

Metabolite profile of 25% ethanol extract of Areca nut (*Areca catechu*) which has potential as an anti-inflammatory

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Abstract

Background: The areca nut plant has been preclinically tested for its pharmacological effects. A 25% ethanol extract is one type of extract suspected to have pharmacological potential. **Objective:** The purpose of this research is to describe the results of secondary metabolite profiles using LC-MS, which hopefully can provide an overview for further development. **Methods:** Extraction using the maceration method, then phytochemical screening, and preparation with methanol and DCM, then injected as much as 5 µl into UPLC-QToF-MS/MS, then analyzed with Masslynx 4.1 and Chemspider software. **Results:** The results have shown that the extract contained 12 compounds, several of which have the potential to be anti-inflammatory. **Conclusion:** Literature studies have several compounds that have the potential to be anti-inflammatory.

Keywords: Metabolites; Extract; Ethanol 25%; Areca catechu.

Cite This Article

Humaryanto, Fairuz, Kasmadi, F. S., Hanina, & Perkasa, T. A. B. (2025). Metabolite profile of 25% ethanol extract of areca nut (*Areca catechu*) which has potential as an anti-inflammatory. *Proceedings Academic Universitas Jambi*, 1(2). 443-451.

Editor

I Made Dwi Mertha Adnyana, M.Ked.Trop.

Article info

Received: October 04, 2025. Revised: October 30, 2025. Accepted: November 09, 2025



INTRODUCTION

The areca nut plant is a plant that is widely cultivated in Southeast Asia. It is also an integral part of customs and herbal medicine across various cultures. One tradition that remains enduring today is the use of Areca nut for chewing betel quid in villages. History also demonstrates that communities have used Areca nut as a herbal remedy for various ailments [1–3]. In previous research, a 25% ethanol extract of areca nut seeds was found to contain a secondary metabolite chemical content comprising alkaloids, flavonoids, tannins, and steroids [4]. Bioactive compounds are active chemical compounds produced by organisms through secondary metabolite biosynthetic pathways. Secondary metabolites are divided into two categories: primary and secondary metabolites. Primary metabolites, such as fats, DNA, proteins, and carbohydrates, are used for the growth and survival of living organisms.

Secondary metabolites, on the other hand, are compounds that are not used for growth but are instead produced by organisms in specific amounts under specific conditions. Examples of the use of secondary metabolites include antibiotics, pigments, toxins, ecological competition effectors, symbiosis, pheromones, enzyme inhibitors, immunomodulating agents, agonist and antagonist receptors, antitumor agents, and others. For plants, secondary metabolite compounds function to deter predator attacks, compete for resources, prevent infections, regulate reproductive processes, and protect against ultraviolet rays [5,6].

Extraction methods play a crucial role in enhancing the potency of a plant. Ethanol extraction is the most commonly used technique due to its ability to dissolve a variety of secondary metabolites [7–10]. This is evidenced by screening the anti-inflammatory potential of various ethanol concentrations, which revealed that a 25% ethanol extract had good potential in inhibiting the 5-lipoxygenase enzyme compared to other variations. This solvent concentration was chosen to optimally isolate compounds with specific polarities, which are expected to exhibit potent biological activity, including anti-inflammatory agents [11]. Although a 25% ethanol extract of areca nut seeds shows promising potential, the profile of its suspected compounds has not been comprehensively identified. Therefore, this research aims to characterize the secondary metabolite profile of this extract using Liquid Chromatography-Mass Spectrometry (LC-MS) technology. It is hoped that these identification results will serve as a basis for further research and development.

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 25% 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 H₂SO₄ 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 FeCl₃. 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.

