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Original Research Article

Phytochemical, Antibacterial, and Cytotoxic Properties of Suji Plant (*Dracaena angustifolia* [Medik.] Roxb.) Nanoemulsion Serum as Potential Anti-Acne

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ABSTRACT

Acne or acne vulgaris is a common skin condition caused by clogging of hair follicles or pores of oily skin. Suji (Dracaena angustifolia [Medik.] Roxb.), an Indonesian herbal plant, is used to prevent bacterial growth. The study was conducted to identify the phytochemicals of ethanol extract of suji leaves and analyze the cytotoxic and antibacterial properties against Propionibacterium acnes and Staphylococcus aureus of its nanoemulsion. The results showed that the ethanol extract of suji leaves contained active compounds of alkaloids, phenols, flavonoids, saponins, triterpenoids, and steroids which are secondary metabolites. Serum nanoemulsion showed strong antibacterial activity against Propionibacterium acnes and Staphylococcus aureus at 500 and 750 ppm. The IC50 value of MTT Assay for the cytotoxic effect of nanoemulsion of ethanol extract of suji leaves was 61.92 µg/mL, which indicated that the extract was moderately active against prepuce cells, which is used as a model of acne prone skin. Nanoemulsion of suji leaf ethanol extract has antibacterial and cytotoxic properties, so it has potential as an anti-acne agent.

***Keywords*:** *Acne vulgaris*, suji plant, *Dracaena angustifolia*, nanoemulsion, *Propionibacterium acnes*.

Introduction

Acne or *acne vulgaris* is a widespread dermatological condition characterized by chronic inflammation of the pilosebaceous units, mostly affecting the face, back areas, and chest. About 85% of the population especially adolescents are affected by this condition.[1] *Acne vulgaris is* caused by excessive sebum production, accumulation of dead skin cells, *Propionibacterium acnes* colonization, and inflammation.[2,3] Both synthetic and herbal medicines are widely used to treat wounds. One of the herbal plants in Indonesia that is used in traditional medicine is suji (*Dracaena angustifolia* [Medik.] Roxb.) leaf to help inhibit antibacterial growth.[4,5,6] Nanoemulsion is one of the drug delivery systems that is currently being developed. This is because the tiny particle size of nanoemulsion (1-100 nm) can improve the solubility of active compounds, increase the stability of active substances, and improve absorption. Nanoemulsions are stable, transparent dispersions of oil and water stabilized by surfactant and cosurfactant molecules.[7,8]

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Nanoemulsions can increase the penetration of active components in the pilosebaceous unit, which are lipophilic. Previous research on the effectiveness of essential oil nanoemulsion preparation against acne showed good results *in vitro* and *in vivo*.[9] Also, previous studies have demonstrated the potential of suji leaf ethanol extract for treating *acne vulgaris*.

The present study determined the phytochemical constituents of suji leaf ethanol extract and investigated the cytotoxic and antibacterial activities of its nanoemulsion serum against *Propionibacterium acnes* and *Staphylococcus aureus*.

Materials and Methods

Ethical approval

This study was approved by the Ethics Committee of the Prima Indonesia University Health Research, Indonesia with the approval No. 036/KEPK/UNPRI/VIII/2023.

Plant Collection and Identification:

The suji leaves were collected from Banten City, West Java, Indonesia with (6.1186111,106.5746111), in August 2023. Each plant sample used for this study was identified at the Andalas University Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang - West Sumatra, and given a voucher number ANDA00052506.

Preparation of Plant Extracts

The leaves were washed with running water and left overnight to dry and the leaves were cut into small pieces. All the samples were dried under the shade for a week and ground to powder, weighing 2 kg each. Leaf samples were pulverized, soaked in 96% ethanol in a ratio of 1:7 for 24 hours, then filtered. The resulting filtrate was extracted with a rotary evaporator until a thick extract was obtained. The yields were computed in percentages from the weight of the extract obtained relative to the weight of the powdered material used.[10,11]

Phytochemical screening of suji leaf ethanol extract
Phytochemical analysis of the extract was conducted to identify the secondary metabolites.[12,13]

