

FORMULATION AND CHARACTERIZATION OF 50% ETHANOL EXTRACT OF ARECA NUT (*Areca catechu*) NANOPARTICLES USING THE IONIC GELATION METHOD

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ABSTRACT

The extract of areca nuts (*Areca catechu*) contains secondary metabolites, such as flavonoids, alkaloids, saponins, tannins, and steroids. These compounds possess pharmacological effects and can be developed into herbal medicine products. The objective of this study is to determine the optimal nanoparticle formulation by varying the concentrations of chitosan and sodium tripolyphosphate. The 50% ethanol extract of areca nuts was obtained through the maceration method. The extract obtained calculation the yield value. Then tested for phytochemical screening. Subsequently, the extract was formulated into nanoparticle colloids with different ratios of extract concentration: chitosan: sodium tripolyphosphate: Tween 80. The resulting nanoparticles were characterized by their particle size, zeta potential, and polydispersity index. The research findings demonstrated that Formula 3 exhibited the best results, with a particle size of $254.2 \pm 0,00$, zeta potential of $12.7 \pm 0,71$, and polydispersity index of $0.436 \pm 0,03$.

Keywords: Formulation, Nanoparticles, 50% Ethanol, Areca Nuts, Ionic Gelation.

INTRODUCTION

Areca nuts (*Areca catechu* L.) is a plant that have been extensively used for the treatment of various diseases. Previous studies have demonstrated its therapeutic properties in the management of diverse illnesses, including immunomodulation (Sari et al., 2020), cytotoxicity (Al-Tayar et al., 2020), anticancer effects (Wei et al., 2021), antibacterial activity (Pradeep et al., 2019), and others. This plant is a prominent commodity readily available in Jambi province, thus offering the potential for local product development.

Initial research has revealed that the 70% ethanol extract exhibits an IC_{50} value of 49.549 $\mu\text{g/mL}$. Researchers commonly employ 50% ethanol as a solvent for extracting bioactive compounds from plant material. This choice aims to determine the solvent variation that can optimally extract active antioxidants, which can then be further developed into traditional medicines capable of addressing various diseases. Phytochemical screening of the 50% ethanol extract of areca nuts has identified the presence of active compounds, including flavonoids, alkaloids, saponins, tannins, and steroids. These secondary metabolites possess pharmacological effects (Arifin and Ibrahim, 2018; Susanti et al., 2021).

Previous studies have provided substantial evidence that the type and concentration of the solvent used have an impact on the extraction rate. The choice of solvent during the extraction process influences the activity of the extract in delivering pharmacological effects. Water is more polar compared to ethanol, resulting in lower solvent polarity as the ethanol

concentration increases (Niawanti and Putri, 2020; Wijayanti and Hasyati, 2018). The use of 50% ethanol offers a new perspective in research, highlighting that variations in solvent concentration can modify the potential or pharmacological effects of a compound.

Nanotechnology is a field that produces molecules ranging in size from 1-1000 nm. Its applications have been extensively explored across various scientific disciplines, particularly in the medical field (Najahi-Missaoui et al., 2021). Research in nanotechnology has great attention from researchers worldwide due to its remarkable effects and wide-ranging applications. Nanoparticles can be employed to deliver small-molecule drugs through their encapsulation within polymers. These polymers can be of synthetic or non-synthetic origin. Chitosan, along with the cross-linking agent sodium tripolyphosphate (NaTPP), is one such polymer used in nanoparticle formulation (Samudra et al., 2022). The ionic gelation method offers several advantages, including simplicity, ease of use, utilization of non-organic solvents, and precise control over the process. Research conducted by Samudra et al. (2022) demonstrated variations in chitosan and Na TPP compositions, resulting in differences across each data set.

Based on the aforementioned issues, this study was conducted to formulate and characterize nanoparticles of 50% ethanol extract derived from areca nuts using the ionic gelation method by varying the concentrations of chitosan and Na TPP.

RESEARCH METHODS

Tools and Materials

Tools

Analytical balance (Ohaus G332), volumetric flask (Pyrex), Erlenmeyer flask 250 mL (Pyrex), stirring rod (Pyrex), UV-VIS spectrophotometer, magnetic stirrer (Pyrex), 250mL beaker glass (Pyrex), and test tubes (Pyrex).

