



Formulation and Effectiveness Test of 96% Ethanol Extract Anti-Acne Serum Cashew Leaves (*Anacardium occidentale* L.)

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ABSTRACT

Introduction: Recently, the use of cosmetics to cure acne made from natural ingredients can have a good effect. Cosmetic preparations that are currently developing rapidly are serum preparations because they have better effects, and the moisturizing technology used is based on skin physiology. The natural ingredient in question is cashew leaves, which treat acne caused by *Propionibacterium acnes*. This research aims to obtain a serum preparation of cashew leaf extract to determine its effectiveness against *P. acne* and optimize the two ingredients, namely carbopol and triethanolamine (TEA) so that the serum preparation has physical stability that meets predetermined standards.

Methods: This research is pure experimental research of a qualitative nature to obtain a serum preparation of cashew leaf extract with effectiveness as an anti-acne and using the factorial design method with the optimization factor for carbopol concentration comparison as a gelling agent and TEA as an alkalizing agent. The physical property parameters observed as a response to the equation are spreadability and viscosity using two-way statistical tests, ANOVA, with IBM SPSS statistics 29.0.1.0 software. **Results:** The research results obtained indicate the effectiveness of cashew leaf extract serum preparations against bacteria, such as *Propionibacterium acnes*. Apart from that, there is no significant difference between the formulas in the spreadability parameters, which means there is no influence from variations in carbopol and TEA concentrations on the physical evaluation of spreadability. Meanwhile, there are significant differences between the formulas in the viscosity parameters, there is an influence of variations in the concentration of carbopol and TEA on the physical evaluation of viscosity. **Conclusion:** Cashew leaf extract serum preparations are effective in treating bacteria *Propionibacterium acnes*, the cause of acne.

1. Introduction

Acne is a skin disease that commonly occurs in teenagers aged 16-19 years and continues until the age of 30 years. The high prevalence of acne is associated with exposure factors, such as nutrition, medication, occupational factors, pollutants, climate, psychosocial, and lifestyle. Although acne is not life-threatening, it can affect your quality of life. The main factors involved in acne formation are increased sebum production, keratinocyte shedding, bacterial growth, and inflammation. The most common treatments used to cure acne are topical and systemic

preparations, but topical agents are most often used, for example, retinoids, which have comedolytic and anti-inflammatory properties. Additionally, benzoyl peroxide is a key component of acne therapy. *Propionibacterium acnes* (*P. acnes*). is on the entire surface of the skin, grows slowly, is rod-shaped, does not form spores, and is an anaerobic Gram-positive bacteria. This bacteria is found throughout the body, including sebaceous follicles on the face and neck.¹⁻³

Other research states that cashew leaf extract is an ingredient that can be used as a natural antibacterial. Additionally, the main ethnopharmacological

applications of cashew leaf extract include venereal and skin diseases, diarrhea, stomatitis, bronchitis, intestinal cramps, muscle weakness, diabetes, toothache, weakness, inflammation, psoriasis, and cough. Phytochemical studies of cashew leaf extract also report the presence of flavonoids, alkaloids, sugars, tannins, steroids, triterpenes, and phenolic compounds, which function as anti-inflammatory, anti-allergic, and anti-microbial. The cashew plant (*Anacardium occidentale* L.) is a family of Anacardiaceae originating from Brazil; this plant also grows abundantly in NTT, especially in Southwest Sumba. It was emphasized that cashew nuts are mostly developed on marginal land with hot and dry climates, such as in NTB and NTT.^{4,5}

Cosmetic preparations that are currently developing rapidly are serum preparations because they have better effects, and the moisturizing technology used is based on skin physiology. Serum is a cosmetic product with low viscosity, which aims to increase its effectiveness. The advantage is that it is comfortable to use because it has a high water content, hydrates the skin, and is easy to spread when used. Serum is usually formulated with a low viscosity and is less clear (semi-transparent), which contains higher levels of active ingredients than topical preparations in general. Important factors in serum formulation, namely gelling agent. Hydrophilic gel base can increase the activity of extracts in a formulation, such as those derived from synthetic polymers, namely carbopol. The advantage of serum in anti-acne preparations is that it is a semi-solid preparation that contains a lot of water so that it can penetrate the cell walls of gram-positive bacteria, which are more polar.⁶⁻⁸ This study aims to find out how to obtain a serum preparation of cashew leaf extract, to determine its effectiveness against *P. acne*, and to optimize the two ingredients, namely carbopol and TEA, so that the serum preparation has physical stability that meets the specified standards.

