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Cytotoxicity activity of 50% ethanol extract of areca nut seeds on RAW 264.7 macrophage cells

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Abstract

Background: Areca nut is a widely cultivated plant, particularly in Jambi province. This presents an opportunity for its development into a herbal product with pharmacological potential. Cytotoxicity testing plays an important role in the in vitro testing process. **Objective:** This aims to determine the level of 50% ethanol extract of areca nut seeds that is safe for cell survival. **Methods:** The concentration of 50% ethanol extract of areca nut seeds that is safe in the cytotoxic test was followed by testing on RAW 264.7 cells (macrophages) stimulated by lipopolysaccharide (LPS) to simulate the inflammatory process. The test concentrations used were 31.25, 62.5, 125, and 250 micrograms/mL. **Results:** The research results have shown that all concentrations of the 50% ethanol extract of areca nut seeds still exhibit non-toxic properties, with cell viability values above 90%. **Conclusion:** Concentrations of 31.25, 62.5, 125, and 250 micrograms/mL can be continued for further in vitro testing using RAW 264.7 macrophage cells.

Keywords: Cytotoxicity; Ethanol 50%; Areca catechu; RAW 264.7 Macrophage Cells.

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INTRODUCTION

Indonesia is a country with the greatest biodiversity in the world, which has extraordinary potential for the discovery and development of new herbal medicines. The empirical use of medicinal plants has been part of people's culture for centuries, with advances in science and research technology[1–3].

One of the plants with significant development potential is the areca nut. This plant grows well in various regions of Indonesia, including Jambi Province, which is a significant producer of plantation commodities. Traditionally, areca nut has been used for a wide range of purposes, from traditional rituals to medicinal uses[4–6]. The results of phytochemical screening of the 50% ethanol extract of areca nut seeds have shown that areca nut contains bioactive compounds, alkaloids, tannins, and flavonoids[7]. These compounds are known to possess diverse pharmacological activities, including antioxidant, antibacterial, and anti-inflammatory agents, thus opening up opportunities for their development as a herbal product with therapeutic potential[8,9].

Inflammation is a fundamental biological response to various harmful stimuli, including pathogens, damaged cells, or tissue irritation. Although inflammation is a defense mechanism for the body, uncontrolled inflammation can trigger various chronic and degenerative diseases[10–13]. In vitro inflammation studies often use RAW 264.7 macrophage cells as a standard model. This is because when stimulated with lipopolysaccharide (LPS) from the cell walls of Gram-negative bacteria, these cells exhibit a characteristic inflammatory response, characterized by increased production of pro-inflammatory mediators (e.g., nitric oxide and cytokines). Therefore, this cellular model is highly relevant for screening compounds or extracts with potential as anti-inflammatory agents[14–17].

Before conducting an in vitro inflammatory efficacy test, a fundamental step that should not be ignored is the cytotoxicity test. This test aims to evaluate the effective dose of a substance at the cellular level and determine the concentration range that will be used to assess the viability or level of life of cells given a treatment. Therefore, determining the cytotoxicity profile is an important initial screening step in the development of natural ingredient-based pharmaceutical products, ensuring that the observed effects are pharmacological and still within the viability range that maintains cell survival during the treatment process[15].

Based on the above issues, researchers conducted a cytotoxic activity evaluation test of the 50% ethanol extract of areca nut seeds on RAW 264.7 macrophage cells. The results of this study will establish a non-toxic concentration range, which will then form the basis for further in vitro research, particularly in testing the anti-inflammatory potential of areca nut extract in the same cell model.

METHODS

Plant materials and extraction

The plant used in this research was the areca nut. The extraction process was carried out using the maceration method. The amount of simplicia used was 500 grams, which was macerated using a 50% ethanol solvent at a ratio of 1:10 for 2 × 24 hours, with 5 to 6 stirrings carried out at room temperature. The maceration results were then filtered using a filter to obtain the filtrate. Repeat or re-maceration was carried out two more times with a maceration process of 1 x 24 hours each. All collected macerate results were concentrated using a rotary evaporator at a temperature of 40 °C. The extract results were subjected to phytochemical screening.

