

Nano Spray From Sambong (*Blumea Balsamifera*) and Cumin (*Coleus Amboinicus*) Leaves As Antibacterial Against Acne and Antioxidant

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Abstract

Acne vulgaris, commonly known as acne, is a prevalent skin problem. The use of antibiotics to treat acne can lead to side effects like antibiotic resistance. Therefore, natural remedies are sought as they avoid the side effects of chemical drugs and do not induce adverse effects. Natural ingredients like sambong leaves (*Blumea balsamifera*) and cumin leaves (*Coleus amboinicus*) can act as antibacterials due to their rich content of secondary metabolites such as flavonoids, tannins, and phenols. The formulation used in this study is a nano spray, which has the advantage of being absorbed into the skin more effectively. Sambong and cumin leaves were dried and macerated for 3 x 24 hours. Nano spray formulations were then made from the extracts of these leaves with three different concentrations. Phytochemical tests were conducted on each extract, and the antibacterial and antioxidant activities were evaluated to assess the potential of the nano spray as an acne treatment. The data were analysed using SPSS software with the one-way ANOVA method. Phytochemical testing showed that the presence of saponin, flavonoid, tannin, and phenol in *B. balsamifera* extracts. While, flavonoid was absent in *C. amboinicus* extract. Antioxidant screening showed that both extracts and their combinations had weak antioxidant activities ($IC_{50} > 250$ ppm). Antibacterial screening invitro against *Propionibacterium acnes* showed that combination of ethanol extract of sambong leaves and cumin Leaves with ratio of 1:3 gave the highest diameter zone of inhibition of $24,94 \pm 0,85$ mm. Overall, the present study provided a preliminary finding of the potential application of *B. balsamifera* and *C. amboinicus* leaves ethanolic extracts to be developed as anti-acne product in the future.

Keywords: nano spray, *Blumea balsamifera*, *Coleus amboinicus*

INTRODUCTION

More than 80% of the population aged 12-44 years experiences acne, especially during puberty (8-9 years old) (Sifatullah & Zulkarnain, 2021). *Acne vulgaris* is an inflammation of the skin caused by excessive oil gland activity, exacerbated by bacterial infections (Gunarti et al., 2021). One of the bacteria causing acne is *Propionibacterium acnes* (Lestari et al., 2021). *P. acnes* damages the sebaceous follicles and causes skin inflammation (Hafsari et al., 2015).

Acne treatment usually involves antibiotics, but these can lead to side effects such as antibiotic resistance, irritation, immune hypersensitivity, and organ damage (Lestari et al., 2021). Other treatments, such as topical retinoids, can cause side effects like dry

and irritated skin in users who are not suited to them (Leyden et al., 2017).

Given these issues, natural remedies are preferable as they avoid the side effects of chemical drugs and do not cause adverse effects (Lestari, 2016; Lestari et al., 2021). Sambong (*Blumea balsamifera*) and cumin (*Coleus amboinicus* L.) leaves are known for their antibacterial and antioxidant properties due to their content of secondary metabolites like saponins, tannins, flavonoids, and phenols (Amalia et al., 2017; Lestari et al., 2021; Rahmi et al., 2021). Sambong is a plant native to Bali, widely used for medicinal purposes and abundantly available (Mantra et al., 2019). Cumin is also frequently cultivated for medicinal use (Mardisiwi, 2018).

Nano spray is a formulation of interest in the medical field because it penetrates body tissues more easily than

conventional sprays with larger particles, enhancing the effect of active compounds on the tissues (Shabri et al., 2019). Therefore, this study was designed to develop an anti acne nano spray by employing sambong and cumin extracts against *P. acnes*, the causative agent of acne. In addition, phytochemical compositions of the combined extracts were evaluated to give an overview of its active compounds.

METHODS

EXTRACT PREPARATION

The preparation began with washing fresh sambong and cumin leaves, which were then dried in an oven at 50°C for 24 hours, ground into a fine powder, and sieved to obtain a simple powder. A total of 500 grams of the powdered sambong and cumin leaves were macerated in 15 liters of 96% ethanol in a closed container for 3 days at room temperature, with daily stirring, followed by filtration. This process was repeated three times, and the solution was evaporated using a rotary evaporator at 60°C with a speed of 100 rpm (Perawati et al., 2018).

NANOSPRAY PREPARATION

The nano spray was prepared by mixing acetic acid solution, NaTPP solution, and a combination of sambong and cumin leaf extracts in three different ratios (1:3, 1:1, and 3:1). The acetic acid solution was made by dissolving acetic acid in distilled water and adding chitosan. The NaTPP solution was prepared by dissolving NaTPP in distilled water.

