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Formulation and Activity Test of Breadfruit Leaves (*Artocarpus altilis* (Parkinson) Fosberg) Extract Serum Spray as Antioxidant

Yola Desnera Putri[1], Deby Tristiyanti[1], Yudhia Delyta Sri[1]
[1] *Sekolah Tinggi Farmasi Indonesia, Bandung, West Java, Indonesia*

*Corresponding author: yoladesnera@stfi.id

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ABSTRACT
Breadfruit leaves (*Artocarpus altilis*) contain bioactive flavonoids known to have antioxidant properties, which can be utilized in the formulation of skin care products. This study aims to develop and evaluate an antioxidant serum spray containing bread1fruit leaf extract. Breadfruit leaves extract was obtained through stepwise maceration using n-hexane, ethyl acetate, and 70% ethanol. Two spray serum formulas with different propylene glycol concentrations (5% in F1 and 3% in F2) were developed and tested for physical stability and antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. Data were analyzed based on IC_{50} values as well as stability tests including organoleptic, pH, viscosity, spray pattern, and adhesion spreadability. The IC_{50} values for n-hexane, ethyl acetate, and ethanol extracts were 595.045 ppm, 47.466 ppm, and 129.177 ppm, respectively, showing significant antioxidant potential in the ethyl acetate extract. Spray serum containing ethyl acetate extract met the physical stability requirements regarding pH, viscosity, and spray pattern. Both formulas showed effective antioxidant activity, with The IC_{50} values of 60.752 ppm (F1) and 71.918 ppm (F2), indicating the potential of the formulas to maintain antioxidant activity over time. The breadfruit leaf serum spray formulation showed strong potential as an antioxidant skin care product with stable physical properties.

Keywords: Antioxidants; free radicals; breadfruit leaves (*Artocarpus altilis*), spray serum; DPPH.

INTRODUCTION
Free radicals (FR) are highly reactive substances with unpaired electrons, generated from both internal metabolic processes and external environmental factors like cigarette smoke and polluted air. They can initiate aggressive oxidation reactions, damaging lipids, proteins, and nucleic acids(1). This instability and reactivity contribute to various health issues, including aging and degenerative diseases such as diabetes mellitus, stroke, cardiovascular disease, cancer, and vascular disorders. Free radical activity that damages important cells in the body needs to be overcome by the use of antioxidants (2).

Good sources of antioxidants include vegetables, fruits, and various plant materials. These antioxidants, such as flavonoids and polyphenols, can effectively neutralize free radicals, preventing oxidative damage and maintaining cellular stability without becoming harmful themselves. One plant that has activity as an antioxidant, *Artocarpus altilis* (Parkinson) Fosberg, well-known in Indonesia as Sukun, is a tropical plant in the family Moraceae. Breadfruit, besides being a food source, is also frequently utilized in traditional medicine. Flavonoids are polyphenolic compounds with antioxidative, anti-inflammatory, memory-enhancing, cardiovascular and gastroprotective effects. According to research conducted on this plant's bioactive compound, secondary metabolites can be found in breadfruit in various forms, including flavonoids, stilbenes, steroids, and lectins. Breadfruit leaves extracts and isolated chemicals have anti-inflammatory, antioxidant, anticholinergic, and cytotoxic properties (3–5).

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According to research (Riasari, 2018), breadfruit attached yellow leaves was given the most excellent antioxidant activity, and isolating the compound. The ethyl acetate fraction showed the best results with an IC50 of 17.11 compared to the IC50 of the n-hexane fraction of 26.16 and the IC50 of the water fraction of 20.68 (6). However, breadfruit attached yellow leaves have never been studied for their cosmetic preparations in the form of spray serum. Therefore, this study aimed to formulation of antioxidant cosmetic preparations in the form of spray serum from breadfruit attached yellow leaves (6).

METHODS

Tool

The tools used were tube macerator, blender, porcelain crucible, filter paper, analytical balance (*OHAUS*®), beaker (*Pyrex*®), vaporizer cup, mortar, test tube (*Pyrex*®), tube rack, furnace (*Branstead Thermolyne*®), oven (*Memmert*®), desiccator, spatula, tube clamp, dropper pipette, UV-VIS spectrophotometry (*Shimadzu UV-1800*®), rotary vaporator (*IKA Basic 05*®), pH meter (*Mettle Taledo*®).