Phytochemical screening

a. Preparation of the Main Solution
   0.5 g of thick extract was placed in a test tube, followed by 5 mL of distilled water and 5 mL of chloroform, and then shaken until the mixture was homogeneous. The solution was then allowed to stand until a water layer and a chloroform layer formed.
b. Alkaloid Test
   A small amount of chloroform was taken from the chloroform layer, then 10 ml of 0.05 N ammoniacal chloroform was added, followed by gentle stirring. 2 to 3 drops of 2N H_2SO_4 were added to the solution and shaken. Let it separate, and then take the acid solution, followed by two drops of Mayer's reagent. Positive samples containing alkaloids were indicated by the formation of white clumps or white mist
c. Flavonoid Test
   Place 1 to 2 drops of water on a dropper plate, then add Mg powder and HCl solution. Observe the changes that occur; if a red color forms, the sample was positive for flavonoids.
d. Saponin Test
   Take the water layer and place it in a test tube, then shake it vigorously. If the foam forms that lasts for approximately 15 minutes, the sample was positive for saponins.
e. Tannin Test
   Add 1 to 2 drops of water to the drip plate, then add $FeCl_3$. Observe the changes that occur. If a blue color forms, it indicates the sample contains tannin.
f. Triterpenoid Test
   A 0.5 mL chloroform layer was taken and transferred to another test tube. Next, 2 mL of concentrated sulfuric acid was added through the tube wall. If a brownish or violet ring forms at the solution border, the sample contains terpenoids, while a blue-green ring indicates steroids.

Cytotoxicity test on RAW 246.7 cells (macrophage cells) using MTT assay

MTT was a yellow dye used to clarify living cells that would be identified in the research process. The full name of the compound is 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. The succinate dehydrogenase enzyme in the mitochondria of living cells can reduce MTT to a water-insoluble, blue-purple formazan, which then precipitates inside the cell. The amount of formazan produced was proportional to the number of living cells, which a microplate reader detects at 560 nm, and then the number of living cells was inferred from the OD value.

The media was discarded, and 10 µL of MTT solution (5 mg/mL) was added to each well. The wells were then incubated for 4 hours at 37 °C, 5% CO_2 (without air bubbles), to reduce MTT to formazan. After the media was discarded, 150 µL of DMSO was added to each well to dissolve the formazan. The plate was then stirred at 25 °C for 10 minutes to dissolve the formazan completely. Finally, absorbance was measured at 560 nm using a microplate reader. Perform three independent repetitions for each experiment. Cells without drug treatment were designated as blank controls.

Cytotoxicity test: Count the number of viable cells using a hemocytometer. Cells were seeded at a density of 5 × 103 cells per well in 96-well plates. Then, incubate for 24 hours at 37 °C and 5% CO_2. The culture medium was replaced with a new one of 180 µL, and 20 µL of a 50% ethanol extract of areca nut was added to each well. The concentrations of the 50% ethanol extract samples used in this test were 31.25, 62.5, 125, and 250 ppm. Then, the cells were incubated for 24 hours. Afterward, 20 µL of

MTS was added to each well, and the cells were incubated for an additional 3 hours. Absorbance was measured using spectrophotometry, with readings at a wavelength of 560 nm (equation 1):

% Viability = (A sample corrected / A control negative corrected) X 100.....(1)

RESULTS

A 50% ethanol extract of areca nut seeds was obtained using the maceration method. This was a simple extraction method used to extract active compounds from natural ingredients. Maceration was carried out by soaking the material in a specific solvent at room temperature for a specified period. The advantage of the maceration extraction method was that the equipment used was simple and did not require heating, so the active ingredients in the plant are not easily decomposed. Several studies have shown that the maceration method can increase the levels of phenolic compounds. Phenolic compounds act as antioxidants, which are useful for healing various degenerative diseases[18,19].

After obtaining the extraction results, phytochemical screening tests were carried out. Phytochemical screening was a preliminary test (qualitative test) carried out to detect the presence of a group of natural chemical compounds found in plant extracts. The results of phytochemical screening of the 50% ethanol extract of areca nut seeds can be seen in Table 1. For the cytotocixity test results againts RAW 264.7 macrophage cells can be seen in Figure 1.

Table 1. Phytochemical Screening Test of 50% Ethanol Extract of Areca Nut Seeds.

