Comparative Analysis of Anti-aging Serum based SNI 16-4399-1996 using *Aloe Vera*, Binahong Leaves (*Anredera cordifolia*), and Mangosteen Peel (*Garcinia mangostana L*) Extracts

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ABSTRACT

The cosmetics industry is seeing rapid growth, particularly in producing anti-aging serums containing plant extracts rich in antioxidants. This serum was created to analyze parameter values, particularly antioxidants, acquired by including extracts of aloe vera, binahong leaves, and mangosteen peel. The extraction process involved soaking dry aloe vera, binahong leaves, and mangosteen peel in a 1:5 (w/v) ratio of 96% ethanol for 3 days. The filtrate from the maceration process was concentrated using a rotary evaporator at 55°C until a thick extract was obtained. The IC50 values obtained for antioxidant test results using the DPPH technique were 10169 ppm for aloe vera extract, 13438 ppm for binahong leaf extract, and 51.034 ppm for mangosteen peel extract. The IC50 values in serum are aloe vera serum at 59681 ppm, binahong leaf serum at 54.652 ppm, and mangosteen peel serum at 43.747 ppm. This research evaluated parameters according to SNI 16-4399-1996. Based on the IC50 antioxidant value and other characteristics, mangosteen peel extract and serum are ideal for development, production, and commercialization as facial care products with anti-aging properties.

1. Introduction

The Taylor Nelson Suffer Research Institute (TNS) conducted a study on 1,800 women between the ages of 20 and 39 in five Asian countries: India, Korea, the Philippines, and Thailand. According to the survey results, Asian women often start to exhibit indications of aging around the age of 25 years [1]. A serum is now being developed as a cosmetic. A serum is a concentrated cosmetic solution that is particularly helpful for treating facial skin due to its high concentration of active ingredients [2]. Kojic acid and niacinamide have reportedly been proven as serum ingredients that can reduce hyperpigmentation on the face, such as melasma [3]. This 12-week clinical study suggests that a facial serum, combined with Purpose Gentle Wash and Purpose Dual Treatment Moisture Lotion, is effective and well-tolerated in treating photodamaged facial and periocular skin, significantly improving fine lines, wrinkles, pigmentation, elasticity, resiliency, radiance, tone, and overall appearance as early as four weeks, with excellent subject satisfaction and cutaneous tolerability, though a controlled trial is needed to confirm these findings [4]. Clinical research shows that the use of facial hydrating serum is statistically very effective in improving the parameters of dry skin obtained from the addition of moisturizers [5].

Serum can enhance skin firmness, smoothness, pore reduction, and moisture levels [6]. Facial serums offer numerous benefits, including soothing irritated skin, reducing fine lines and wrinkles, and protecting against free radicals with their lightweight and quickly absorbed formula. These qualities make them a popular choice for achieving optimal skincare and a youthful appearance [7].

Skincare products that possess elevated concentrations of antioxidants can counteract the process of skin aging. Antioxidant chemicals have numerous advantages for the health of the skin. Antioxidants have the dual function of preventing premature aging [8], shielding the skin from oxidative stress and damaging UV radiation [9]. Antioxidants inhibit oxidation reactions by binding to free radicals and substances that induce cellular damage. Black pepper oil leaves contain natural antioxidants [10]. The leaves of Binahong (*Anredera cordifolia*) contain oleanolic acid. Oleanolic acid belongs to the triterpenoid category of antioxidants that are present in plants [11]. Binahong leaves often consist of flavonoids, alkaloids, phenols, and saponins [12]. The peel of the mangosteen fruit, scientifically known as *Garcinia mangostana L.*, is utilized as a traditional medicinal remedy and possesses notable benefits for the skin, particularly the facial area, due to its abundant antioxidants. Elevated vitamin C inhibits premature aging, eliminates deceased skin cells, and stimulates cellular regeneration. Additionally, it enhances the radiance of lackluster skin [13]. The methanol extract derived from the peel of the mangosteen fruit exhibits a significant ability to remove harmful free radicals. Even at low concentrations of 44.49 mg/L and 54.45 mg/L, it can neutralize 50% of these radicals [14]. The phytochemical screening experiments indicate that the ethanol extract of mangosteen rind (*Garcinia mangostana L.*) includes alkaloids, triterpenoids, saponins, flavonoids, tannins, and polyphenols [15]. *Aloe vera* is a plant that is extensively utilized in the fields of health food, cosmetics, and medicine. It is reputed to possess anticancer, antidiabetic, and moisturizing properties. Additionally, aloe vera includes flavonoid molecules that consist of antioxidant chemicals. The highest yield of antioxidants from aloe vera was achieved using the extraction method utilizing n-hexane 60% at a temperature of 50^{o}C and an extraction period of 5 hours, resulting in a yield of 56.29% [16].

