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RESEARCH ARTICLE

# Formulation and *in vitro* study of nanoparticles loaded *Anredera cordifolia* leaf extract as anti-acne

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## Abstract

**Background:** The ethanolic extract of *Anredera cordifolia* (AC) leaves has previously been investigated to have antibacterial activity against *Propionibacterium acnes*. An active antibacterial compound against *propionibacterium acnes* was flavonoid. **Objective:** This study aimed to compare antimicrobial activity differences between nanoparticles and extract alone. **Method:** AC leaves were extracted and then formulated into nanoparticles using chitosan as the polymer and sodium tripolyphosphate (Na-TPP) as a crosslinker. The nanoparticle-preparation method was ionic gelation, while the antibacterial study used the disc method. **Result:** Nanoparticle evaluations showed an average size of  $84.93 \pm 15.36$  nm, a polydispersity index of 0.22, a percent entrapment efficiency of  $55.06 \pm 1.05\%$ , and an average yield of  $11.00 \pm 1.27\%$ . Based on statistical t-test analysis, nanoparticles differed significantly from extract alone in antimicrobial activity. **Conclusion:** Nanoparticles significantly increase the antibacterial effectiveness of extract through the synergism effect between chitosan and flavonoid content in the extract. Hence nanoparticles serve good potency to deliver extract for acne therapy.

## Introduction

Acne is a disorder caused by excessive accumulation of sebum or by a bacterial infection (Pelen *et al.*, 2016). Bacteria that can infect acne include *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* (Darmawati, 2015). Antibiotics are the most frequently used option for treating acne. However, antibiotics alone can cause some side effects, such as irritation and antibiotic resistance. Another alternative treatment to avoid antibiotics' adverse side effects is herbal plants (Utami, 2012). *Anredera cordifolia* (AC) leaf has an anti-acne effect. The secondary metabolite content of AC leaf includes flavonoids, saponins, alkaloids, polyphenols, and monosaccharides, and the leaf also contains L-arabinose, D-galactose, and L-rhamnose (Rachmawati, 2008; Hasbullah, 2016; Alba *et al.*, 2020).

Many drug delivery systems developed through the skin are modified into nanoparticles to take advantage

of their use as a drug delivery system. This allows them to increase drug penetration through the stratum corneum. Conventionally, acne is treated with either creams or gels. Recent advancements use nanotechnological carriers to effectively treat acne (Verma *et al.*, 2018) because they can quickly penetrate cells and tissues and increase drug stability (Emeje *et al.*, 2012). These carriers show controlled drug release and improved drug penetration even up to the pilosebaceous unit of the skin (Verma *et al.*, 2018).

Ionic gelation is often used in manufacturing nanoparticles because the process is simply by ionic interactions between polymers with a positive charge and a negative charge of polyanion cross-linking (Mardiyati *et al.*, 2012). In this study, the nanoparticles containing extracts were dried to improve long-term stability, characterised, and subjected to an antibacterial test.

## Methods

### Material

AC leaves were obtained from Jember, East Java. Chitosan (C<sub>6</sub>H<sub>11</sub>NO<sub>4</sub>)<sub>n</sub> with a degree of deacetylation of 96.24% was obtained from Commanditaire Vennootschap (CV) Chimultiguna, Cirebon. Sodium tripolyphosphate (Na TPP) food grade was purchased from Thailand, Tween 80 from Sigma, and Glacial acetic acid from a Limited Liability Company (PT). Brataco Chemika, and Mueller Hinton Agar (MHA) media from Merck. *Propionibacterium acnes* were supplied from the author's laboratory.

### AC leaf extraction

AC leaf extraction was performed by maceration with 96% ethanol, then concentrated using a rotary evaporator and an oven.

### Phytochemical screening

#### Flavonoid test

A total of 2mL of AC leaf extract and 1mL of 1% HCl were mixed into a test tube with magnesium (Mg) powder. The result is positive if forming a yellow color (Nafisah et al., 2017).