Determination of total flavonoid content in ethanol extract of suji leaves
The number of flavonoids in the ethanol extract of suji leaves was determined using UV-visible spectrophotometry. Quercetin was employed as a reference standard at various concentrations (30, 40, 50, 60, and 70 ppm). The calibration curve approach was used to determine the total flavonoid content (TFC). Each concentration of the standard solution was combined with 0.2 mL of 10% $AICI_3$, 0.2 mL of 10% sodium acetate, 3 mL of 96% ethanol, and distilled water. Then, the solution was incubated in the dark at room temperature for 30 minutes. The absorbance readings of the standard solution were recorded at a wavelength of 371.50 nm using a UV-visible spectrophotometer. Absorbance to concentration calibration curves were plotted by repeating the procedure using 0.5 mL of 1 mg/mL extract solution as a substitute for quercetin with results expressed as a percentage of total flavonoid content.[14]

Nanoemulsion serum base formulation
Tween 80 and lecithin were added to the nanoemulsion serum base, and the mixture was then blended for 10-15 minutes at 400–2,000 rpm using a planetary centrifugal mixer. Then olive oil was added to the oil phase and the mixture was stirred for 10-15 minutes at the same speed, creating a mixture of surfactant and oil. Subsequently, the mixture was progressively supplemented with distilled water while being continuously mixed until the volume reached 100 mL. The mixture was then subjected to additional mixing for 10-15 minutes under the same conditions to ensure homogeneity and stability of the nanoemulsion base.[9]

Evaluation of the nanoemulsion base
Evaluation of the nanoemulsion was done by sprinkling methylene blue, a water-soluble dye on the surface of the nanoemulsion. If the nanoemulsion was an oil-in-water type, the methylene blue dye would dissolve in it and diffuse evenly throughout the water. Conversely, if the nanoemulsion was water-in-oil type, the methylene blue dye particles would be clumped together.[15]

Characterization of the nanoemulsion serum
An organoleptic test was conducted on the nanoemulsion serum by visual examination, including colour, aroma, consistency, and uniformity as described by Damayanti (2019).[9] The pH of the nanoemulsion serum was determined with a calibrated pH meter. The pH test measures the pH level at room temperature aiming to obtain a pH range of 5-7, comparable to the skin pH to ensure comfort and avoid irritation during usage.[15] Stability testing of nanoemulsion was performed using the freeze and thaw method by storage at 25 and -5^{o}C for 24 hours and repeated three times.[16] Turbidity testing was carried out by measuring the absorption of the nanoemulsion serum at a wavelength of 502 nm. Emulsion stability is indicated by turbidity values below 1%.[15] Viscosity was measured using a Brookfield DV2T viscometer and spindle number 3 at 100 rpm, This measurement was carried out in triplicates.[17]

Preparation of nanoemulsion formula of suji leaf ethanol extract
The suji leaf ethanol extract was dissolved in 2.3 % oil (VCO), 1% lecithin, and 28.3% tween 80. Then, the mixture was stirred with a planetary mixer at 75^{o}C at a speed of 1,500 rpm for 15 minutes. After that, the oil containing ethanol extract of suji leaves was mixed in the planetary centrifugal mixer, and distilled water was added dropwise until blended.[17]

Antibacterial activity testing of suji leaf ethanol extract nanoemulsion formula
Propionibacterium acnes and *Staphylococcus aureus* bacteria were cultured on nutrient agar (NA) medium by sub-culturing bacteria from pure cultures and inoculating them on the surface of agar slants, and then incubated at 30ºC for 24 hours. The optimal concentration of the inoculum was from the isolated colony was prepared using the McFarlan standard.[1819] *Propionibacterium acnes* and *Staphylococcus aureus* bacterial suspensions containing 100 µl each were added to NA media and shaken to ensure even spread. Sterile discs that had been soaked in a nanoemulsion formulation of suji leaf ethanol extract at concentrations of 250, 500, and 750 ppm were placed on the agar surface. Nanoemulsion was used as the negative control and clindamycin as a positive control. The cultures were then incubated for 24 hours at 30°C. The antibacterial activity of the nanoemulsion formula of suji leaf ethanol extract was determined by measuring the diameter of the inhibition zone with a caliper.[20]

Cytotoxicity assay on the suji leaf ethanol extract nanoemulsion formula
Assessment of the cytotoxic effects of the nanoemulsion formula of suji leaf ethanol extract was performed on fibroblast derived from human foreskin. Cells were initially distributed in 96-well plates, and incubated at 30°C for 24 hours under optimal conditions of 5% CO_2 and 95% ambient air. Then, the culture medium was systematically replaced with a solution of progressively decreasing concentration. Following an additional 72-hour incubation period, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to evaluate the cell survival rate.[21]

Data analysis
Data obtained from the phytochemical, antibacterial, and cytotoxic tests were subjected to statistical analysis. The differences in bacterial inhibition were assessed using an independent T-test. The one-way analysis of variance (One-Way ANOVA) was used to determine whether there was a difference in the mean of data from more than five groups or to compare the means of populations represented by multiple sample groups collectively.[22] Decision-making was based on the assumption that data were normally distributed if the significance was $p > 0.05$, and abnormally distributed if the significance was $p < 0.05$.