Prolonged exposure to UV radiation from the sun leads to alterations in the structure and composition of the skin, as well as oxidative stress in the skin. The observed consequences encompass erythema, pigmentation, photosensitivity, and enduring repercussions in the guise of premature aging. Chronic UV exposure has also been associated with photoaging skin aging. For example, UV irradiation increases the expression of matrix metalloproteinase (MMP, an enzyme that damages skin collagen and contributes to photoaging) in the skin connective tissue and outer skin layers, compared to unexposed skin layers [17].

Anti-aging serums have been researched using different plant extracts, including tamarind seeds (*Tamarindus indica*) [18], moringa leaves (*Moringa oleifera*) [19], grape seed oil (*Vitis vinifera*) [20], tomato (*Solanum lycopersicum L.*), and cinnamon juice (*Cinnamomum verum*) [8], black pepper essential oil (*Piper nigrum* L.) [10], gotu kola (*Centella asiatica L. Urban*) [6], black cumin oil (*Nigella sativa L.*) [21], cermai fruit (*Phyllanthus Acidos*), and watermelon rind (*Citrullus lanatus*) [22], red beetroot (*Beta vulgaris L.*) [23], china cherry fruit (*Muntingia calabura L.*) [24], green coffee (*Coffea canephora* var. Robusta) [25], *Hancornia speciosa* [26] and algae [27]. It was reported that mangosteen peel and white rice serum with additional ingredients of phenoxyethanol, allantoin, and hydroxyethyl cellulose proved to be able to overcome the problem of premature aging and dull skin, based on the average results of 30 panelists [28].

The application of sunscreen is crucial for providing chemical protection against the harmful effects of sunlight [29]. Sunscreen agents work by blocking, reflecting, and scattering sunlight, with chemical sunscreens absorbing high-energy UV rays and physical blockers reflecting or scattering the light. Typically, multiple organic compounds are included in chemical sunscreens to provide broad-spectrum UV protection [30]. This research will conduct antioxidant tests using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, SPF tests for the three serums, and tests that adhere to the SNI 16-4399-1996 standard for serums made from aloe vera extract, binahong leaves (*Anredera cordifolia (Ten) Steenis*), and mangosteen peel (*Garcinia mangostana L.*). This research aims to facilitate the advancement of skin care products that are more efficacious in preserving the vitality and attractiveness of facial skin.

2. Research Methodology

2.1. Materials

The materials used in this research include Mangosteen peel, Binahong leaves, Aloe Vera, Ethanol 96%, DPPH, Aquades, Propylene Glycol, Refined Glycerin, Carbomer 940, D-Glucitol, Dimethyol-dimethyl (DMDM) hydantoin, Rose Hydrosol, L-Ascorbic Acid (LAA), Niaciamide, perfume. Meanwhile, the tools used in this research were measuring flasks, pipettes, measuring cups, glass bottles, and test tubes.

2.2. Procedures

The maceration of aloe vera, binahong leaves, and mangosteen peel procedure begins by washing each ingredient with water and cutting them into small pieces, then drying them in the sun for approximately 3 days. Then, the ingredients are macerated with 96% ethanol at a ratio of 1:5 for 3 days. Subsequently, the macerated extract was evaporated again to concentrate using a rotary evaporator at a temperature of 55ºC until the extract was obtained.

