ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY TESTS OF BANGKAL LEAF (*Nauclea subdita* Leaf.) SERUM EXTRACTS

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ABSTRACT

Bangkal leaves contain polyphenols, alkaloids, flavonoids, and quinone compounds that have the potential to act as antioxidants and antibacterial agents. To maximize the use of bangkal leaves, a cosmetic preparation, serum, was prepared. Serum is composed of 3 formulas: formula 1 (3% extract concentration), formula 2 (4% extract), and formula 3 (5% extract). Serum was tested for antioxidant activity using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method at a wavelength of 518 nm. Ascorbic acid was used for comparison. Serum was also tested to evaluate its physical properties including organoleptics, pH, spreadability and viscosity. Antibacterial activity was determined using the well diffusion method. The research results showed that the IC_{50} value of the extract was 171.18 ppm, the bangkal leaf extract serum preparation for formula I was 174.26 ppm, formula II was 209.97 ppm, and formula III was 237.91 ppm. The antibacterial activity of bangkal leaf extract serum (*Nauclea subdita*) against *Staphylococcus aureus* formulas 1, 2, and 3 had an average zone of inhibition of 10.67, 12.00, and 14 mm. Based on the antioxidant and antibacterial activity values, bangkal leaf extract serum has moderate antioxidant and antibacterial abilities.

Keywords : Bangkal leaf extract, antioxidant activity test, antibacterial test

INTRODUCTION

Bangkal plants (*Nauclea subdita*) are tropical plants of the Rubiciae family, which are widely found in lowland to mountain forests, swamp areas, streams, and rivers, and are generally planted to stabilize slopes and river banks (Asmiyarti & Wibowo, 2014). Based on a study (Ariessanty *et al.*, 2020), the ethanol extract from the leaves of *Nauclea subdita* by the maceration method has antibacterial activity against *Propionibacterium acnes* with the highest concentration of 80%, resulting in an inhibition zone with a diameter of 8.56 mm. According to (Rahmawanty *et al.*, 2017), ethanol leaf bang can be used as a veil sun and antioxidant, which can be used as an alternative to natural ingredients.

To maximize treatment against cellular damage to facial skin caused by free radicals due to UV rays and *S. aureus*, skincare cosmetics are needed (Deni *et al.*, 2017). The selected skin cosmetic preparation is serum because it has better penetration of the skin (Ojha et al., 2019). Study This aim For determine the effect of variations in the concentration of bangkal leaf extract (*Nauclea subdita* Leaf) 3%, 4% and 5% on antioxidant activity as well as For to determine the effectiveness of antibacterial serum gel extract of bangkal leaves on *staphylococcus aureus* bacteria

RESEARCH METHODS
Tools and materials

Ultrasound Assisted Extraction (GT Sonic), Waterbath (Memmert), Cuvette (Hellma), UV-Vis double beam spectrophotometry (Shimadzu UV 1800), LAF (Biolus CLB-201), calipers (Tricke brand). Bangkal leaf, Carbophol 934 (Merck), ethanol 96% pa (Merck),

methanol pa (Merck), and propylenglykol (PT. Brataco), and methyl paraben (PT. Sumber Berlian Kimia), propyl paraben (Alpha Chemika), sodium metabisulfite, sodium benzoate (PT. Smart-Lab Indonesia), triethanolamine (Merck), and technical distilled water (Waterone)

Research procedure

1. Bangkal Leaf Extraction

The process of making bangkal leaf extract begins with preparing bangkal leaves (*Nauclea subdita* Leaf.) that have been made into simplicia. The extraction process used the ultrasound-assisted extraction method (UAE). It takes 500 gram of simplicia powder was extracted with 96% ethanol solvent at a ratio (1:5) into the extraction vessel. In this extraction, the Erlenmeyer flask was then covered with aluminum foil, after which it was placed in a sonicator, and extraction was carried out for 30 minutes at a temperature of 40°C and a wave frequency of 40 kHz. After this process, filtering was carried out to separate the filtrate from the dregs. Evaporation was performed in a glass container at room temperature to obtain a thick extract of bangkal leaves (Buanasari *et al.*, 2019). The moisture content of the extract was 2.12%.