Results and Discussion

Phytochemical constituents of suji leaf ethanol extract revealed that the extract contained active compounds of alkaloids, phenols, flavonoids, saponins, triterpenoids, and steroids, as listed in Table 1. Table 2 displays the results of the maximum wavelength of quercetin solution, absorbance value, and total flavonoid content. The test results showed that the flavonoid levels in suji leaf extracts of 250, 500, and 750 ppm were 84.5, 125, and 140.83%, respectively.
The characterization of nanoemulsion from ethanol extract of suji leaves which includes determination of organoleptic properties, pH, freezing and thawing, density, viscosity, and turbidity are presented in Table 3. The polydispersion index values of the three formulations (250, 500, and 750 ppm) are less than 0.5. Therefore, it can be concluded that the nanoemulsion formula has a particle size distribution that tends to be homogeneous and more stable over a long period of time. Studies conducted by Prihantini *et al*. (2020) showed variations in risperidone after intraperitoneal administration in certain nanoemulsions, most likely due to different droplet surface properties (different stabilizer layer compositions).[23]

The evaluation of the antibacterial properties of the nanoemulsion formulations showed antibacterial activity on the test bacteria (Table 4). All the formulas had a strong antibacterial activity on both the *Propionibacterium acnes* and *Staphylococcus aureus* bacteria, except formula 2, which had a moderate antibacterial activity on *P. acnes* with a mean diameter of zone of inhibition value of 9.973 ± 0.461 compared to formulas 1 and 3, which, had values of 11.00 ± 1.00 and 11.00 ± 1.00, respectively, exhibited high antibacterial activity.

No	Phytoconsitutents	Test
1	Alkaloids	+
2	Terpenoids/steroids	+
3	Saponins	+
4	Flavonoids	+
5	Phenol	+

Table 1. Compounds contained in ethanol extract of suji leaves

(+) Indicates the presence and (-) Indicates the of phytoconstitutents

Table 2: Absorbance and Flavonoid Content of Ethanol Extract of Suji Leaf (*Dracaena angustifolia* [Medik.] Roxb.)

Concentration of suji leaf ethanol extract (ppm)	Absorbance	Total flavonoid content
250 µg/ml	0.550	84.7 %
500 µg/ml	0.695	125 %
750 µg/ml	0.752	140.83 %

Table 3: Characteristics of nanoemulsion made from ethanol extract of suji leaves (*Dracaena angustifolia* [Medik.] Roxb.)

Characteristics	Formulation			Mean ± SD
	F1 (250 ppm)	F2 (500 ppm)	F3 (750 ppm)	
pH	6.7	6.7	6.8	6.70±0.00
Viscosity (Cp)	284	284	284	284.00 ± 0.00
% transmittance (%)	91.568	91.514	91.475	91.519 ±0.381
Freeze and Thaw	NC	NC	NC	-
Density (g/L)	1.137	1.133	1.134	1.135
Particle size (nm)	9.8	11.5	13.5	11.6
Polidispersion index	0.148	0.260	0.444	0.284
Zeta potential (mV)				-29.7

Table 4. Antibacterial activity of nanoemulsion of ethanol extract of suji leaves (*Dracaena angustifolia* [Medik.] Roxb.)

Bacteria	Formula	n	Mean diameter of inhibition zone	Activity
Propionibacterium acnes	1	3	11.00 ± 1.00	Strong
	2	3	9.973 ± 0.461	Medium
	3	3	11.00 ± 1.00	Strong
	Clindamycin (300 mg)	3	23.57 ± 0.00	Very Strong
Staphylococcus aureus	1	3	10.161± 0.764	Strong
	2	3	10.240± 0.659	Strong
	3	3	11.000 ± 1.00	Strong
	Clindamycin (300 mg)	3	19.700 ± 0.00	Very Strong

1: Nanoemulsion of 250 ppm ethanol extract of suji leaves; 2: Nanoemulsion of 500 ppm ethanol extract of suji leaves; 3: Nanoemulsion of 750 ppm ethanol extract of suji leaves.

