is mangrove plant used generally as traditional medicine, in particular the fruit and leaves which are utilized as medicinal powder and scar remover [20]. Moreover, the fruit, bark, and leaves of the Sonneratia species have been used in traditional medicine to treat asthma, fever, ulcers, hepatitis, sprains, hemorrhoids, and bleeding [13].

It is plant, traditionally applied as a cold powder composition on the face, in particular most used in South Kalimantan for years exposure to the sun [9], [26]. Plenty of isolated studies explained that Red Pidada leaves contain fatty acid compounds, sterol hydrocarbons, and two flavonoids, namely luteolin and luteolin

7-O-β glucoside which has a high antioxidant effect [20], [21].

[7], stated that this plant contains alkaloids, flavonoids, glycosides, saponins, and phenols, while [1] also reported that extracted Red Pidada has highly antioxidant activity of 21.62 ppm due to its secondary metabolites which are one of the medicinal ingredients completed diverse biological activities.

Recent developed cosmetic dosage forms is serum preparations and supported by. Mardhiani et al. (2018), stated serum is a preparation with low viscosity and high concentration of active ingredients. Rapid absorption supported by several advantages of the skin, provides a more comfortable effect, and is easily applied on skin surface due to its relatively low thickness. Finally concluded that aims of this study to formulate serum nanoparticles based on extracted red pidada as an antioxidant preparation with three base variations that meet the requirements for physical stability. The particle size, organoleptic, homogeneity, pH, spray pattern, viscosity, dispersion, irritation, and hedonic test were carried out to obtain a serum extract formula that is nanoparticle-sized, stable, safe, effective, comfortable, and highly preferred.

2. Experimental Section

2.1 Materials

The used of materials include Red Pidada leaves, ABTS powder, $K_2S_2O_8$ powder, ascorbic acid (vitamin C), distilled water, 70% ethanol, n-hexane, ethyl acetate, aluminum foil, filter paper, tissue, HPMC, carbopol, Na CMC, triethanolamine, propylene glycol, BHT, methylparaben, propylparaben, STPP, DMSO 10%, glacial acetic acid 1%, chitosan, ethanol 96% and ethanol 70%.

2.2 Methods

2.2.1 Plant Determination

Plant determination was carried out to ensure correctness and avoid mistakes in obtaining the materials and the process of determining names and species. This was conducted at the Wana Research Herbarium, Samboja Natural Resources Conservation Center, East Kalimantan. The taxonomic identity of the plant was confirmed by Bina Swasta Sitepu, M.Sc on October 20, 2020. Meanwhile, the voucher specimens were stored in the Wanariset herbarium (WNA) number: 130/AK/2020 in comparison of AA548 and AA416052. The finding indicated that the tubers used were correct and namely *Sonneratia alba* (L) Engl from the Iridaceae Menispermaceae family. The plant was purchased of the Samboja Special Purpose Forest Area (KDKT), Kutai Kartanegara Regency, East Kalimantan, Indonesia.

2.2.2 Simplicia Production

The Red Pidada leaves were separated from the attached dirt, washed by clean and flowing water, chopped and dried by airing in shady places, while the drying process was carried out for within aweek. The dried Simplicia was weighed and then pulverized into powder and sieved using a 60 mesh sieve to reduce the size of the powder.

2.2.3 Red Pidada Leaf Extraction and Fractionation (Sonneratia caseolaris L.)

The extraction was carried out using simple maceration method and it caused minimizes the possibility of damage to the compounds contained in the sample due to the heating process. The solvent used was 70% ethanol which is semipolar hence, it produced the optimal amount of active ingredients.

The extracted ethanol was fractionated gradually by the liquid fractionation method using n-hexane and ethyl acetate as solvents. The ethanol extract was dissolved in distilled water, added with n-hexane, and

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shaken/separated using a separating funnel procedure. Furthermore, the mixture was allowed to stand forwhile formed into two layers, namely the n-hexane which is the supernatant and the water which is the bottom layer. The n-hexane fraction was separated to evaporate the solvent, while water was placed back into separating funnel for subsequent fractionation to obtain ethyl acetate and the ethanol fraction.

2.2.4 Phytochemical Screening

Phytochemical screening was carried out on the ethanol extract including the examination of chemical compounds of alkaloids, flavonoids, saponins, tannins, and steroids [2].