#### Alkaloids test

Mayer's reagent was added to 2mL of the sample extract with 5mL of ethanol. The result is positive if white sediment is present (Parwati et al., 2014).

#### Identification of saponins

Foam tests will identify saponin. The result is positive if foam with a constant height is maintained for 30 minutes after adding 2N iodine (Helmidanora et al., 2020).

### Standardisation of extracts

Extract standardisation determined the total ash, water, and Rutin content.

#### Rutin determination

Rutin concentrations of 50, 100, 200, 300, and 400 g/ml were used to create a calibration curve. Furthermore, the percentage of Rutin content was calculated using the standard curve equation.

#### Determination of ash content

The total ash content was calculated by dividing the ash weight by the weight of the original sample multiplied by 100% (Kemenkes RI, 2017).

#### Determination of water content

Samples were heated in the oven for five hours at 105°C. The percent of water content is calculated as the

initial sample's weight (Kemenkes RI, 2017).

### Nanoparticles preparation

Chitosan was dissolved in 100mL of 1% glacial acetic acid to obtain a chitosan concentration of 0.04%. An amount of 75mg of AC ethanol extract was added with 70% ethanol until the concentration of extracts was 0.2%. The chitosan and extracts are stirred using a magnetic stirrer for 90 minutes. Furthermore, 6mL of 0.05% Na-TPP and 3.6mL of 1% Tween 80 were stirred at 1000 rpm to form nanoparticles (Table I) (Lazaridou et al., 2020).

**Table I: Formulation design**

Materials	Function	Volume	%
<i>Anredera cordifolia</i> leaf extract	Active ingredient	3mL	0.20
Chitosan	Polymer	30mL	0.04
Na TPP	Crosslinker	6mL	0.05
Tween 80	Surfactant	3.6mL	1.00

Note: The formula was prepared in triplicate

### Particles size

The size average and size distribution of ethanolic extract of AC leave nanoparticles were analysed using a Particle Size Analyser.

### Entrapment efficiency (EE)

Free rutin was measured using a spectrophotometer after nanoparticle centrifugation for an hour at 12000 rpm. The percentage of EE can be calculated by the formula equation below.

$$\%EE = \frac{\text{Initial concentration} - \text{Concentration in supernatant}}{\text{Total initial concentration}} \times 100\%$$

### FTIR (Fourier transform infrared) analysis

FTIR analysis was carried out on the ethanolic extract of AC leaves, chitosan, Na TPP, and nanoparticles in powder.

### Antibacterial activity

Paper discs previously soaked with samples equivalent to Rutin of 1500, 2000, and 2500 mcg/mL were placed into the MHA (Mueller Hinton Agar) media and incubated for 24 hours at 37°C. The positive control was tetracycline, and distilled water was the negative control.

### Data analysis

The data obtained were analysed using one-way ANOVA followed by a t-test if a significant difference ( $p < 0.05$ ) was found between the analysed group.

## Results

### Phytochemical screening and an extract standardisation

AC leaf powder was extracted with 96% ethanol solvent to produce a viscous extract yield of  $13.50 \pm 0.51\%$ . The yield value meets the requirements of the AC viscous extract, which is not less than 11.9% (Kemenkes RI, 2017). Phytochemical screening gave a positive result for alkaloids, flavonoids, and saponins (Table II),

following the previous finding of Basyuni and the authors (2017). Rutin content in the ethanolic extract of AC leaves was  $2.32 \pm 1.46\%$ , which exceeded the requirement in the Indonesian Herbal Pharmacopoeia (no less than 1.74%). The thick extract of the AC leaf should contain a total water content of less than 8.9% and total ash of not more than 7.2% (Kemenkes RI, 2017). The water content was  $7.71 \pm 0.98\%$ , and the total ash was  $0.70 \pm 1.04\%$  which met the requirements (Table II).