2. Methods

The tools used for this research were analytical scales (OHAUS® ST 1), blender (Philips), 40 mesh sieve, overhead stirrer mixer, rotary evaporator

(BUCHI Rotavapor R-300), hot plate (IKA C-MAG HS 7), maceration vessel, jar, glass object, scale watch glass, filter paper, pH meter (Kedida® CT-6020A), ostwald viscometer, micropipette (SOCOREX®), parchment paper, horn spoon, stirring rod, porcelain cup, caliper, incubator, paper disk, tweezers, sterile test pad, spirit lamp, tube needle, and glassware (Pyrex). The ingredients used are cashew leaves from Southwest Sumba Regency, East Nusa Tenggara, Indonesia. Ethanol 96%, alcohol 70%, carbopol, triethanolamine (TEA), ascorbic acid (Vitamin C), propylene glycol, distilled water, nutrient agar (Merck Granucult®), *Cutibacterium acnes* ATCC® 11827™*, and benzoyl peroxide (Benzolac® 5%).

Cashew leaf plants were taken in Southwest Sumba with specifications in the form of fresh green leaves, intact without holes, clean, the fourth leaf from the shoot, and the fourth from the base of the stem. Washing is done first to remove stuck-on dirt, such as dust. The leaves of the cashew nut plant are dried under sunlight and then followed by drying using an oven at a temperature of 40°C. The dried leaves of the cashew plant are then powdered using a blender and then sieved using a 30-mesh sieve. Extraction is carried out using the maceration method using 96% ethanol solvent. 500 g of simplicia powder is put into a glass jar that has been covered with black tape, and 96% ethanol solvent is added until the simplicia is completely submerged. The extract was left for 3x24 hours, stirring occasionally. After 3 days, the macerate was removed and collected. Remaceration is carried out for 3 days until the macerate becomes clear. The resulting solvent reservoir is mixed, and then the extract is concentrated using a rotary evaporator vacuum device followed by a water bath at a temperature of 70°C.

Before the treatment was carried out, the *P. acnes* culture was first taken using a loop needle and then dissolved in 0.9% NaCl. The effectiveness test of cashew leaf extract was carried out using the disc diffusion method with treatments including a concentration of 10%, benzoyl peroxide as a positive control, and distilled water as a negative control. Prepare 4 petri dishes and label each petri dish for each treatment. Then, sterilize the mouth of the petri

dish using a spirit lamp. Pour 10 ml of Nutrient Agar (NA) into the petri dish and leave it until it solidifies. Dip a sterile cotton swab into the *P. acnes* suspension, then rub it on the surface of the solidified agar medium, then leave it for 1-5 minutes so that the suspension enters the agar. Next, the paper discs were soaked in the cashew leaf extract to be tested at a concentration of 10%. Then, dip the paper disc into the positive control and negative control. Remove the paper disc using sterile tweezers, then wait until the cashew leaf extract, positive control, and negative control no longer drip from the paper disc. Then, the disc paper was placed on the NA medium and

incubated at 37°C for 24 hours, and the inhibitory power was measured in the form of a clear zone using a caliper (mm).

Carbopol is developed in hot water for 15 minutes and then mixed using an overhead stirrer mixer at a speed of 1000 rpm for 15 minutes. During this mixing process, dissolve the ascorbic acid with distilled water, then add propylene glycol (mixture 1). Put mixture 1 in a container containing carbopol and continue mixing. Add TEA, and then mix for 10 minutes. Adjust the addition of the concentration series solution of 96% ethanol extract of cashew leaves to each formulation.

Table 1. Serum reference formula.

Material	Total (%)	Function
Cashew leaf extract	1	Active substance
Viscolam MAC 10	3	Gelling agent
Ascorbic acid	0.05	Antioxidant
Propylene glycol	15	Humectant
Methylparaben	0.18	Preservative
Propylparaben	0.02	Preservative
Triethanolamine (TEA)	Qs	pH adjuster
Aquadest ad	100	Solvent

Table 2. Modified formula.