2.2.5 Production of Ethyl acetate fraction nanoparticles

1 gram of chitosan was dissolved in 100 mL of 1% glacial acetic acid using a magnetic stirrer to obtain a 1% chitosan concentration. The entirely of 500 mg of the ethyl acetate fraction extract of Red Pidada leaves was added by mixed solvent consisting of 20 mL propylene glycol: 20 mL ethanol 70: 20 mL DMSO 10%, and 100 mL aqua dest. 40 mL of 1% chitosan solution was then added and concentration of chitosan consequently became 0.2%. The mixture was stirred using magnetic stirrer for 10 minutes long, then 20 mL of 0.4% STPP was dripped at speed of 1 drop/3 second with burette and in magnetic stirrer at 300 rpm in speed until nanoparticles were formed which were characterized by homogeneous turbidity. Moreover, magnetic stirrer was used for 15 minutes to obtain stable solution of the ethyl acetate fraction nanoparticles. The stability was observed for 5 days including color, turbidity, and precipitate [23].

Table 1. Formulation of serum nano extract of ethyl acetate fraction pidada red [3]

	Formulation of serum nano extract of ethyl acetate fraction pidada red						
Material	Function	F1	F2	F3	F4	F5	F6
Pidada Merah Ethyl Acetate Fraction Extrac	Active Substance	0,05	0,05	0,05	0,05	0,05	0,05
HPMC	Base	0,5	0,6	0,7	0,8	0,9	1
Propilen Glikol	Humectant	16	16	16	16	16	16
Metil Paraben	Preservative	0,18	0,18	0,18	0,18	0,18	0,18
Propil Paraben	Preservative	0,03	0,03	0,03	0,03	0,03	0,03
BHT	Moisturizing	0,2	0,2	0,2	0,2	0,2	0,2
Aquades	Solvent	100	100	100	100	100	100

HPMC was dispersed in aqua distillate to form serum mass, while Methylparaben, BHT, and propylparaben were dissolved in propylene glycol. The solutions were mixed in the serum mass in a mortar, while the serum base formed was then added with the active substance, and ground becomes homogeneous. The remaining aqua dest pounded until homogeneous.

3. Stock evaluation

3.1 Particle Size Test

This was carried out with Size Analyzer (Delsa Max PRB-Beckman Coulter, United States) which measures the size distribution in the 2-7000 nm range using dynamic light scattering and Brownian motion [11].

3.2 Organoleptic Test

Organoleptic test was carried out by observing the visible appearance of the preparation such as color, odor, clarity, separation, and other changes that might occur passing manufactured process [14].

3.3 Homogeneity Test

The homogeneity test was carried out by applying the serum samples to pieces of glass or other suitable transparent material, the preparation is expected to show homogeneous composition combined no coarse grains [14].

3.4 pH test

This was carried out to observe the pH stability in relation to the required range for topical preparations namely 4.5-7 to avoid irritation to the skin. The pH was measured using universal indicator paper according to [14].

3.5 Viscosity Test

Viscosity measurements were carried out using Brookfield viscometer. The preparations were placed into a beaker and the appropriate spindle was lowered until the limit is submerged into the preparation at 50 rpm and the results are recorded [8].

4. Results and Discussion

Based on the screening phase of phytochemical, the ethanol extract of Red Pidada leaves was positively for secondary metabolites of alkaloids, flavonoids, saponins, and tannins. Ethyl acetate is included in solvent that has semipolar properties, therefore, it is able to attract compounds supported polar and nonpolar properties. Ethyl acetate will extract active compounds that are soluble in intracellular and extracellular fluids in plants [15]. Factors that can affect the yield value include the extraction method used, sample particle size, storage conditions and time, the length of the extraction process, the ratio of the number of solvents and the number of samples, and the use of the type of solvent (Satriani & Burhanuddin, 2018).

The main reason is the ethyl acetate solvent is able to attract more bioactive compounds. Based on the phytochemical test produced, the compounds contained in the ethyl acetate extract include alkaloids, steroids/triterpenoids, and tannins. These compounds have toxic properties that have the ability to kill Artemia salina larvae which work as stomach poisons [17]. Bioactive compounds have the ability for human health, among others, as source of antioxidants, antibacterial, anti-inflammatory, and anticancer [5]. The result of phytochemical screening show in table 2.

Table 2. Phytochemical Screening Result

Phytochemical Compound	Test	Result
Alkaloids	Mayer	+
	Bouchardat	+
	Dragendorf	+
Flavanoids	HCL $_{(P)}$, Mg, Amyl alcohol	+
Saponin	HCL 2N	+
Tannin	FeCl 1%	+
Steroid	n-Hexane, acetat acid anhidrat, H2SO4 $_{(p)}$	-

+ = positive result

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- = negative result

4.1 Particle Size Test

The particle size test was carried out using the Particle Size Analyzer (PSA) tool to produce the expected characteristics. Particle characteristics greatly affect the effectiveness, stability, safety, solubility properties, and drug penetration (Ningsih, 2018). The desired size in nanoparticle preparations ranges of 1 to 1000 nm. Based on the analysis results, the obtained particle size was 308 nm.