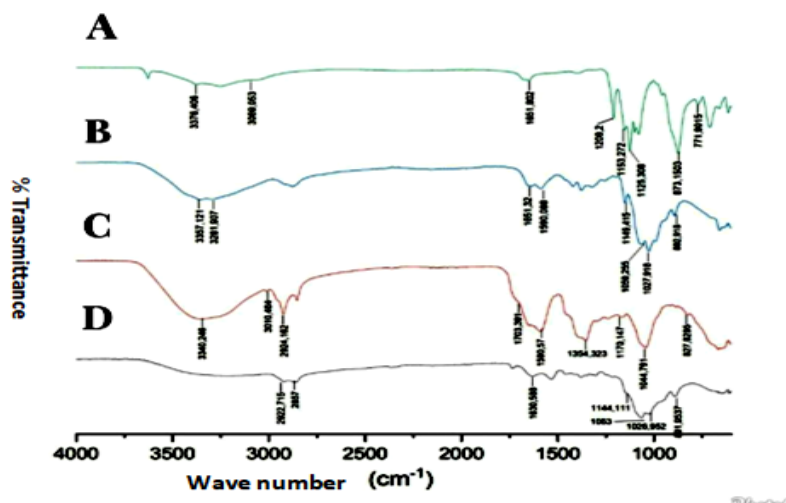
**Table II: Results of phytochemical screening and extract standardisation**

Test	Result	Conclusion
Alkaloids	Formation of orange sediment	(+)
Triterpenoids	Formation of brown ring	(+)
Flavonoids	Formation of a red or orange colour on the amyl alcohol layer	(+)
Rutin content	$2.32 \pm 1.46\%$	Met the requirement
Water content	$7.71 \pm 0.98\%$	Met the requirement
Ash content	$0.70 \pm 1.04\%$	Met the requirement

### Formulation and evaluation of preparations

The average particle size of nanoparticles was  $84.93 \pm 15.36\text{nm}$  with a polydispersity index of 0.22. It indicates that the particle size had a monodispersed system (Worldwide, 2011). The nanoparticles have a

reasonably good percentage entrapment efficiency of  $54.89 \pm 0.01\%$ . The formation of a nanoparticle through the cross-linking reaction between chitosan and sodium tripolyphosphate was determined from the FTIR analysis (Figure 1).



**Figure 1: Spectra of (A) Na TPP, (B) Chitosan, (C) *Anredera cordifolia* leaf extract, (D) *Anredera cordifolia* leaf nanoparticles**

### Antibacterial activity

The results of the one-way ANOVA on the inhibition zone revealed a difference between groups with a

significant value of 0.022 ( $p < 0.05$ ), indicating a significant difference in the moderate antibacterial activity based on the provided treatment. The results of

the t-test analysis displayed a significant difference ( $p < 0.05$ ) between the positive control group and the negative control, extract alone, and nanoparticles

extract. The nanoparticles inhibition zone was more excellent and gave a significant difference ( $p < 0.05$ ) with the AC leaf extract alone in all doses (Figure 2).