Ingredients	F1	FA	FB	FAB
Ethanol extract 96% Cashew leaves 10% w/v	0,025 % b/v	0,045 % b/v	0,500 % b/v	10 % b/v
Carbopol (g)	0.5	1	0.5	1
Ascorbic acid (g)	0.5	0.5	0.5	0.5
Propylene glycol (g)	15	15	15	15
TEA (g)	0.12	0.12	0.24	0.24
Aquadest add (mL)	100	100	100	100

Serum preparations were evaluated using organoleptic tests, homogeneity tests, spreadability tests, pH tests, viscosity tests, and the effectiveness of serum preparations against *P. acnes* bacteria. Optimization in this research was carried out using the factorial design method. Analysis of physical property data (viscosity and spreadability) used IBM SPSS statistics 29.0.1.0 software with the two-way ANOVA method with a confidence level of 95%. The use of this method can show the effects of the two ingredients (carbopol and TEA) and their interactions.

3. Results and Discussion

Cashew leaf simplicia was extracted using the maceration method with 96% ethanol solvent, and a yield of 81.34 grams (16.27%) was obtained. The results obtained from this test are a clear zone shown in Figure 1. Aquadest does not display a clear zone because it is a negative control that does not contain any antibacterial substances. A clear zone of 9.5 mm \pm 0.057 was shown in 5% benzoyl peroxide, which is a positive control and is a drug used to kill acne-causing bacteria, and antibacterial activity was found with a clear zone of 9.4 mm \pm 0.1 in cashew leaf extract

concentration of 10 %. From the results obtained, it was concluded that cashew leaf extract with a concentration of 10% and benzoyl peroxide 5% had almost the same strength in inhibiting the growth of *P. acne* bacteria.

The results obtained prove that cashew leaf extract has antibacterial benefits; this is because it contains

flavonoids and tannins, which are included in the group of phenolic compounds. Other research states that the presence of flavonoids and tannins can provide antibacterial activity against *S. aureus* bacteria. This flavonoid compound has an -OH group, which can coagulate proteins so that bacteria cannot grow.⁹⁻¹¹



Figure 1. Effectiveness test results of cashew leaf extract concentration 10% w/v. (a: positive control; b: cashew leaf extract; c: negative control.)

Table 3. Effectiveness test results of cashew leaf extract concentration 10% w/v.

Treatment	Inhibition zone diameter (mm)			Average
	Replication I	Replication II	Replication III	
Control (-) Aquadest	0	0	0	0
Control (+) Benzoyl peroxide 5%	9.5	9.6	9.6	9.5 ± 0.057
Concentration 10% Cashew leaf extract	9.3	9.4	9.5	9.4 ± 0.1

Table 4. Organoleptic test results.

Formula	Color	Aroma	Texture
F1	Clear yellowish	Distinctive	Watery and not sticky
FA	Clear greenish	Distinctive	Slightly thick and not sticky
FB	Green	Distinctive	Watery and not sticky
FAB	Deep green	Distinctive	Slightly thick and not sticky

From the results obtained, the serum is not sticky with the distinctive aroma of cashew leaves, is watery to slightly thick, and produces a clear yellowish to dark green color. Serum is a slightly thick liquid product that has a transparent or semi-transparent color that is light on the skin. It can be concluded that the serum produced in the F1 and FA formulas is

suitable because it gives a semi-transparent color. However, the FB and FAB formulas give a darker color because they have a higher concentration of cashew leaf extract. Apart from color, it can be concluded that the serum from all formulas produces an appropriate and good texture.



Figure 2. Formula F1 cashew leaf extract serum.

Table 5. Homogeneity test results.

Formula	Homogeneity test
F1	Homogeneous
FA	Homogeneous
FB	Homogeneous
FAB	Homogeneous



Figure 3. Homogeneity test.

In this research, the serum produced in all formulas is homogeneous, and there are no clumping

particles, so it can be concluded that this serum is good in terms of homogeneity.

Table 6. Spreadability test results.

	F1	FA	FB	FAB
Replication 1	5	5	4	3
Replication 2	5.5	5.5	5	4.9
Replication 3	5.45	5.55	6.8	5
Mean \pm SD	5.5 cm \pm 0.26	5.35 cm \pm 0.30	5.27 cm \pm 1.42	4.3 cm \pm 1.13

The results obtained from measuring the spreadability of the formulas F1, FA, FB, and FAB were 5.5 cm \pm 0.26, 5.35 cm \pm 0.30, 5.27 cm \pm 1.42, and 4.3 cm \pm 1.13. These results have met the requirements for the spreadability of serum preparations, namely in the F1, FA, and FB formulas, namely in the range of