Anti-aging serum was made by weighing the following components (in grams): distilled water (60.16%), propylene glycol (10%), refined glycerin (12%), D-glucitol (2.19%), DMDM (0.5%), carbomer 940 (0.15%), rose hydrosol (6.35%), LAA (3%), niacinamide (2.5%), perfume (0.15%), and aloe vera extract (3%). Next, combine all of the ingredients until homogenous. Transfer to a tight, dark container and let settle for 1-3 hours until foaming ceases. After that, verify the serum's pH. These methods were repeated to produce serum from binahong leaf extract and mangosteen peel, using 3% by weight of the extract.

Standard serum tests were performed following SNI 16-4399-1996 [11], which included pH, viscosity, density, organoleptic, and homogeneity testing performed in the UAD chemical engineering laboratory. The total plate number test (ALT) is a microbiological analysis method (MA PPOM 61/MIK/06) performed at the Health and Calibration Laboratory Center, Yogyakarta Special Region Health Service. Meanwhile, the UAD Pharmacy integrated laboratory conducts antioxidant testing utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Antioxidant Activity Test was measured using the DPPH Method

The antioxidant test method with DPPH for extract and serum involves several important steps. First, the DPPH solution is prepared, and the maximum wavelength of DPPH is determined by measuring the absorbance in the range of 450-600 nm. The absorbance of DPPH was assessed using extracts from plants and serum, respectively, and then the IC50 data was interpreted. The necessary tools and materials were collected, and DPPH (20 mg) and ethanol (10 ml) were carefully weighed. A 0.15 mM DPPH reagent solution was prepared, and the negative control was set at the peak wavelength. The sample stock solution was prepared by dissolving 200 mg of the sample in 5 ml of ethanol p.a., sonicating for 15 min, filtering, and adding 10 ml of ethanol p.a. for homogenization. The duration of operation was determined by measuring the absorbance at intervals of 0 to 60 min at a wavelength of 516 nm. Finally, antioxidant activity curves were constructed using six different volumes of the sample mother liquor.

Sun Protection Factor (SPF) testing on three serums was done through several steps. Samples were dissolved in pro-analyzed ethanol (p.a.) and prepared at specific concentrations. The spectra of the sample solutions were then measured at wavelengths of 290-320 nm in 5 nm increments using a UV-Vis Spectrophotometer, with ethanol p.a. as a blank. The SPF value was then calculated. Replication was done 3 times. The spectrophotometric method uses the dilution method with the calculation of SPF value using the Mansur method [31].

3. Results and Discussion

To produce a filtrate, the serum-making process begins with the maceration of each ingredient: aloe vera, binahong leaves, and mangosteen peel. Each ingredient above is thickly extracted in a rotary evaporator at 55°C, as shown in Fig. 1.

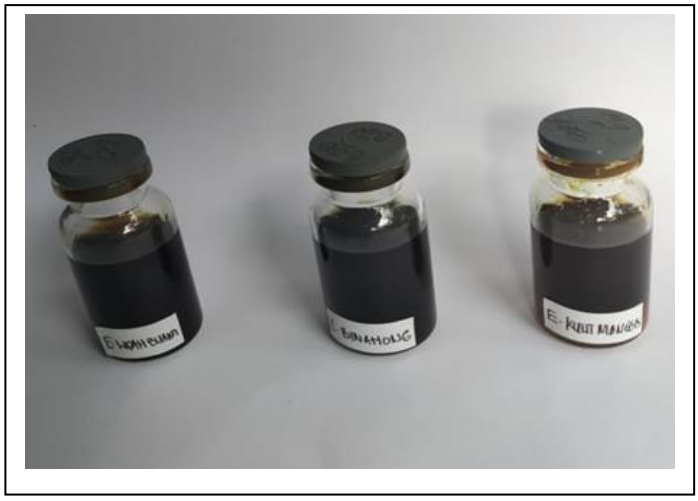

Fig. 1. Aloe vera extract, binahong leaf extract, and mangosteen peel extract (from left to right)

After preparing the extract, make the serum. Fig. 2 depicts the appearance of the finished serum. The results showed that the aloe vera serum is orange, the binahong leaf serum is light green, and the mangosteen peel serum is milky yellow. Each extract and serum was evaluated for antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) technique. Table 1. shows the antioxidant value of each extract and serum.