Phytochemical Test	Results
Flavonoids	+
Alkaloids	+
Saponin	-
Tannin	+
Steroids	+
Phenol	+

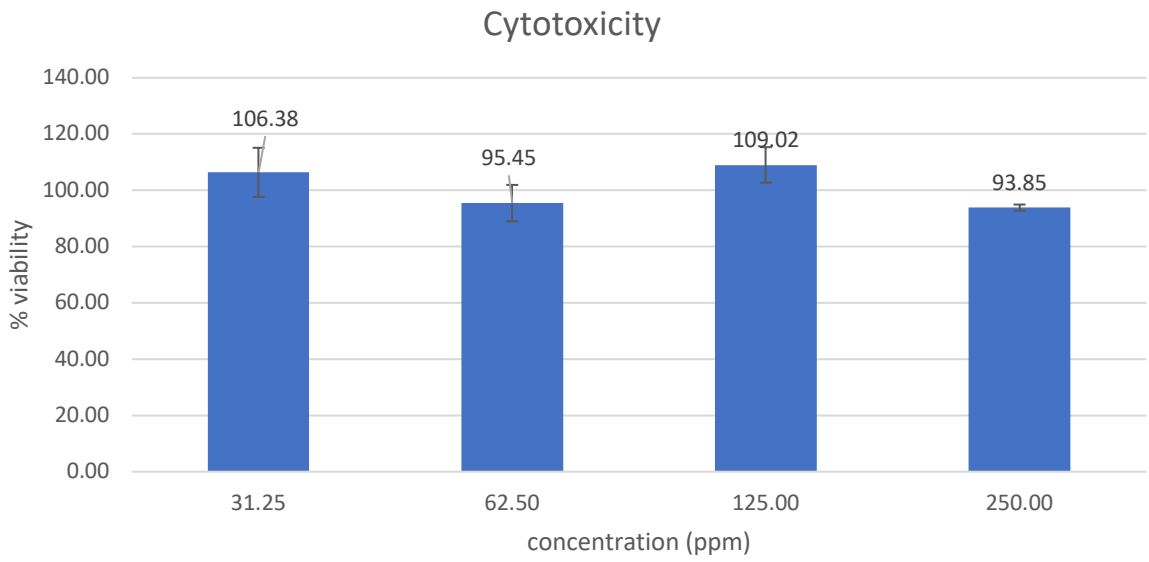

Figure 1. Cytotoxicity test results against RAW 264.7 macrophage cells

DISCUSSION

Inflammation is the immune system's response to various stimuli, including pathogens, toxins, or radiation. Although inflammation is a marker of the activation of the body's defense mechanisms, it can sometimes cause significant damage, triggering various diseases such as autoimmune diseases, diabetes, Alzheimer's disease, chronic kidney disease, and others. Therefore, monitoring the inflammatory response and promoting the appropriate use of cellular and organism-based inflammatory therapies was increasingly important[10,11,13].

Inflammatory conditions trigger immune cells, such as macrophages, monocytes, and neutrophils, to release large amounts of pro-inflammatory cytokines, including TNF-alpha, IL-1β, and IL-6. If the inflammatory response becomes excessive, it triggers the release of anti-inflammatory cytokines, such as IL-10, IL-4, and transforming growth factor-β. In addition, inflammation also involves the regulation of several other genetically encoded proteins, including the structural domain of nucleotide receptor binding oligomerization 2, MCP-1, iNOS, and others. Overall, the role and mechanism of these molecules in regulating the progression of inflammation were unclear. Therefore, it was important to build a foundation to be able to assess the effects of drugs or harmful agents on the anti-inflammatory function of cells and animals quickly, efficiently, and consistently[10,20].

Lipopolysaccharide compounds and RAW 264.7 cells act as mimics of the inflammatory response by releasing or increasing various inflammatory mediators. Therefore, RAW 264.7 can be used as the most common in vitro test cell model for screening anti-inflammatory active substances. Drug cytotoxicity testing was the first step to consider when assessing anti-inflammatory activity. Commonly used methods were MTT and CCK-8. The tools used to detect the expression levels of specific inflammation-related proteins in cells were ELISA and Western blotting. Both were working based on the principle of specific antigen-antibody binding to detect the presence and content of target proteins[21,22].