Materials

Plant materials used in this study were yellow breadfruit leaves (*Artocarpus altilis* (Parkinson) Forberg) obtained from the Kopo area, Bandung. The chemicals used were distilled water, chloroform (*Merck*), n-hexanes (*Merck*), ethyl acetate (*Merck*), 70% ethanol (*Brataco)*, methanol (*Merck*), ammonia (*Merck*), HCl (*Merck*), Dragendorff reagent, Mayer reagent, magnesium metal (*Merck*), amyl alcohol (*Merck*), iron (III) chloride reagent (*Merck*), ether (*Merck*), Lieberman-Burchard reagent, NaOH (*Merck*), DPPH (2,2- diphenyl-picrylhydrazyl) (*Sigma*), vitamin C (*Sigma*).

Characterization of Simplisia and Phytochemical Screening

Phytochemical screening was carried out on simplisia and extracts of breadfruit attached yellow leaves to determine the content of secondary metabolite compounds. The compounds tested include alkaloids, flavonoids, phenolics, tannins, steroids, triterpenoids, quinones, saponins, monoterpenes and sesquiterpenes (2).

Extraction

Extraction was carried out by multistage maceration method. The solvents used were solvents with increasing polarity, namely n- hexanes, ethyl acetate, and 70% ethanol. Each solvent used as much as 2 liters (1:10). The simplisia powder was weighed as much as 200 grams, macerated with 75 parts of n-hexan solvent (1500 mL) for 24 hours while occasionally shaking, after 24 hours the filtrate was separated and the residue was macerated again with 25 parts (500 mL) of n-hexane for 24 hours while occasionally shaking, after maceration with n-hexane was complete, the simplisia was dried and then macerated with different solvents on increasing solubility, namely ethyl acetate and 70% ethanol. The liquid extract obtained was concentrated in a rotary evaporator until a thick extract was obtained.

Antioxidant Activity Test

The antioxidant activity test was conducted using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. A 50 ppm DPPH solution was prepared by dissolving 5 mg of DPPH in 100 mL of methanol. As a positive control, 2.5 mg of vitamin C was dissolved in methanol and diluted to 100 mL, followed by the preparation of various concentrations. Sample extract solutions were prepared from thick extracts of yellow

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breadfruit leaves using n-hexane, ethyl acetate, and ethanol. Each extract was dissolved in methanol, homogenized, and diluted to a final volume of 50 mL, with further dilution to obtain different concentrations. For the blank measurement, 3 mL of DPPH solution was diluted to 10 mL with absolute methanol, incubated in the dark for 30 minutes, and measured using UV-Vis spectrophotometry. The antioxidant activity of vitamin C and the extracts was assessed by mixing 1 mL of each solution with 2 mL of DPPH, incubating for 30 minutes in the dark, and measuring the absorbance at 516 nm. All measurements were performed in triplicate (7,8).

Antioxidant *Serum Spray* Formulation

The preparation of *serum spray* was carried out by means of natrosol developed on Aqua DM stirred until a gel base was formed, then added yellow attached breadfruit leaf extract (*Artocarpus altilis* (Parkinson) Fosberg) which had been dissolved in DMSO, then added methyl paraben which had been dissolved with propylene glycol, stirred again until homogeneous and added Aqua DM ad 150 mL stirred until homogeneous. The formulas showed in table 1.

Table 1. *Spray* formula of yellow attached breadfruit leaf extract *serum*

Material	Function	Concentration (% w/v)			
		B1	B2	F1	F2
Extract	Antioxidants	-	-	5	5
Natrosol	*Gelling Agent*	0.1	0.1	0.1	0.1
Propylene glycol	Humectants	5	3	5	3
Methyl paraben	Preservatives	0.3	0.3	0.3	0.3
DMSO	Penetrant	1	1	1	1
Aqua DM	Solvent (add)	100	100	100	100

Description:
B1 : Base 1 *spray serum*
B2 : Base 2 *spray serum*
F1 : Formula 1 ethyl acetate extract *serum spray*
F2 : Formula 2 ethyl acetate extract *serum spray*

Evaluation of the preparation

Evaluation was carried out organoleptic, homogeneity, pH, viscosity, spray pattern, *cycling test* and antioxidant activity testing *of serum spray* preparation for 28 days (9).

Data Analysis

The data to be analyzed is presented in the form of tables and graphs and then described in narrative form. Data processing in this study was carried out theoretically, namely the results of the evaluation test of serum spray preparations compared with existing literature. Antioxidant data were analyzed using a linear equation: $y = bx + a$, so that the IC_{50} value was obtained (7). Antioxidant activity against DPPH free radicals can be calculated by the formula:

$$\% \text{ Inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \times 100$$

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RESULT AND DISCUSSION

Yellow attached breadfruit leaves were determined in the Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Padjadjaran University, Jatinangor to determine that the plants used for the study were true breadfruit leaf plants that included the species *Artocarpus altilis* (Parkinson) Fosberg.