There was no significant ($p = 0.648$) difference in the mean inhibition with the nanoemulsion formula between the *Propionibacterium acnes* and *Staphylococcus aureus* (Table 5). The inhibition against *P. acnes was* slightly higher than that of *S. aureus*. The zone of inhibition in this formula had a moderate to strong ability to inhibit bacterial growth. In the present study, the inhibition zone was higher than that in research conducted by Pratiwi *et al.* (2019).[24] Nanoemulsion can penetrate the microbial cytoplasmic membrane more efficiently, thus increasing the antimicrobial effectiveness of suji leaf ethanol extract. The production of clear zones is most likely caused by secondary metabolites, namely alkaloids, flavonoids, phenols, saponins, and terpenoids/steroids that have antibacterial properties. According to Godoy-Gallardo *et al*. (2021), antibacterial compounds usually involve interference with protein biosynthesis, cell wall construction, bacterial membrane integrity, metabolism in bacteria, and bacterial DNA replication.[25]

The cytotoxic effect of suji leaf ethanol extract nanoemulsion on fibrous derived from human foreskin was evaluated by MTT assay with the result that all formulas showed cytotoxic activity against the test cell cultures in a dose-dependent pattern. The higher the concentration, the lower the cell viability. The results were computed from absorbance readings of the ELISA plates at a wavelength of 595 nm against the four MTT gram control reactions (Table 6). The absorption value demonstrated that the absorption value decreased with increased concentration. A high concentration of suji leaf ethanol extract nanoemulsion preparations resulted in a drop in live cells or an

Table 5: Difference in bacterial inhibition zone of ethanol extract of suji leaves (*Dracaena angustifolia (Medik.)*.

Bacteria	n	Mean diameter of inhibition zone	p-value
Staphylococcus aureus	9	10.469 ±0.815	0.648
Propionibacterium acnes	9	10.658 ± 0.904	

Table 6: The IC_{50} values result of MTT Assay

Concentration (µg/ml)	Mean Absorbance	Cell Viability (%)
10	0.29 ± 0.0235	80.96
20	0.25 ± 0.0181	67.82
30	0.24 ± 0.0071	62.14
40	0.22 ± 0.0071	57.80
Medium	0.05	
IC_{50}	61.92	

increase in fibroblast cells. The ELISA reader results and the percentage of living cells were calculated by linear regression at the four concentrations (10, 20, 30, and 40 µg/mL) of suji leaf ethanol extract. The concentration needed to prevent 50% of the growth of the cell population in comparison to the growth of cells that are not exposed to the chemical is represented by the IC_{50}. This change in the size of the preputium cell population can lead to cell death or decreased cell proliferation. When analyzing drug reactions to ascertain the compound's effect, the IC_{50} value is important. The extract is classified as cytotoxic active if $IC_{50} \geq 30$ µg/ml, moderately active if IC_{50} between ≤ 30-100 µg/ml, and inactive if $IC_{50} \geq 100$ µg/ml, according to the U.S. National Cancer Institute. A comparison between the IC_{50} values of the nanoemulsion of suji leaf ethanol extract and that of the standard (61.92 µg/ml), showed that suji leaf ethanol extract was effective in inhibiting 50% of prepuce cell proliferation. According to Berrouet *et al*. (2020), the IC_{50} value of the active medium showed that the formula was effective at low concentrations so it is safer due to less systemic toxicity.[26] Cytotoxic activity can be influenced by the phytochemical content in suji leaf extract. At low concentrations, flavonoids are antioxidants and at high concentrations flavonoids are prooxidants because they can initiate the formation of reactive oxygen species (ROS), causing toxic effects on cells.[27]

Conclusion

The total flavonoid content of suji leaf ethanol extract was highest at a concentration of 750 ppm. The suji leaf ethanol extract nanoemulsion preparation's IC_{50} value was 61.92 µg/mL, which is active against prepuce cells, such as acne skin. The findings of this study revealed that nanoemulsion of ethanol extract of suji leaves has antibacterial and cytotoxic properties as an anti-acne agent.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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