This research used five concentration variations for the cytotoxic test of RAW 264.7 cells, namely the control group, 50% ethanol extract of areca nut seeds with doses of 31.5 ppm, 62.5 ppm, 125 ppm, and 250 ppm. Observations of the five groups were conducted using an inverted microscope (Olympus CKX41-F32FL) at 40x magnification. Figure 1 shows the cytotoxic test results of a 50% ethanol extract of areca nut seeds, where all treatment concentrations yielded viability values above 90%. These findings provide important evidence that the concentration range tested was safe and suitable for use in further efficacy studies.

Cytotoxicity testing was a crucial indicator for evaluating in vitro assays. Cell viability or cell proliferation rate was an indicator of cell health. The purpose of cytotoxicity testing before in vitro anti-inflammatory testing was to determine a safe concentration of the sample, ensure that the cells remained viable, and verify that the observed anti-inflammatory effects were truly due to the biological activity of the compound and not from toxic effects on the cells. Agents can mechanically and chemically affect cell health and metabolism. These agents cause toxicity to cells through various mechanisms, including cell membrane damage, inhibition of protein synthesis, irreversible binding to receptors, inhibition of polydeoxynucleotide elongation, and interference with enzymatic reactions[23–25].

The content of secondary metabolite compounds in the 50% ethanol extract of areca nut seeds, such as flavonoids, alkaloids, tannins, and steroids, which have the potential as anti-inflammatory agents, is very worthy of further testing the potential of this extract using a therapeutic window of 31.25 to 250 ppm, which can work

pharmacologically without causing cell damage. This data supports efforts to develop standardized herbal products, or phytopharmaceuticals, from Indonesia's rich biodiversity, which is a crucial step in enhancing the added value of local natural resources and promoting independence in the health sector.

CONCLUSIONS

Based on the research results, it can be concluded that the 50% ethanol extract of areca nut (Areca catechu) did not show toxic properties against RAW 264.7 macrophage cells in the test concentration range of 31.25 µg/mL, 62.5 µg/mL, 125 µg/mL, and 250 µg/mL. This was supported by cell viability values, which consistently exceeded 90%. Therefore, all concentrations were deemed safe and can be recommended for further in vitro testing stages using RAW 264.7 macrophage cells.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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DECLARATION OF ARTIFICIAL INTELLIGENCE USE

The authors declare that this article was not created using Artificial Intelligence (AI) tools. All scientific content, findings, and data interpretation are entirely the work and responsibility of the authors.

REFERENCES

[1] Pratama AB. Khasiat Tanaman Obat Herbal. 2021.
[2] Contents C. Herbal therapeutic systems. 2013. https://doi.org/10.1016/b978-0-443-06992-5.00001-3.
[3] Elfahmi, Woerdenbag HJ, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. J Herb Med 2014;4. https://doi.org/10.1016/j.hermed.2014.01.002.
[4] Sun H, Yu W, Li H, Hu X, Wang X. Bioactive Components of Areca Nut: An Overview of Their Positive Impacts Targeting Different Organs. Nutrients 2024;16. https://doi.org/10.3390/nu16050695.
[5] Nelson Anthikat R, Michael A. Study on the areca nut for its antimicrobial properties. Journal of Young Pharmacists 2009;1. https://doi.org/10.4103/0975-1483.51874.
[6] Jam N, Hajimohammadi R, Gharbani P, Mehrizad A. Evaluation of Antibacterial Activity of Aqueous, Ethanolic and Methanolic Extracts of Areca Nut Fruit on Selected Bacteria. Biomed Res Int 2021;2021. https://doi.org/10.1155/2021/6663399.
[7] Humaryanto, Sani K F, Yuliawati Y, Rahman AO, Muhaimin M. Uji aktivitas antioksidan ekstrak etanol 50% biji pinang (areca catechu) dengan metode dpph (2,2-diphenyl-1-picryhydrazyl). Jurnal Ilmiah Manuntung 2023;9. https://doi.org/10.51352/jim.v9i1.659.
[8] Jing W, Xiaolan C, Yu C, Feng Q, Haifeng Y. Pharmacological effects and mechanisms of tannic acid. Biomedicine and Pharmacotherapy 2022;154:113561. https://doi.org/10.1016/j.biopha.2022.113561.