Materials

Absolute ethanol (Merck), areca nuts, glacial acetic acid (Merck), chitosan (Sigma Aldrich), sodium tripolyphosphate (Brataco), distilled water, Tween 80 (Brataco), FeCl₃ (Merck), Mg powder (Sigma Aldrich), Mayer's reagent, Dragendorff's reagent, sulfuric acid (Merck).

Areca Nut Extraction

The extraction of areca nuts was carried out using a maceration method with 50% ethanol as a solvent. A total of 500 grams of powdered raw material was placed in a container, and the solvent was added at a ratio of 1:10, ensuring that the powder was fully immersed. The mixture was allowed to stand for two days while stirring occasionally. Next, the mixture was filtered to separate the liquid extract. The residue was employed for a second maceration using 50% ethanol, and allowed to stand for an additional 24 hours to yield a second macerate. The macerate was concentrated using a rotary evaporator at a temperature of 40-50°C until a concentrated extract was obtained.

1. Phytochemical Screening

A qualitative test of 50% ethanol extract of areca nuts was carried out using the phytochemical screening method.

a. Tannins

A small extract that had been added 10 mL of distilled water was boiled, and a few drops of FeCl₃ were subsequently added. The development of brownish green or bluish black color indicates the presence of tannin compounds.

b. Flavonoids

A small extract was mixed with mg powder and a few drops of concentrated HCl. The development of pink, magenta, or orange color indicates the presence of flavonoid compounds.

c. Alkaloids

A small sample extract was added with 1% HCl, followed by the addition of 1 mL of Mayer's reagent. The development of a precipitate or turbidity indicates the presence of alkaloids.

d. Saponins

A small amount of extract sample was added to 10 mL of distilled water and then shaken for 30 seconds. The formation of permanent foam indicated the presence of saponin compounds.

e. Steroids

A small amount of extract sample was added with anhydrous acetic acid and one drop of H_2SO_4 (Liebermann-Burchard reagent). The development of a blue-green color indicated the presence of steroid compounds.

f. Terpenoid

A small amount of extract sample was added with anhydrous acetic acid and one drop of H_2SO_4 (Liebermann-Burchard reagent). The development of brownish red or brownish pink color indicates the presence of terpenoid compounds.

2. Nanoparticle Preparation

The preparation of nanoparticles involved several stages. In stage 1, chitosan solutions were prepared by combining 0.1 and 0.2 grams of chitosan with 100 mL of a 1% acetic acid solution in distilled water. The mixtures were stirred using a magnetic stirrer at speed of 1500 rpm. In stage 2, sodium tripolyphosphate solutions were prepared by weighing 0.1 and 0.2 grams of sodium tripolyphosphate and adding 100 mL of distilled water. The solutions were stirred using a magnetic stirrer. Stage 3 comprises preparing a Tween 80 solution by dissolving 0.5 mL of Tween 80 in 100 mL of distilled water, mixed using a magnetic stirrer. Stage 4 followed the method described by Gredi et al. (2017) for nanoparticle formation. As much as 0.5 grams of the extract was mixed with the chitosan solution in varying concentrations and stirred using a magnetic stirrer of speed 2500 rpm for 30 minutes. Then, 9 mL sodium tripolyphosphate solution was added dropwise with continuous stirring at 2500 rpm for 30 minutes. Finally, dropwise addition of 3 mL was performed using a magnetic stirrer at 2500 rpm for 30 minutes until a nanoparticle suspension was formed. The formulation of the nanoparticle suspension of areca nut 50% ethanol extract can be found in [Table I](#).

Table I. Formulation of Nanoparticle Containing 50% Ethanol Extract of Areca Nuts

	Extract	Chitosan	Na TPP	Tween 80
Formula 1	0.5 gram	0.1%; 18 mL	0.1%; 9 mL	0.5%; 3 mL
Formula 2	0.5 gram	0.2%; 18 mL	0.2%; 9 mL	0.5%; 3 mL
Formula 3	0.5 gram	0.1%; 18 mL	0.2%; 9 mL	0.5%; 3 mL
Formula 4	0.5 gram	0.2%; 18 mL	0.1%; 9 mL	0.5%; 3 mL