The results of phytochemical screening can be seen in Table 2. Table 2 shows that the compounds contained in the yellow breadfruit leaf simplisia are flavonoids, phenolics, tannins, steroids and quinones. Flavonoids are polyphenolic compounds that have the ability to donate hydrogen atoms to free radical compounds, then antioxidant activity of polyphenols can be produced in the neutralization reaction of free radicals. free radical neutralization reaction or at the termination of the chain reaction that occurs (10).

Table 2. Phytochemical Screening Results of Yellow Attached Leaf Simplisia

Testing	Simplisia	Extract		
		N hexanes	Ethyl acetate	70% Ethanol
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Phenolics	+	-	+	+
Tannins	+	-	-	-
Steroids	+	+	+	+
Triterpenoids	-	-	-	-
Monoterpenes and Sesquiterpenes	-	-	-	-
Quinones	+	+	+	+
Saponins	-	-	-	-

Description: (+) Identified (-) Not identified

Furthermore, the three extracts were tested for antioxidant activity and vitamin C as a comparison. Testing the antioxidant activity of the extracts was carried out using the DPPH method. The DPPH method was chosen because this method is a common method used as a radical to test antioxidant activity due to its stable nature in the form of free radicals and is a simple, fast and inexpensive method (8).

Measurement of sample absorbance was carried out with a UV-Vis Spectrophotometer at a wavelength of 515 nm. Before checking the absorbance, the sample and DPPH were incubated for 30 minutes(11). This incubation aims to form free radicals which are then marked by a change in the purple color of DPPH to yellow, this color change is used to determine the activity of antioxidant compounds. Percent Inhibition of the three extracts can be seen in Table 3.

Based on the percent inhibition and concentration data obtained, a liner regression equation showing the highest percent antioxidant activity is found in the ethyl acetate extract so that the ethyl acetate extract is used for the preparation of *serum spray* preparations.
The IC_{50} value obtained from ethyl acetate extract is 47.466 ppm. So that the dose of ethyl acetate extract used in the preparation of serum spray is 7.1199 mg/150 mL. The IC_{50} value of vitamin C was obtained at 4,460 ppm with very strong activity.

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Table 3. Percent antioxidant inhibition of extracts

Extract	Concentration	Absorbance	Inhibition (%)
n-hexane	30	0.479	55.149
y= 0.044x + 53.818	70	0.461	56.835
R^2= 0.9981	80	0.455	57.397
	90	0.451	57.771
Ethyl acetate	30	0.299	72.003
y= 0.4701x+ 57.686	40	0.258	75.842
R^2 = 0.9917	50	0.194	81.835
	60	0.153	85.674
70% Ethanol	30	0.416	61.142
y= 0.1873x+ 55.805	40	0.390	63.483
R^2 = 0.9926	50	0.369	65.449
	70	0.334	68.726

The evaluations carried out were organoleptic, homogeneity, pH, viscosity, spraying pattern, *cycling test* and antioxidant activity testing *of serum spray* preparation for 28 days. Organoleptic examination can be seen in table 4.

Table 4. Results of Organoleptic Examination of Serum Spray Preparations

Formula	Inspection Components	Organoleptic on Day-				
		1	7	14	21	28
F1	Color	TY	TY	TY	TY	TY
	Smell	BK	BK	BK	BK	BK
	Texture	D	D	D	D	D
F2	Color	TY	TY	TY	TY	TY
	Smell	BK	BK	BK	BK	BK
	Texture	D	D	D	D	D

Description: TY (Transparent Yellow), BK (Special Odor), D (Dilute)

Organoleptic examination aims to see changes in color, odor and texture of the preparation. Organoleptic observation of this preparation is done visually. Based on organoleptic examination data from day 1 to day 28, there were no changes. Homogeneity observations can be seen in table 5.

Table 5. Results of Homogeneity Observation of Serum Spray Preparations

Formula	Homogeneity on Day-				
	1	7	14	21	28
F1	H	H	H	H	H
F2	LH	LH	LH	LH	LH

Description: H (Homogeneous), LH (Less Homogeneous)

Homogeneity observation aims to determine the presence or absence of unmixed particles in the preparation or the presence of gel lumps. The results of this homogeneity observation show that formula 1 has good homogeneity while formula 2 has poor homogeneity, this is because when evaluating the preparation of formula

2, it shows the presence of white particles that are suspected to be methyl parabens to find out for sure further testing needs to be done.