4.2 Organoleptic Test

The results of the organoleptic test on the texture, color, and odor of each serum nanoparticle preparation formula carried out for 28 days are shown in table 3.

Table 3. Organoleptic Test Results

Observation	Preparation	0	1	7	14	21	28
Texture	**F1**	K	K	K	K	K	K
	F2	K	K	K	K	K	K
	F3	K	K	K	K	K	K
	F4	K	K	K	K	K	K
	F5	K	K	K	K	K	K
	F6	K	K	K	K	K	K
Colour	**F1**	B	B	B	B	B	B
	F2	B	B	B	B	B	B
	F3	B	B	B	B	B	B
	F4	B	B	B	B	B	B
	F5	B	B	B	B	B	B
	F6	B	B	B	B	B	B
Odor	**F1**	Typical	Typical	Typical	Typical	Typical	Typical
	F2	Typical	Typical	Typical	Typical	Typical	Typical
	F3	Typical	Typical	Typical	Typical	Typical	Typical
	F4	Typical	Typical	Typical	Typical	Typical	Typical
	F5	Typical	Typical	Typical	Typical	Typical	Typical
	F6	Typical	Typical	Typical	Typical	Typical	Typical

K = Thick
B = Clear

4.3 Homogeneity Test

The aim of homogeneity test is to identified mixed and homogeneity of active material and its additional of emulgel formed (Nurdianti et al., 2018). The results of the homogenity test on the texture, color, and odor of each serum nanoparticle preparation formula carried out for 28 days are shown in table 4.

Table 4. Homogeneity Test Results

Observation	Preparation	0	1	7	14	21	28
Texture	**F1**	H	H	H	H	H	H
	F2	H	H	H	H	H	H
	F3	H	H	H	H	H	H
	F4	H	H	H	H	H	H
	F5	H	H	H	H	H	H
	F6	H	H	H	H	H	H

H = homogeneity

4.4 pH test

The pH measurement targeted to identified part of sunscreen cream is made acidic or alkaline. According to the standard of SNI 16-4399- 1996 the pH value of leather products for sunscreens ranges from 4.5-7.5. Products that have a pH too high or too low will cause irritation to the skin and pH value below 4.5 will irritate skin, while a pH value above 6.5 will caused scaly skin [24]. This test targeted to evaluate the pH of serum preparations to avoid irritation of the skin. The result pH are shown in table 5.

Table 5. pH Test Result

Observation	Preparation	0	1	7	14	21	28
pH	**F1**	7,00	7,00	6,60	6,50	6,40	6,10
	F2	7,00	7,00	6,80	6,70	6,60	6,50
	F3	7,00	7,00	6,70	6,30	6,30	5,90
	F4	7,00	7,00	6,50	6,30	6,20	5,80
	F5	7,00	7,00	6,60	6,40	6,40	6,10
	F6	7,00	7,00	6,40	6,30	6,30	5,80

4.5 viscosity Test

The viscosity test results for the four formulas showed that the blanks had the lowest viscosity, while formulas 1, 2, and 3 had the highest. Based on the Indonesian National Standard (SNI) 16-4399-1996 regarding sunscreen preparations, a good viscosity value of a preparation ranges from 200-1500 cP. Viscosity value indicated thickness of matterials. Topical viscosity which are fit is 50-1000 cP. The test results indicate that formulas 1, 2, 3, 4, 5, and 6 met the SNI viscosity value.

Table 6. viscosity Test Results

Formula	Viscosity Days to				
	0	**1**	**7**	**14**	**28**
1	401cP	421cP	497cP	515 cP	601 cP
2	402 cP	404cP	400cP	535cP	635 cP
3	402 cP	445cP	414cP	541cP	616 cP
4	403 cP	444 cP	400cP	535 cP	635 cP
5	401 cP	445cP	514 cP	641 cP	741cP
6	404cP	445cP	541cP	641cP	751cP

Red Pidada has been shown to contain bioactive compounds such as flavonoids, steroids, phenol hydroquinone, tannins, and two flavonoids namely luteolin and luteolin 7-O-β glucoside [20]. Meanwhile, [30], reported that the Sonneratia alba species contains tannins, steroids, phenols, and saponins. The results of this study shows that Red Pidada Extract in the form of a nanoparticle serum has great potential as a sunscreen.

5. Conclusion

Based on research finding, the serum nanoparticles obtained from Red Pidada leaves extract had a particle size of 308 nm with a transparent white color, and a distinctive smell. Moreover, the dosage forms semisolid gel, the preparation is homogeneous, the pH is stable ranging from 7.6 - 5.7, while the viscosity is between 401-751 cps. The entirely formulas of this study met and fit through the criteria for the physicochemical stability test

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