Metabolite profiling using LCMS-MS

A total of 0.5 grams of 25% ethanol extract of areca nut seeds was placed in a 10 mL volumetric flask, then methanol was added. Sonicate for 30 minutes. Then, it was filtered using a 0.22 µm PTFE membrane filter. The filtered sample was injected into a Waters Acquity Tandem Xevo G2S QTOF UHPLC system. Analysis using LCMS/MS equipped with a binary pump and a Quadropole Time of Flight (MS-QTOF) mass spectrometer detector with Electro Spray ionization (ESI) mode. Sample analysis using MS-QTOF with positive and negative ionization modes, acquisition range 50-1200 Da, non-polar column C18 XTerra® RP 18 3.5 µm, column temperature 40 °C. The eluent used was 0.1% formic acid in acetonitrile (A) and 0.1% acid in distilled water (B) with an isocratic elution system. Isocratic elution system was carried out at 0-1 min 95: 5 ratio, 0 min linear gradient elution A from 95% to 5%, 6-7 min isocratic elution 0: 100 ratio, 6-7 min linear gradient elution solvent A from 0% to 100%, 7.5-9 min isocratic elution 95: 5 ratios, to 7.5-9 min linear gradient elution solvent A from 95% to 5%. The eluent was set at a flow rate of 0.6 mL/min and an injection volume of 10 µL.

Data processing and compound identification

Raw data obtained from LCMS/MS results were processed and analyzed using the Masslynx 4.1 software suite. Metabolite identification was performed by comparing experimental mass spectral data with the comprehensive database, ChemSpider. This process enabled the identification of compounds suspected to be present in a 25% ethanol extract of areca nut (*Areca catechu*).

RESULTS

A 25% ethanol extract of areca nut seeds was obtained using the maceration method. This is a simple extraction method used to extract active compounds from natural ingredients. Maceration was performed by soaking the material in a specific solvent at room temperature for a specified period. The advantage of maceration extraction is

that the equipment used is simple and does 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 [7,12–14].

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 25% ethanol extract of areca nut seeds can be seen in Table 1.

Table 1. Phytochemical screening results.

Phytochemical Tests	Results
Flavonoids	+
Alkaloids	+
Saponins	-
Tannin	+
Steroids	+
Phenol	+

DISCUSSION

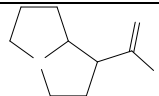
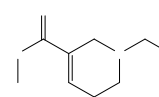
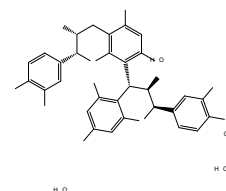
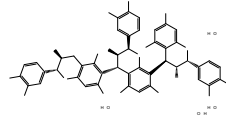

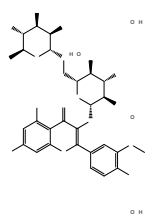
Bioactive compounds are compounds found in animals and plants. These compounds offer numerous benefits to human life, including acting as antioxidants, anti-inflammatory agents, antibacterial agents, and anticancer agents [15,16]. LCMS stands for Liquid Chromatography-Mass Spectrometry, and in Indonesian, it is known as Kromatografi Cair-Spektrofotometri Massa. This tool is used to detect suspected compounds by separating them in a mixture based on their polarity, size, or interaction with the stationary phase. Compounds are identified by determining the molecular mass of the separated compounds [17–19].

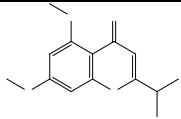
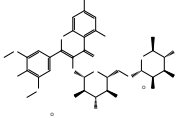
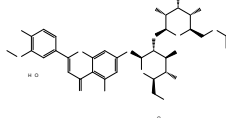
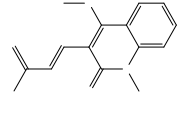
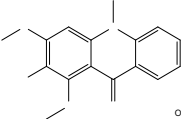
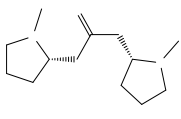
The LCMS chromatogram results revealed 12 different suspected compounds in the 25% ethanol extract of areca nut. These molecules were identified, ranging from simple phenols to secondary metabolites (alkaloids, flavonoids, tannins, and steroids). The variation in the suspected chemical content will serve as the basic data for identification or screening on official websites regarding its potential as an anti-inflammatory agent (PubMed and NCBI). Quantitative data have shown the relative percentage of each compound, allowing an assessment of which molecules were most likely to contribute to the sample's overall biological activity, indicating its potential function as a natural anti-inflammatory agent.

Inflammation is the body's natural immune system response to injury or infection. Healing inflammation is part of the body's complex healing process [20,21]. This process will produce typical inflammatory symptoms such as redness, swelling, and infection. The secondary metabolite compound content in the 25% ethanol extract of areca nut seeds is expected to provide the basis for identifying possible compounds through LC-MS examination.