Fig. 2. Aloe vera serum, binahong leaf serum, and mangosteen peel serum (from left to right)

Metabolic processes play a major role in the aging of living organisms, forming free radical atoms, ions, or molecules containing unpaired electrons. Oxygen is an element that can easily accept single electron transfer and can then bind hydrogen to form water:

$$O_2 + 4e^- + 4H^+ + 2H_2O \quad (1)$$

or can form a superoxide-free radical:

$$O_2 + e^- \rightarrow O_2^- \quad (2)$$

Oxide free radicals can form other active oxygen species that tend to interfere with cellular enzyme activity, destroy DNA, and damage membrane lipids [32].

The antioxidant activity test using the DPPH method is reported by Inhibition Concentration 50% (IC50), which is the concentration of the extract that can inhibit DPPH activity by 50%; the lower the IC50 value, the higher the antioxidant activity. Based on Table 1., the antioxidant value gained is quite good, as evidenced by the modest antioxidant value achieved. The antioxidant value of mangosteen peel extract (51.034 ± 0.068 ppm) and mangosteen serum (43.747 ± 0.046 ppm) is higher than that of aloe vera extract and serum, as well as binahong leaves. Antioxidants are very strong if the IC50 value is less than 50 ppm, strong if the IC50 is 50-100 PPM if the IC50 value is 100-150 ppm, and weak if the IC50 value is more than 150 ppm [13]. If the IC50 level of a sample is low, the antioxidant activity is stronger or higher so that the concentration of the sample needed to

reduce 50% of free radicals is less [10]. *Mangosteen* has been shown in-vitro and in-vivo to promote health, which includes anti-inflammatory antioxidants, anti-inflammatory antioxidants, and anticancer activities [33],[34],[35],[36],[37].

Table 1. DPPH Antioxidant Test Results

Materials	Unit	Result
Aloe Vera Extract	ppm	10169 ± 65
Aloe Vera Serum	ppm	59681 ± 134
Binahong Leaves Extract	ppm	13438 ± 27
Binahong leaves Serum	ppm	54.652 ± 0.195
Mangosteen Peel Extract	ppm	51.034 ± 0.068
Mangosteen Peel Serum	ppm	43.747 ± 0.046

Serum quality testing based on SNI 16-4399-1996 on aloe vera serum, mangosteen peel, and binahong leaves was carried out using several parameters, namely appearance, pH, specific gravity, viscosity, active ingredients, preservatives, and microbial contamination. Table 2. demonstrates that the outcomes of the three serum formulations are homogeneous, indicating that the ingredients have been evenly combined and there are no visible coarse grains. The pH test determines if the preparation is acidic or alkaline. If the gel preparation is too acidic in comparison to the skin's pH, it is likely to irritate the skin; if it is too alkaline, the skin may dry out [1],[38]. The three serum values meet the standards of SNI 16-4399-1996. Specific gravity testing determines the effect of components used in facial serum formulations on the specific gravity of the facial serum generated. Table 2. shows that the three serums' specific weights are slightly heavier than the SNI standard of 0.95 - 1.05 g/ml. Viscosity testing is used to determine the consistency of a preparation, which affects its spreadability and application such that it is easy to remove from the container but does not flow freely from the hands [19],[39]. The viscosity of the three serums complies with SNI 16-4399-1996.

Table 2. Test Results for Serum Parameters based on SNI 16-4399-1996

Test criteria	SNI standard	Test result Aloe Vera Serum	Test results Binahong Leaves	Test Results Mangosteen Peel
Appearance	Homogeneous	Homogeneous	Homogeneous	Homogeneous
PH	4.5 -8.0	4.5	4.5	4.5
Specific Gravity 28^0C (g/ml)	0.95 -1.05 g/ml	1.0812 g/ ml	1.0793 g/ml	1.0816 g/ml
Viscocity 28^0C	2000-50000 Cp	3286 cp	3323 cp	2643 cp
Sun Protection Factor (SPF)	Minimal 4	8.534 ± 0.006	8.731 ± 0.025	20.709 ± 0.012
Active ingredients	According to the Minister of Health Regulation. No.376/Menkes/Per/VIII/1990	suitable	suitable	suitable
Preservative	According to the Minister of Health Regulation. No.376/Menkes/Per/VIII/1990	suitable	suitable	suitable
	Microbial contamination			
Fungus	Negatif (colony/gr)	Negative	Negative	Negative
Total Plate Number (ALT)	Maximal 1.0 x 10^2 colony/g (colony/g = cfu/ml)	3.0 x 10^1 cfu/ml	7.0 x 10^1 cfu/ml	< 10 cfu/ml
Pseudomonas Aeruginosa	Negative	Negative	Negative	Negative
Staphylococcus Aeureus	Negative	Negative	Negative	Negative
APM *Coliform*	<3 APM/g	<3.6 APM/ml	<3.6 APM/ml	<3.6 APM/ml