PHYTOCHEMICAL TESTING

Phytochemical tests were conducted to determine the flavonoid, tannin, and phenol content of the sambong and cumin leaf extracts qualitatively. The results were indicated by color changes in the test.

ANTIBACTERIAL TESTING

The method began with the

preparation of Mueller Hinton Agar (MHA) media. After 24 hours, 200 µL of *Propionibacterium acnes* was suspended in sterile MHA media and spread using a cotton swab. Each plate contained six sterile disc papers, each soaked in 20 µL of the test substance, which included sambong extract, cumin extract, nano spray with three different extract ratios (1:3, 1:1, and 3:1), 96% ethanol as a negative control, and clindamycin as a positive control. The disc papers were then placed on MHA media and incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the clear zone around the disc paper using an analytical caliper, and the results were categorized into four categories based on inhibition zone diameter: weak (0-5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) (Davis and Stout, 1971).

ANTIOXIDANT ACTIVITY TESTING

The method involved adding 4 mL of nano spray with concentrations of 20-100 ppm to test tubes. Each tube was then added with 1 mL of 0.25 mM DPPH, homogenized using a vortex, incubated for 30 minutes, and the absorbance of the solution was measured using UV-visible spectrophotometry. Antioxidant activity was expressed as IC₅₀, which indicates the concentration needed to inhibit 50% of DPPH activity. A sample is considered to have very strong antioxidant activity if the IC₅₀ value is less than 50, strong (50-100), moderate (100-250), and weak (251-500) (Oey et al., 2022).

PHYTOCHEMICAL TESTING RESULT

The results of the phytochemical screening of the ethanol extracts of sambong leaves and cumin leaves can be seen in the following table.

Table 1. Phytochemical Screening Results of Sambong and Cumin Leaves.

Extract	<i>B. balsamifera</i>	<i>C. amboinicus</i>

Saponin	+	+	Sambong 1: 538.121	Weak
			3 Cumin	
			Sambong 1: 403.692	Weak
			1 Cumin	
Flavonoid	+	-	Sambong 3: 361.023	Weak
			1 Cumin	

ANTIBACTERIAL SCREENING TEST

Table 3. Antibacterial Activity Test Result

Bacteri	Sample	Average Diameter of Inhibition Zone (mm) ± SD	Interpretation
	Ethanol Extract of Sambong Leaves	8,56±0,76	Moderate
	Ethanol Extract of Cumin Leaves	30,78±0,79	Very Strong
	Combination of Ethanol Extracts of Sambong Leaves and Cumin Leaves 3:1	13,84±3,24	Strong
	Combination of Ethanol Extract of Sambong Leaves and Cumin Leaves 1:1	18,13±0,13	Strong
	Combination of Ethanol Extract of Sambong Leaves and Cumin Leaves 1:3	24,94±0,85	Very Strong
<i>P. acne</i>	Nano Spray Ethanol Extract of Sambong Leaves and Cumin Leaves 3:1	1,37±0,03	Weak
	Nano Spray Ethanol Extract of Sambong Leaves and Cumin Leaves 1:1	4,38±0,08	Weak
	Nano Spray Ethanol Extract of Sambong Leaves and Cumin Leaves 1:3	2,4±0,24	Weak
	Negative Control (Ethanol)	0	-
	Negative Control (nano spray without extract)	0	-
	Positive Control (Clindamyci)	51,3±0,4	Very Strong

Explanation:

(+): Active compounds are present

(-) : Active compounds are not present

ANTIOXIDANT TESTING RESULT

The antioxidant activity test of the nano spray combining sambong leaf extract and cumin leaf extract was conducted using the DPPH method. The antioxidant activity of a compound can be expressed by its IC₅₀ (inhibition concentration), which indicates the concentration of the compound needed to inhibit 50% of the DPPH activity. A compound is considered to be a very strong antioxidant if the IC₅₀ value is less than 50, strong (50-100), moderate (100-250), and weak (251-500) (Oey et al., 2022). The greater the antioxidant activity of a compound, the lower the IC₅₀ value. The results of the antioxidant activity test of the nano spray combining sambong and cumin leaf extracts can be seen in Table 2 below.

Table 2. Antioxidant Test Results of Nano Spray Combining Sambong and Cumin Leaf Extracts.

Sample	IC ₅₀ (ppm)	Antioxidant Activity
Negative control	3518.218	Weak

DISCUSSION

PHYTOCHEMICAL TEST

Phytochemical tests showed the presence of flavonoids, saponins, tannins, and phenols in the sambong leaf extract. This was evidenced by the color change and the formation of a precipitate in the tested solution. In contrast, the ethanol extract of cumin leaves showed the presence of saponins, tannins, and phenols but did not show the presence of flavonoids.