Based on data from the 12 suspected compounds in the chromatogram above, two dominant groups of compounds were identified: alkaloids and flavonoids. These two compounds play a role in inhibiting inflammatory mediators through various mechanisms. Firstly, they act as inhibitors of the cyclooxygenase and lipoxygenase enzymes, thereby reducing the levels of prostaglandins and leukotrienes, which are inflammatory mediators. This effect can lead to a reduction in pain, redness, and

Table 2. Profile of secondary metabolite compounds as a result of examination using LC-MS.

No	RT (min)	m/z measured	m/z fragment	MW	Molecular Formula	% Composition	Prediction Compound Name	Compound Group	Structure
1	3.10	172.0971	156.1020; 140.0707	171.19	C ₈ H ₁₃ NO ₃	18.29	6-hydroxy-hexahydro-1h-pyrrolizine-1-carboxylic acid	Alkaloid	
2	4.16	170.1177	156.1021; 142.0863	169.22	C ₉ H ₁₅ NO ₂	10.46	Ethyl 1-methyl-5,6-dihydro-2h-pyridine-3-carboxylate	Alkaloid	
3	4.40	579.1506	367.1505; 349.1396	578.52	C ₃₀ H ₂₆ O ₁₂	22.58	Procyanidin B2	Polyfenol	
4	4.60	867.2100	579.1506; 391.2079; 291.0880	866.77	C ₄₅ H ₃₈ O ₁₈	1.65	Arecatannin B1	Tannins	
5	4.70	291.0866	231.1700; 156.1017; 139.0391	290.27	C ₁₅ H ₁₄ O ₆	13.57	Catechol	flavonoids	
6	5.94	625.1749	401.1594; 317.0648; 279.1700	624.55	C ₂₈ H ₃₂ O ₁₆	4.53	Narcissin	Flavonoids	

7	6.24	249.1117	211.0957; 183.0649; 156.1013	248.27	$C_{14}H_{16}O_4$	3.42	2-isopropyl-5,7-dimethoxychromen-4-one	Flavonoids	
8	7.78	669.2010	639.1909; 361.0916; 331.0814	668.60	$C_{30}H_{36}O_{17}$	4.12	Syringetin 3-O-rutinoside	Flavonoids	
9	8.26	667.1866	523.1461; 361.0924; 319.1159	666.58	$C_{30}H_{34}O_{17}$	2.14	Stachyspinoside	Flavonoids	
10	8.84	256.1333	238.1229; 156.1015	255.31	$C_{16}H_{17}NO_2$	10.59	4-methoxy-1-methyl-3-(3-methylbuta-1,3-dien-1-yl)quinolin-2-one	Alkaloids	
11	8.97	270.1126	256.1335; 156.1018	269.30	$C_{16}H_{15}NO_3$	1.42	1,3-dimethoxy-10-methylacridin-9-one	Alkaloids	
12	10.48	225.1962	156.1017; 123.0787	224.34	$C_{13}H_{24}N_2O$	7.23	Cuscohygrine	Alkaloids	

edema in inflamed tissue. The second mechanism was to suppress the production of inflammatory cytokines (TNF- α , IL-6, IL1 β) produced by macrophages or other immune cells. The third mechanism works by inhibiting the NF- κ B and MAPK signaling pathways, which play a crucial role in regulating the transcription of various inflammatory mediators. The fourth mechanism was through antioxidant activity and free radical scavenging. Alkaloids and flavonoids were potent antioxidants that can reduce oxidative stress, which damages tissue and thereby halts the oxidative-inflammatory cycle. Lastly, the working mechanism involves the activation of immune cells, including macrophages and neutrophils [22–25]. The tannin compound content also works as a stabilizer of lysosomal membranes and capillary permeability in inflamed tissue, thereby inhibiting the occurrence of edema in the inflamed area. These compounds work together to combat inflammation in the body [26,27].

CONCLUSIONS

The results of screening suspected secondary metabolite compounds using LC-MS from a 25% ethanol extract of areca nut seeds revealed 12 suspected compounds. Of these 12 compounds, four groups of secondary metabolites were found: alkaloids, flavonoids, tannins, and polyphenols.

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.

FUNDING

The author would like to thank the Faculty of Medicine and Health Sciences, University of Jambi, for the research grant fund applied for from the Faculty's PNPB funds, with contract number 360/UN21.11/PT01.05/SPK/2025.

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.

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