[9] Fajaryantie AR, Erwin, Pasaribu SP. Uji Fitokimia Dan Uji Toksisitas Ekstrak Kasar Daun, Batang Dan Kulit Batang Tanaman Durian (Durio Zibethinus Murray). Prosiding Seminar Nasional Kimia 2021 2021.
[10] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2018;9. https://doi.org/10.18632/oncotarget.23208.
[11] V. Stankov S. Definition of Inflammation, Causes of Inflammation and Possible Anti-inflammatory Strategies. Open Inflamm J 2012;5:1–9. https://doi.org/10.2174/1875041901205010001.
[12] Fehrenbacher JC, McCarson KE. Models of Inflammation: Carrageenan Air Pouch. Curr Protoc 2021;1. https://doi.org/10.1002/cpz1.183.
[13] Ashley NT, Weil ZM, Nelson RJ. Inflammation: Mechanisms, costs, and natural variation. Annu Rev Ecol Evol Syst 2012. https://doi.org/10.1146/annurev-ecolsys-040212-092530.
[14] Sagbo IJ, Otang-Mbeng W. Evaluation of the efficacy of ethanol leaf extract of Helichrysum petiolare Hilliard and B.L. Burtt against skin aging. Tropical Journal of Pharmaceutical Research 2020;19. https://doi.org/10.4314/tjpr.v19i12.22.
[15] Sabi EM, Mujamammi AH, Sumaily KM, Dahman LS Bin, Althafar ZM. Anti-inflammatory and Anti-hyperuricemic Effect of Ficus benghalensis Bark Extract in Raw 246.7 Cell Line. Indian Journal of Pharmaceutical Education and Research 2022;56. https://doi.org/10.5530/ijper.56.2.74.
[16] Varghese AA, Anton Smith A, Sreejith M. Phytochemical analysis, in-vitro antioxidant and cytotoxicity activities of Flaveria trinervia ethanolic extract. Biomedicine (India) 2023;43. https://doi.org/10.51248/.v43i3.2526.
[17] Jalava PI, Hirvonen MR, Sillanp M, Pennanen AS, Happo MS, Hillamo R, et al. Associations of urban air particulate composition with inflammatory and cytotoxic responses in RAW 246.7 cell line. Inhal Toxicol 2009;21. https://doi.org/10.1080/08958370802695710.
[18] Ćujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G, Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. Food Chem 2016;194. https://doi.org/10.1016/j.foodchem.2015.08.008.
[19] Velarde-Salcedo AJ, De León-Rodríguez A, Calva-Cruz OJ, Balderas-Hernández VE, De Anda Torres S, Barba-de la Rosa AP. Extraction of bioactive compounds from Rubus idaeus waste by maceration and supercritical fluids extraction: the recovery of high added-value compounds. Int J Food Sci Technol 2023;58. https://doi.org/10.1111/ijfs.16687.
[20] Tampa M, Mitran MI, Mitran CI, Sarbu MI, Matei C, Nicolae I, et al. Mediators of inflammation – A potential source of biomarkers in oral squamous cell carcinoma. J Immunol Res 2018;2018. https://doi.org/10.1155/2018/1061780.
[21] Wu X. Guidelines for anti-inflammatory assays in RAW264.7 cells. Food Safety and Health 2025;3:128–36. https://doi.org/10.1002/fsh3.12073.
[22] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105. https://doi.org/10.1161/hc0902.104353.
[23] Alvarez-Suarez JM, Carrillo-Perdomo E, Aller A, Giampieri F, Gasparrini M, González-Pérez L, et al. Anti-inflammatory effect of Capuli cherry against LPS-induced cytotoxic damage in RAW 264.7 macrophages. Food and Chemical Toxicology 2017;102. https://doi.org/10.1016/j.fct.2017.01.024.
[24] Sahlan M, Mahira KF, Pratami DK, Rizal R, Ansari MJ, Al-Anazi KM, et al. The cytotoxic and anti-inflammatory potential of Tetragonula sapiens propolis from Sulawesi on raw 264.7 cell lines. J King Saud Univ Sci 2021;33. https://doi.org/10.1016/j.jksus.2020.101314.
[25] Dewi IP, Wahyuni F, Aldi Y, Dachriyanus. Garcinia cowa roxb. Ethanol extract inhibits inflammation in lps-induced raw 264.7 macrophages. International Journal of Applied Pharmaceutics 2023;15. https://doi.org/10.22159/ijap.2023.v15s1.01.