2. Bangkal Leaf Extract Serum Formulation

Table I. **Extract Serum Gel Base Composition Leaf Bangkal**

Material	Formulas (%)			
	0	I	II	III
Bangkal Leaf Extract	-	3	4	5
Carbopol	0.5	0.5	0.5	0.5
Propylene glycol	10	10	10	10
TEA	ad pH 7	ad pH 7	ad pH 7	ad pH 7
Nipagin	0.18	0.18	0.18	0.18
Nipasol	0.02	0.02	0.02	0.02
Na. Metabisulfite	0.075	0.075	0.075	0.075
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

Description :
Formula 0   : Without Material Active Extract Leaf Bangkal
Formula 1   : Extract Leaf Bangkal 3%
Formula 2   : Extract Leaf Bangkal 4%
Formula 3   : Extract Leaf Bangkal 5%

3. Evaluation of Serum Preparations

Organoleptic Test

Each serum preparation formula was observed visually to cover color and scent on the skin using the method of observing visual appearance and sensation on the skin when applied (Fikayuniar *et al.*, 2021).

pH Test

The pH test was carried out using a pH meter for the three formulas and a placebo. The recommended pH value is 4.5 – 6.5 (Ojha *et al.*, 2019).

Viscosity Test

The serum was placed in the viscometer until the spindle was submerged, and the spindle was then inserted into a beaker glass with a regulated spindle and adjustable speed. The range of requirements for testing the viscosity of serum preparations is 800 – 12000 CPs (Thakre, 2017).

Spreadability Test

Serum was placed on a glass plate and added to the preparations, which were squeezed for 1 minute. Then, burden addition was added, and the cells were silenced for 1 minute.

The desired range is 5 – 7 cm, which is very convenient for serum preparation (Fikayuniar *et al*., 2021).

4. Determination of Antioxidant Activity

Making DPPH solution

Eight grams of DPPH dissolved with methanol pro analysis to 50.0 mL in a volumetric flask then covered with aluminum foil and tightly closed so protected from light (Djamil & Wijiastuti, 2015).

Control Positive (Vitamin C)

The positive control solution was prepared by weighing 0.1 grams of vitamin C, dissolving it with 50.0 mL of methanol pa in a volumetric flask to obtain a concentration of 200 ppm. Pipette 5.0 mL and then diluted with methanol in a volumetric flask to a volume of 50.0 mL to obtain a concentration of 20 ppm. Again concentrations of 1,2,4,6,8, and 10 ppm were used.

Making Solution Bangkal Leaf Extract

Fifty milligrams of extract dissolved in methanol p. a ad 50.0 mL) was added to the flask, which was shaken ad libitum. The extract leaf bang was tested at various concentrations (20, 50, 100, 250, and 500 ppm).

Making Bangkal Leaf Extract Serum

Fifty milligrams of serum was dissolved in methanol p. a ad 50.0 mL in the flask measure, shaken ad homogeneous. The serum was tested at various concentrations (20, 50, 100, 250, and 500 ppm).

5. Determination of Antibacterial Activity

Making Media

Five grams of Nutrient Agar (NA) medium and then dissolve it in 180 ml of sterile distilled water, after which the agar medium is heated to boiling. Nutrient agar was stirred using a magnetic stirrer to ensure that the media was completely dissolved. If the media was completely dissolved, the media was sterilized using an autoclave at 121°C for 15 minutes, and then left until the temperature was warm (40ºC–45ºC). The Prepared Nutrient Agar was then poured into a sterile petri dish (20 ml with a horizontal surface level to provide a uniform depth of ±0.5 cm. Then, let it stand until the media solidifies (Kosasi *et al*., 2019).

Antibacterial Activity

Testing process activity antibacterial extract serum preparations bangkal leaf with use method well , with procedure measure the diameter of the resistance from growth bacteria to bacteria *S. aureus* . The test was performed on a test medium dripped with the test solution using a micropipette. After incubation process in an incubator at 37°C for 24 hours, the diameter of the inhibition zone around it was measured using a period shove (Fikayuniar *et al.*, 2021; Kindangen *et al*., 2018).

Data Analysis

Antioxidant and antibacterial activity data were analyzed using SPSS with a one-way Annova test. From the resulting data, statistical analysis was performed with degrees of confidence ($\alpha = 0.05$). To determine which formula has a significant difference, it can be determined based on the p and α values. If the results obtained are $p < \alpha$, indicating a significant difference, then proceed with using the Honestly Significant Difference (HSD) test to determine which data obtained are different.

RESULTS AND DISCUSSION

Formulation and Evaluation of Bangkal Leaf Extract Serum

The preparation evaluation test was carried out to determine the quality of serum preparation before use. Good serum preparations have characteristic properties according to predetermined parameters. The preparation evaluation tests included organoleptic, pH, spreadability, and viscosity tests (Kamal & Rusdi, 2018).