The flavonoid test indicated a color change to yellow-orange in the ethanol extract of sambong leaves, which signified a positive result for flavonoids. However, the ethanol extract of cumin leaves did not show any color change, indicating a negative result. The saponin test showed a 1 cm layer of foam formed in both extracts, indicating a positive result. The tannin test showed a color change to greenish-brown in both extracts, indicating a positive result for tannins. The phenol test showed a color change to dark blue in both tested samples, indicating a positive result for phenols (Ergina et al., 2014).

ANTIOXIDANT TEST

The test results showed that the nano spray combining sambong leaf extract and cumin leaf extract has very weak antioxidant activity, with IC₅₀ values varying for each combination ratio. The nano spray combining sambong and cumin leaf extracts in ratios of 1:3, 1:1, and 3:1 yielded IC₅₀ values of 538.121 ppm, 403.692 ppm, and 361.023 ppm, respectively. This indicates that the combination with the highest antioxidant activity is the 3:1 ratio, while the combination with the lowest antioxidant activity is the 1:3 ratio. Therefore, it can be concluded that the higher the concentration of sambong leaves, the higher the antioxidant activity. This is likely due to the more diverse secondary metabolites in sambong leaves compared to cumin

leaves. One of the secondary metabolites found in sambong leaves but not in cumin leaves is flavonoids, as indicated by the phytochemical test results from this study. Additionally, this study only used qualitative phytochemical tests, so the exact quantity of secondary metabolites is unknown. It is possible that the amount of secondary metabolites in sambong leaves is higher than in cumin leaves, which could influence the resulting antioxidant activity. Nevertheless, there is a significant difference in the antioxidant activity of the nano spray combining sambong and cumin leaf extracts when compared to the negative control. Thus, it can be concluded that sambong and cumin leaf extracts have antioxidant activity, although it is categorized as very weak..

ANTIBACTERIAL TEST

The antibacterial activity test of the nano spray combining ethanol extracts of sambong and cumin leaves showed antibacterial activity against *P. acnes*. The nano spray with sambong and cumin leaf extracts in ratios of 3:1, 1:1, and 1:3 showed average inhibition zone diameters of 1.37 ± 0.03 mm, 4.38 ± 0.08 mm, and 2.4 ± 0.24 mm, respectively. These three ratios indicated low antibacterial activity.

The antibacterial activity test of the ethanol extract of sambong leaves against *P. acnes* showed moderate antibacterial activity, with an average inhibition zone diameter of 8.56 ± 0.76 mm. In contrast, the ethanol extract of cumin leaves showed an average inhibition zone diameter of 30.78 ± 0.79 mm, which is categorized as very strong antibacterial activity.

The antibacterial test results of the combination of ethanol extracts of sambong and cumin leaves in ratios of 3:1, 1:1, and 1:3 showed inhibition zone diameters of 13.84 ± 3.24 mm, 18.13 ± 0.13 mm, and 24.94 ± 0.85 mm, respectively. The extract combinations with ratios of 3:1 and 1:1 exhibited strong antibacterial activity, while the combination with a ratio of 1:3 showed very strong antibacterial activity. The positive control using Clindamycin showed

an average inhibition zone diameter of 51.3 ± 0.4 mm, which is classified as very strong antibacterial activity. The negative control using nano spray without extracts and ethanol did not show any antibacterial activity.

The antibacterial activity observed in each group is due to the secondary metabolites present in the extracts of sambong and cumin leaves, such as flavonoids, saponins, tannins, and phenols. These secondary metabolites function to kill *P. acnes* bacteria. However, the varying levels of secondary metabolites result in different inhibition zones in each group. The single extract of cumin leaves had the largest inhibition zone, likely due to a higher concentration of secondary metabolites compared to the other groups. In contrast, the single extract of sambong leaves had a smaller inhibition zone diameter. This pattern is also seen in the extract combinations: the larger the concentration of cumin leaves compared to sambong, the larger the resulting inhibition zone diameter. Therefore, it can be concluded that the greater the concentration of cumin leaf extract compared to sambong leaf extract, the larger the inhibition zone that forms.

CONCLUSION

The extracts of sambong and cumin leaves contain bioactive compounds, including flavonoids, saponins, tannins, and phenols. The nano spray combining sambong and cumin leaf extracts has very weak antioxidant activity. Additionally, the nano spray combining sambong and cumin leaf extracts also exhibits antibacterial activity, although it is considered weak. Combination of ethanol extracts of sambong and cumin leaves at ratio 1:3 provided the highest antibacterial activity against *P. acnes*. Further study should be focused to develop an anti-acne product based on the combination between sambong and

cumin leaves.

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