The pH measurement was carried out to determine the pH of the preparation during the storage period. Based on the measurement results, the pH value of the preparation is in the range of 4.95 - 5.28. Table 6 showed that the pH of the preparation from week to week has increased and decreased but still meets the pH requirements of the skin, namely 4.5 -6.5 (12).

Table 6. Results of pH Measurement of Serum Spray Preparations

Formula	pH on Day-				
	1	7	14	21	28
F1	5.18	5.28	5.07	5.18	5.04
F2	5.05	5.16	5.06	5.24	4.95

Measurement of the viscosity of the preparation is carried out to determine the viscosity of the preparation, the viscosity of the preparation made can affect its use because it has an important role in spraying patterns, the requirement for good viscosity of the preparation is less than 150 cPs. The results of measuring the viscosity of the preparation during 28 days showed a result of 10 cPs. This indicates that the consistency of the preparation during 28 days of storage is stable and has not changed. The higher the viscosity of the preparation, the more difficult it will be to spray, if the lower the viscosity of the preparation, the easier it will be to spray. The viscosity results can be seen in table 7.

Table 7. Viscosity Measurement Results of Serum Spray Preparations

Formula	Viscosity on Day				
	1	7	14	21	28
F1	10 cPs	10 cPs	10 cPs	10 cPs	10 cPs
F2	10 cps	10 cPs	10 cPs	10 cPs	10 Ps

The spraying pattern examination was carried out to see the spraying pattern and size produced by the preparation. This demonstrates the effectiveness of the applicator used in delivering amount of the gel preparation formula per spray. The spray pattern results can be seen in table 8.

Table 8. Results of spray pattern of Serum Spray Preparations

Formula	Spraying Distance	Spraying Pattern on Day				
		1	7	14	21	28
F1	3 cm	5.5	5.5	6	6	6
	5 cm	6	6.5	9	8	9.5
	10 cm	10.5	10	12.5	11.5	12.5
	15 cm	12.5	13	16.5	15.5	15.5
F2	3 cm	5.5	5.5	5.5	5.5	5.5
	5 cm	6	6.5	6	7.5	7.5
	10 cm	8.5	8.5	9	10.5	11
	15 cm	11.5	11.5	13	13	14

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The cycling test aims to see the stability of the preparation when it is at a low temperature of 4°C and a high temperature of 40°C. The observations made, namely observing physical changes in the preparation including organoleptic, homogeneity and pH at the beginning and end of the test can be seen in table 9.

Table 9. The Cycling Test Results

	Testing Components	Formula	
		F1	F2
Start of Testing	Organoleptic	Yellow Transparent Odor Dilute	Yellow Transparent Odor Dilute
	Homogeneity	Homogeneous	Less Homogeneous
	pH	5.65	5.57
End of Testing	Organoleptic	Yellow Transparent Odor Dilute	Yellow Transparent Odor Dilute
	Homogeneity	Homogeneous	Less Homogeneous
	pH	5.87	5.83

Antioxidant activity test of spray serum can be seen in Table 10.

Table 10. Antioxidant Activity Test Results of Spray Serum

Formula	IC_{50} Serum Spray Preparation (ppm)	
	Day 1	Day 28
F1	60.752	75.877
F2	71.918	80.933

The antioxidant activity test results of the spray serum, as shown in Table 10, indicate a decrease in antioxidant potency over time for both formulas. On Day 1, Formula F1 exhibited an IC_{50} value of 60.752 ppm, which increased to 75.877 ppm by Day 28, suggesting a reduction in antioxidant effectiveness. Similarly, Formula F2 showed an IC_{50} value of 71.918 ppm on Day 1 and increased to 80.933 ppm on Day 28. These findings suggest that the antioxidant activity of both serum spray formulations declines with storage time, potentially due to the degradation of active antioxidant compounds over time.

CONCLUSION

Based on the research conducted, the IC_{50} values of the *n*-hexane, ethyl acetate, and ethanol extracts of yellow breadfruit leaves were 595.045 ppm, 47.466 ppm, and 129.177 ppm, respectively. The IC_{50} values of the formulations in Formulas 1 and 2 increased after 28 days of storage, indicating a decrease in antioxidant activity. The physical stability of the preparations including organoleptic properties, homogeneity, pH, viscosity, and spray pattern remained within acceptable limits.

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