Table II. Evaluation Test Results Extract Serum Preparations Leaf Bangkal

Testing (Mean±SD)	placebo	F1	F2	F3
Organoleptic	Colorless, odorless	Greenish brown, characteristic odor	Greenish brown, characteristic odor	Greenish brown, characteristic odor
pH	7.09±0.21	6.85±00,01	6.68±0.01	6.66±0.04
Spread Power	6.77±0.14	6.62±0.19	6.70±0.09	6.58±0.14
Viscosity	7786.67±92.38	1362.67±8.33	1794.67±2.31	2800.00±80.00

The results of the pH test aimed to determine the safety of bangkal leaf serum preparations (*Nauclea subdita* leaf) when used so that they do not cause irritation to the skin (Fikayuniar *et al*., 2021). The recommended pH value is 4.5 – 6.5 (Ojha *et al*., 2019). From the above data, it can be seen that the *gelling agent* carbopol added at a concentration of 0.45% has an acidic pH. This could occur because of the release of the carboxylic group of the carbopol polymer from the acrylic acid monomer, which has acidic properties (Tsabitah *et al*., 2020). Carbopol is extremely acidic (Rowe *et al*. 2009). TEA (triethanolamine) has been added to the serum formulation but has not been able to reduce or neutralize the acidity of carbopol because the amount of TEA required to bind the carboxylic group is insufficient.

Viscosity testing aims to determine the thickness of the liquid (Fikayuniar *et al.*, 2021). From the data above placebo and formulations (1,2,3) obtained results that meet the requirements, where the range of requirements for testing the viscosity of serum preparations is 800 – 12000 CPs (Thakre, 2017).

The spreadability test aims to determine the spread of the preparation when it is applied or smeared so that it is easy to apply the serum preparation to the skin (Fikayuniar *et al.*, 2021). From the data above, it can be concluded that the preparation of bangkal leaf serum (*Nauclea subdita* leaf) meets the requirements, where the spreadability range is 5 – 7 cm (Septiyanti *et al*., 2019).

Antioxidant Activity

Antioxidant activity was measured using the DPPH method in the dark because DPPH is sensitive to light, which causes instability in DPPH. To test antioxidant activity, incubation was first carried out at 37°C for 30 minutes in the dark (Sutriningsih, 2017). The purpose of this incubation process is to optimize the activity of DPPH so that a reaction occurs between the DPPH and the test sample before measuring the antioxidant activity (Toripah Susanti *et al*., 2014).

Table III. Antioxidant Activity of Vitamin C and Extracts

Sample	IC value $_{50}$
Vitamin C (Positive Control)	3.43
Bangkal Leaf Extract	171.18

The antioxidant activity test was performed using vitamin C as a positive control. The test involving a positive control aimed to determine whether the procedure was used to determine the role of the antioxidant activity in a sample. The IC_{50} values obtained were 3.43 ppm. From the results of the IC_{50} of the positive control when seen in the table of the category of strength of antioxidant activity by Sadhiutami NMD (Desmiaty Y, 2016), the IC_{50} value produced by the positive control, namely vitamin C, was included in the very active category, namely <50 ppm. If a sample is equal or nearly close to the antioxidant activity of the positive control, it can be said that the sample has great potential as an alternative antioxidant in preventing exposure to free radicals (Marliani et al., 2014).

The antioxidant activity test on bangkal leaf extract (*Nauclea subdita* leaf) using the DPPH method was carried out at a maximum wavelength of 518 nm, and the absorbance value decreased with increasing concentration in the process of testing bangkal leaf extract samples (*Nauclea subdita* leaf), but the value The % inhibition that was obtained actually increased. To determine the antioxidant activity of bangkal leaf extract (*Nauclea subdita* leaf) was replicated 3 times and an average IC_{50} value of 171.18 ppm. From these results, when referring to the table of categories for the strength of antioxidant activity presented by (Sadhiutami NMD, Desmiaty Y, 2016), the bangkal leaf extract was in the medium category, namely from the range of 101 – 250 ppm. The replication process 3 times is to be thrice to determine whether the results produced were consistent and accurate.