Sunscreens were developed to protect the skin from damaging radiation. Sunscreens can be expanded foam lotions, whipped lotions, gel lotions, creams, or sprays that absorb (chemical

sunscreens) or reflect (physical sunscreens) UV light to help prevent sunburn. In this study, the serum also functions as a sunscreen whose value is measured in SPF units [40][41]. SPF is measured as the ratio of UV radiation needed to burn skin protected with sunscreen compared to unprotected skin. So, a product with SPF 20 will protect skin until it is exposed to 20 times more UVB radiation compared to that needed to burn unprotected skin [30]. The sun protection factor (SPF) values of aloe vera serum, binahong leaves, and mangosteen peel satisfied the standards of SNI 16-4399-1996, which specified a minimum SPF of 4. Mangosteen peel has the highest SPF value (20.7), followed by binahong leaves (8.7) and aloe vera (8.5). The sunscreen protection category is based on the SPF value, according to Damogalad [42]. It can be categorized as binahong leaf and aloe vera serum with maximum protection SPF 8-15, while mangosteen peel serum has ultra protection (SPF ≥ 15). SPF values range from 0 to 100, and sunscreen properties that are considered good are above 15 [43],[44].

The Total Plate Number (ALT) test on the ethanol extract facial serum preparation aims to determine the number of mesophyll aerobic bacteria in each 1 ml of serum preparation observed [45]. Based on the results, the three facial serum formulations with 96% ethanol extract have ALT values below 1.0 x 10^2 cfu/ml, so these three serums meet the requirements of SNI 16-4399-1996. The results of microbial contamination, such as fungi, Pseudomonas aeruginosa, and Staphylococcus Aureus, showed negative results, meaning no microbial contamination was found in the three serums. The APM coliform testing is carried out using the Most Probable Number (MPN) method or APM (most likely number), which is based on statistical methods [46]. APM testing is carried out using the Presumptive Test and the Confirmative Test. The coliform APM results from the three serums showed a < 3.6 APM/ml limit, where the standard limit value is < 3 APM/ml. The presence of coliform bacteria in serum indicates the possibility of enteropathogenic and toxigenic microbes that are dangerous to health [47]. Coliform is a group of bacteria that is used as an indicator of fecal pollution. This APM value can be improved by using more sterile water in the future.

4. Conclusion

Based on our research on the IC50 antioxidant value of extracts and serums from aloe vera plants, binahong leaves, and mangosteen peel, the most powerful antioxidants are mangosteen peel extract and serum. However, the antioxidant value of aloe vera extract and serum and binahong leaves is also considered good. The results of testing the sun protection factor (SPF) value show that the mangosteen skin serum has a very good SPF value compared to the other two serums. In general, the test results for aloe vera serum, binahong leaves, and mangosteen peel have met the criteria of SNI 16-4399-1996, with the need to improve the specific gravity values and APM coliform limits. This study has limitations regarding the amount of data, such as not repeating experiments and testing samples. Serums containing extracts of mangosteen peel, binahong leaf, and aloe vera contribute significantly to skincare and anti-aging research by providing antioxidants, stimulating skin cell regeneration, improving elasticity, and providing hydration and protection against skin damage, making them beneficial for an effective skincare routine. Based on the results of this research, we recommend the development of mangosteen peel serum for commercial production as an anti-aging facial serum, and patent application is currently underway. Our future research will explore variations in plant ingredients, formulations, stability studies, consumer sensory evaluation, and in-vitro anti-aging tests for the formulated products.

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