Characterization of Nanoparticles

The formed nanoparticles were subsequently characterized, including their particle size, polydispersity index, and zeta potential. This characterization was conducted using a Particle Size Analyzer with the Dynamic Light Scattering method ([Nugroho et al., 2020](#)).

a. Particle Size and Polydispersity Index

Characterization was carried out using the HORIBA Nano Particle Analyzer SZ-100. The average diameter of the nanodispersion particles was measured using a dynamic particle size analysis of light scattering with a measuring instrument in the range of 0.3 nm – 8 μm . Samples diluted using a ratio of 1:5 to prevent multiple scattering. The temperature used for measurement is 25°C.

b. Zeta Potential

The analysis used a zeta potential measurement range of -200 mV to 200 mV. The laser beam is divided into two beams like the input beam and the reference beam. The modulator modulates the light scattered by the sample particles and the reference beam. The detected signal is converted into a digital signal calculated and translated as zeta potential units.

3. Data Analysis

The obtained data were subjected to descriptive analysis, which included the results of phytochemical screening, particle size, and zeta potential of the 50% ethanol extract from areca nuts.

RESULTS AND DISCUSSION

1. Phytochemical Screening Results

Phytochemical screening was conducted to initially identify secondary metabolite compounds present in the raw material used. The results of phytochemical screening can be seen in [Table II](#). The research findings indicate that the 50% ethanol extract of areca nuts contains flavonoids, alkaloids, tannins, steroids, phenols, and terpenoids.

Table II. Results of phytochemical screening of areca nut extract

Phytochemical Testing	Observation Results
Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	-
Steroids	+
Terpenoids	+

Note: +: indicating the presence of compounds; - : indicating the absence of compounds

2. Particle Size, Zeta Potential, and Polydispersity Index Results of Nanoparticles from 50% Ethanol Extract Areca Nuts

The results of producing nanoparticles from a 50% ethanol extract of areca nuts using the ionic gelation method with variations of areca nut 50% ethanol extract, chitosan, Na-TPP, and Tween 80 showed that all nanoparticle formulations formed particles in the nano-sized range of 1-1000 nm. However, among all the formulations, the most stable nanoparticle formulation was found in Formula 3. This is attributed to its zeta potential value, which is close to ± 30 mV.

Table III. Particle Size, Zeta Potential, and Polydispersity Index Results

	Nano Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index
Formula 1	267.5 ± 0.28	10.0 ± 0.07	0.383 ± 0.00
Formula 2	316.9 ± 1.48	4.1 ± 0.14	0.520 ± 0.02
Formula 3	254.2 ± 0.00	12.7 ± 0.71	0.436 ± 0.03
Formula 4	365.6 ± 2.83	4.3 ± 0.07	0.313 ± 0.09

The particle size distribution is expressed by the polydispersity index. The Polydispersity Index (PDI) indicates the level of particle homogeneity. If the PDI value exceeds 0.7, it means that the produced nanoparticles are less homogeneous. The results of this study show that all formulations have PDI values below 0.7, indicating that the formulations are in a homogeneous condition ([Fitri et al., 2021](#)).



Figure 1. Result of 50% Ethanol Extract Nanoparticle Formula from Areca Nuts

The production of nanoparticles using the ionic gelation method involved the use of Na-TPP as a low-concentration crosslinker agent to inhibit the crosslinking between the polyanion in TPP and the amino groups in chitosan. This countercharge condition promotes the formation of coiled chitosan polymer chains, resulting in the generation of nanoparticles with specific sizes. A study conducted by Samudra et al. (2021) reported significant differences in particle size and zeta potential due to variations in chitosan and Na TPP during the nanoparticle production process. Tween 80 was used as a surfactant in the research to stabilize the formation of the extract nanoparticle.

In clinical pharmacy, the particle size of a drug can affect the release of the active substance from preparations that are given orally, parenterally, rectally, and topically. Reducing the particle size can have a role in increasing therapeutic effects since there is increase in the degree of solubility of a drug compound (Zhang et al. 2021).

CONCLUSION

The results of the study indicate that all nanoparticle formulations are able to produce nanoparticles within the size range of 200-400 nm. Among the formulations, Formula 3 exhibits the best performance, with a particle size of 254.2 nm and the most stable zeta potential value of 12.7 mV.

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