Table IV. Activity Test Results Antioxidant Serum Preparations

Formulas	Concentration (%)	IC_{50}
1	3	174,26
2	4	209.97
3	5	237.91

In the activity test, antioxidants in bangkal leaf serum preparations (*Nauclea subdita* leaves). From these results, referring to the table of categories for the strength of antioxidant activity presented by (Sadhiutami NMD, Desmiaty Y, 2016), the preparation of bangkal leaf extract serum (*Nauclea subdita* leaf) is included in the medium category, namely from the range of 101 – 250 ppm. Based on the results of the IC_{50} with the addition of variations in the concentration of the active ingredient bangkal leaf extract (*Nauclea subdita* leaf), formulas 1, 2, and 3 can increase the IC_{50} value. Rahmayani *et al*. (2013) stated that the IC_{50} value of mangrove snail extract in methanol solvent is 2329.81 ppm which is included in the category of weak antioxidant activity because it is more than the range of > 500 ppm. This can happen because the extract being tested is still in the form of a crude extract, so it is necessary to carry out a purification process.

In a study conducted by (Agustina *et al*., 2013), several extraction methods were carried out, including one-stirring maceration, maceration every one hour of stirring, and soxhletation, which had different antioxidant activity values, and the best was the soxhletation method. With this method, the extraction temperature can be regulated so as not to damage the antioxidant components, and with the addition of the extraction temperature, the required antioxidant components can be extracted perfectly so that the more dissolved the components, the greater the antioxidant activity. According to (Sayuti, 2017) the average IC_{50} value for methanol solvent with the ultrasonic extraction method was lower than that of the maceration method. The different extraction times are thought to be the cause of the differences in the IC_{50} values for the antioxidant activity of sea bamboo. The longer the extraction process time, the longer the contact between the solvent and the material, causing mass deposition by diffusion until there is an equilibrium concentration of the solution inside and outside the extraction material.

In the UAE extraction method, the process of using a long time can cause an increase in the temperature of the solution, which can accelerate the antioxidant oxidation process, and the extract obtained is low (Sholihah, 2016). According to Ibrahim *et al*. (2015), the extraction process of the UAE method using an extraction time that is too long and exceeds the optimum limit can cause bioactive compounds to experience changes in chemical structure. This was due to the occurrence of an oxidation process, so that the extract obtained was low. Meanwhile, the extraction process, which is too short, causes not all bioactive compounds to be extracted from the material (Marlina Kristina *et al*., 2022).

Antibacterial Activity

The serum gel preparation of Bangkal leaf extract (*Nauclea subdita*) obtained from the extraction process was tested against *Staphylococcus aureus*. *Staphylococcus aureus* can

cause frequent infections with typical signs, such as inflammation, necrosis, and abscess formation (Sri Harti, 2015).

Table VInhibitory Activity Results

Formulas	Concentration (%)	Inhibitory Activity (Mean±SD)
Placebo	0	9.57±0.13
1	3	10.67±0.12
2	4	12.00±0.20
3	5	14.00±-0.16
Clindamycin	-	16,23±0.23

In this study, we found that the serum gel extract of Bangkal (*Nauclea subdita*) leaves had inhibitory activity on the growth of *Staphylococcus aureus* bacteria, with the diameter of the inhibition zone formed at concentrations of 3%, 4%, and 5% with an average diameter of 10.67 mm, 12.00, and 14 mm, respectively. There were differences in the diameters of the inhibition zones formed, which can be seen from the variations in the zones for each test material. This difference is influenced by several factors, including the concentration of the extract, inoculum size, and antibacterial ability of the efficacious substance. The larger the inoculum, the lower the inhibitory power produced, and thus, the smaller the zone formed. The extract concentration affects the diffusion rate of the nutritious substances. The greater the concentration of the extract, the faster it diffuses, which results in greater antibacterial inhibition and a wider diameter of the inhibition zone. This is in accordance with the results of the study that the extract with a concentration of 5% had a larger inhibition zone than the concentrations of 3% and 4 %. The results of this study indicate that the serum gel of bangkal leaf extract at different concentrations had different inhibitory effects on the *Staphylococcus aureus* test. Variations in the inhibitory effect of bangkal leaf extract were due to the *Staphylococcus aureus* bacteria test used in this study derived from three concentrations of bangkal leaf extract gel serum, namely concentrations of 3%, 4%, and 5%.

CONCLUSION

With variations in the concentration of the active ingredient in bangkal leaf extract (*Nauclea subdita* leaf) in serum preparations, the effect of antioxidant activity was quite good. In addition, the inhibition obtained in the antibacterial activity test of Bangkal leaf extract gel serum preparation (*Nauclea subdita*) against *Staphylococcus aureus* was 3%, 4%, and 5% with average inhibition zone of 10.67 mm, 12.00, and 14 mm.

Based on this research, it is hoped that further research will be carried out regarding the irritation test and preparation stability test, so that it can be used by the public for skin care.

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