5-7 cm, while FAB is not included in this range due to the concentration gelling agent and alkalizing agent which is larger than other formulas, resulting in the serum becoming thick and unable to spread properly. The addition of the amount of carbopol will determine the spreadability and viscosity response because the

two responses are correlated with each other. That is, if the spreadability produced is small, the viscosity will be greater and vice versa. The more carbopol added, the spreadability will decrease, while decreasing the amount of carbopol will increase the spreadability. Carbopol will form hydrogen bonds with water when mixed and will be dispersed in water. To prevent carbopol from completely dissolving in water, TEA is

needed as a neutralizing agent so that it can form a serum mass.¹²⁻¹⁴ After testing two-way ANOVA, a significance result of 0.486 (>0.05) means that there is no significant difference between the formulas F1, FA, FB, and FAB, which means there is no influence from variations in carpool and TEA concentrations on the physical evaluation of spreadability.

Table 7. Serum pH test of cashew leaf extract.

	F1	FA	FB	FAB
Replication 1	5.5	5.64	6	6.1
Replication 2	5.62	5.69	6.11	6
Replication 3	5.7	5.52	6	6.15
Mean ± SD	5.61 ± 0.1	5.62 ± 0.08	6.04 ± 0.06	6.08 ± 0.08

The results obtained from cashew leaf extract serum in formulas F1, FA, FB, and FAB were 5.61 ± 0.1, 5.62 ± 0.08, 6.04 ± 0.06, and 6.08 ± 0.08 so it can be concluded that the pH of this serum meets the requirements for a serum preparation that is good for the skin. Providing that the pH of the topical

preparation matches the pH of the skin greatly influences the skin's acceptance of the preparation. If the pH of the preparation is too acidic, then the skin's pH will irritate the skin, but if it is too alkaline, the skin will be dry or scaly.^{15,16}

Table 8. Viscosity test results.

	F1	FA	FB	FAB
Replication 1	548	1382	790	1240
Replication 2	870	1180	840	1300
Replication 3	780	1140	980	1350
Mean ± SD	732.67 cPs ± 166.14	1234 cPs ± 129.72	870 cPs ± 98.49	1296 cPs ± 55.07

Testing the viscosity of the cashew leaf extract serum preparation was then continued with statistical analysis. After the two-way ANOVA test, the significance result of 0.001 (<0.05) means that there is

a significant difference between the formulas F1, FA, FB, and FAB, which means that there is an influence of variations in the concentration of carbopol and TEA on the physical evaluation of viscosity.

Table 9. Results of serum effectiveness test of cashew leaf extract concentration 10%.

Treatment	Inhibition zone diameter (mm)			Average (mm)
	Replication I	Replication II	Replication III	
Control (-) Serum without cashew leaf extract	0	0	0	0
Control (+) Benzoyl Peroxide 5%	9,8	9,9	9,8	9.83 ± 0.06
FAB	2	2,1	2	2.03 ± 0.06

From the results of this test, it can be concluded that serum containing cashew leaf extract with a concentration of 10% (FAB formula) is effective in inhibiting the growth of *P. acne* bacteria, although the

clear zone formed was only 2.03 mm ± 0.06 and could be categorized as weak. The formation of this small clear zone can be caused by the turbidity of the bacterial suspension. If the suspension is less turbid,

the diameter of the inhibition zone will be larger, and conversely, if the suspension is more turbid, the diameter of the inhibition zone will be smaller. Apart from that, incubation temperature can also be a factor that influences the diameter of the bacterial growth inhibition zone and the effective thickness of the agar medium, namely 4 mm. Researchers also believe that the inhibitory zone diameter results obtained by cashew leaf extract serum (FAB formula) have a high viscosity so that it can slow down the diffusion of the serum's active ingredients into the medium and result in reducing the ability of high concentration serum to inhibit the growth of *P. acne* bacteria.¹⁷⁻¹⁹

4. Conclusion

It was found that cashew leaf extract could be formulated as an anti-acne serum preparation. In addition, the cashew leaf extract serum preparation has antibacterial activity against bacteria, such as *Propionibacterium acnes*, which is the bacteria that causes acne. Apart from that, there is no significant difference between the formulas in the spreadability parameters, which means there is no influence from variations in carbopol and TEA concentrations on the physical evaluation of spreadability. Meanwhile, there are significant differences between the formulas in the viscosity parameters, which means that variations in carbopol and TEA concentrations influence the physical evaluation of viscosity.

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