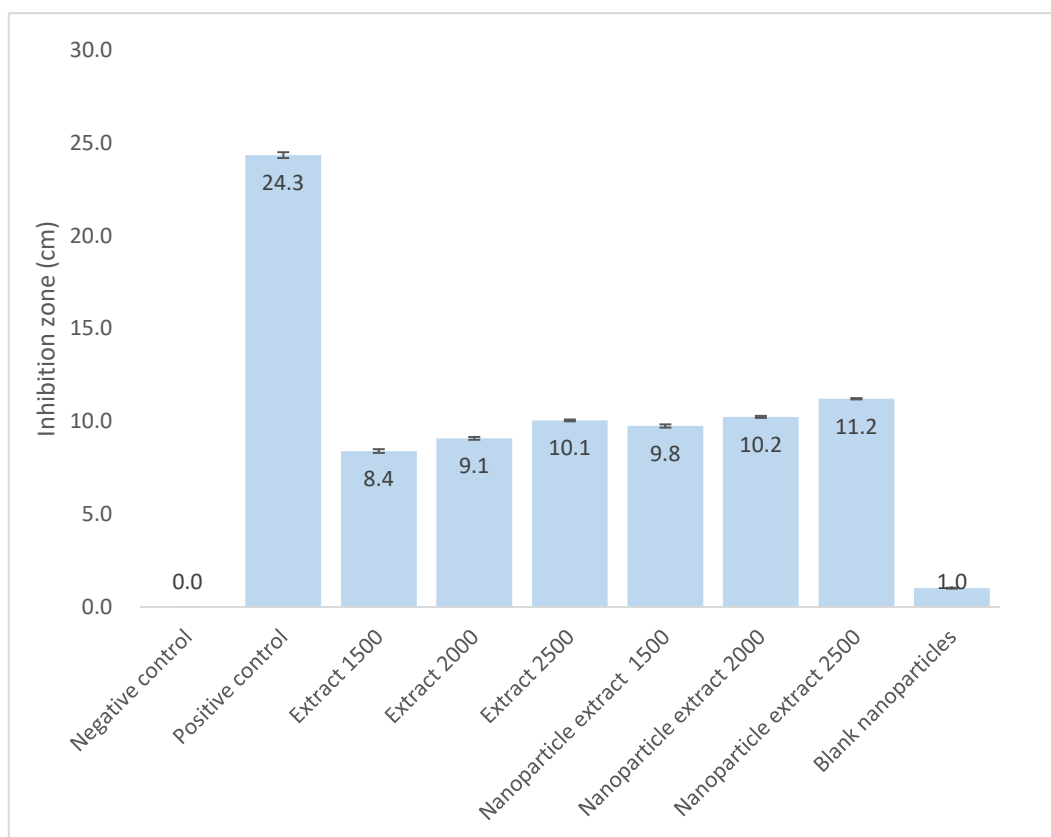


Figure 2: Inhibition zone between group

## Discussion

Ionic gelation occurs through the interaction of chitosan cations and Na-TPP anions to produce nano-sized coacervates that encapsulate the extract. The entrapment of the resulting extract is quite good, with a relatively high percentage of entrapment efficiency. A study of Chitosan-alginate matrices loaded with leaf extracts of AC resulted in an encapsulation efficiency of a phenolic compound of 90% (Krisanti *et al.*, 2019). This study's Entrapment Efficiency (EE) results were lower because rutin was used as a standard. Rutin is a type of phenolic compound instead of total phenolic compounds, which are more numerous in an extract. The extract nanoparticle FTIR (Figure 1) showed a shift in the wave number of chitosan. At the -NH bending of chitosan,  $1651.32\text{cm}^{-1}$  became  $1630.588\text{cm}^{-1}$ . In addition, a new peak was found at wave number  $1703.391\text{cm}^{-1}$  in the -C=O group. The peak at wave number  $1155.201\text{cm}^{-1}$  appeared in the extract

nanoparticles. The peak indicated the presence of a phosphate group from sodium tripolyphosphate. The peak shift was caused by ionic interactions between ammonium ions in chitosan and phosphate ions in sodium tripolyphosphate to form nanoparticles through the ionic interaction.

In all doses, the inhibition zones showed a significant difference between the extract nanoparticles and the extract alone. It was caused by the antibacterial synergism effect between chitosan and flavonoid. Besides, nanoparticles will quickly penetrate cells (Emeje *et al.*, 2012). The proposed antibacterial mechanisms of flavonoids are the inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function, inhibition of energy metabolism, inhibition of attachment and biofilm formation, and alteration of the membrane permeability (Xie *et al.*, 2015). In comparison, the antibacterial activity of chitosan is by

binding to the negatively charged bacterial cell wall and disrupting the cell, altering the membrane permeability, followed by attachment to DNA, causing inhibition of DNA replication and, subsequently, cell death (Nagy *et al.*, 2011). The results showed that nanoparticles could increase the effectiveness of acne therapy using AC extract.

## Conclusion

The results showed that nanoparticles significantly increase the effectiveness of inhibition produced by the ethanolic extract of AC leaves through a synergistic antibacterial effect between chitosan and flavonoids.

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