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Phytochemical Composition of 70% Ethanol Extract of Noni Fruit (*Morindacitrifolia*L.) Using Ultrasound-Assisted Extraction Method and LC-MS Q-ToF

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ABSTRACT

Noni (*Morindacitrifolia*L.) is a traditional medicinal plant that is widely used in the treatment of various degenerative diseases. However, scientific data regarding the composition of its bioactive compounds are still limited, especially those analyzed using a high-precision instrument approach. This study aims to identify the phytochemical composition in 70% ethanol extract of noni fruit using Liquid Chromatography-Mass Spectrometry (LC-MS) method. Extraction was performed by Ultrasound-Assisted Extraction (UAE) method and compound analysis using Q-ToF type LC-MS instrument with positive ionization mode. The results showed that noni extract contained 28 compounds, with 13 compounds successfully identified consisting of 7 alkaloids, 3 glycosides, 1 phenolic, 1 amino acid, and 1 aminoglycoside. The major compound identified was Methyl (5R)-2,3,4-tris-O-(methoxymethyl)-5-{2-[(2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-piperidinyl]ethyl}-α-D-xylopyranoside, belonging to the glycoside group with a molecular weight of 485.25 g/mol. These results indicate that noni fruit contains diverse secondary metabolite compounds with the dominance of alkaloid and glycoside compounds. This study contributes to providing primary data based on LC-MS technology on the chemical composition of Indonesian natural ingredients and can be a scientific basis for the development of medicinal plant-based pharmaceutical preparations with noni active ingredients.

Keywords: *Morindacitrifolia*,UAE, LC-MS, *phytochemical composition*.

Introduction

Indonesia is a megabiodiversity country with an abundance of biological resources. Various endemic flora are widespread throughout the archipelago and are widely used as traditional medicinal plants. The use of natural materials for medicine has become a cultural heritage inherent in the lives of Indonesian people. It is estimated that more than 1,000 plant species have been used as medicine by various ethnic groups in Indonesia, both in fresh form, simplisia, and galenic preparations(Kementerian Kesehatan Republik Indonesia, 2016). However, most of these utilizations are still traditional and not fully supported by strong scientific data.

One plant that is very popular in traditional medicine is noni (*Morindacitrifolia*L.). This plant is widely known in Southeast Asia and the Pacific region, including Indonesia. In traditional medicine practices, noni is often used to treat hypertension, indigestion, joint pain, as well as to help increase endurance. Various preparations such as juice, capsules, and noni-based herbal tea have been widely consumed, not only in Indonesia, but also globally(Abou Assi et al., 2017). A number of reports mention that noni fruit contains bioactive compounds that have potential as antioxidants, anticancer, antihypertensive, and immunomodulators(Lee et al., 2020). However, in-depth chemical identification of noni extracts from local Indonesian sources is still very limited.

In terms of phytochemistry, *Morindacitrifolia*is known to contain various secondary metabolites such as alkaloids, iridoid glycosides, flavonoids, coumarins, and phenolic compounds(Hou et al., 2025). These compounds play an important role in various biological activities that form the basis of the plant's traditional efficacy claims. However, the chemical profile of medicinal plants is highly influenced by factors such as geographical origin, harvesting season, type of solvent, and the extraction and analysis methods used(Dewi et al., 2022). Therefore, to ensure the consistency and effectiveness of natural ingredient-based preparations, a scientific approach is needed that allows for comprehensive and standardized chemical characterization.

One of the important stages in the scientific validation of natural materials is the evaluation of chemical composition through modern analytical approaches. Conventional phytochemical analyses such as Thin Layer Chromatography (KLT) or UV-Vis spectrophotometry are only capable of detecting general groups of compounds, not providing specific structural information. Technologies such as Liquid Chromatography-Mass Spectrometry (LC-MS) are becoming superior options as they are able to detect and identify compounds with very high sensitivity and accuracy, even in small amounts and complex matrices(Malm et al., 2021).

LC-MS, especially the Q-ToF type, is widely used in metabolomics studies due to its ability to generate high-precision mass data, identify minor metabolites, and map compound fragmentation. With this method, it is possible to obtain a chemical fingerprint of a plant that is useful for standardization, quality control, and mapping compound variations between locations or varieties (Gautam et al., 2023). LC-MS-based studies on medicinal plants are also an important approach in the development of evidence-based phytopharmaceuticals. A number of previous studies have applied LC-MS to the analysis of noni fruit. Nuengchamnong et al. (2023) used LC-MS/MS and successfully identified more than 50 compounds in noni fermentation products, including iridoid glycosides and scopoletin(Nuengchamnong et al., 2023). Dewi et al. (2022) analyzed several noni extracts from various locations in Indonesia and found significant differences in metabolite profiles, signaling the importance of modern analytical approaches to ensure the quality of raw materials. However, until now there have not been many studies that specifically profile the phytochemical composition of 70% ethanol extracts of noni fruit from Indonesia using Q-ToF type

LC-MS. In fact, 70% ethanol solvent is an ideal solvent that is widely used in the herbal industry because of its ability to extract polar and semi-polar compounds efficiently and safely for human consumption(Plaskova&Mlcek, 2023). The choice of solvent and extraction method greatly affects the success of the analysis. One of the modern extraction methods that is now increasingly used is Ultrasound-Assisted Extraction (UAE). UAE uses high-frequency ultrasonic waves to accelerate the separation of compounds from the plant cell matrix via cavitation mechanisms. This method is proven to increase extraction efficiency, decrease solvent usage, and maintain the stability of temperature-sensitive compounds(Gadjalova&Mihaylova, 2019).

The use of UAE in the extraction of medicinal plants, including noni, has shown extraction results that are richer in active compounds than conventional methods such as maceration or reflux (Gadjalova&Mihaylova, 2019). The advantages of UAE lie not only in its efficiency, but also in conformity with the principles of green chemistry. Extraction using low temperatures minimizes the degradation of thermolabile compounds such as flavonoids and glycosides, and is more environmentally friendly due to reduced solvent and energy requirements. Therefore, UAE is very suitable for the extraction of bioactive compounds from noni fruit, especially when combined with high-precision analytical techniques such as LC-MS.

The importance of metabolite mapping in natural materials has also become a concern on an international scale. Organizations such as WHO and ASEAN have encouraged the standardization of herbal raw materials through an evidence-based herbal medicine approach. In this context, analytical methods such as LC-MS have a crucial position because they can provide in-depth information about the presence and amount of active compounds, while supporting the validation process of safety and effectiveness of preparations(Organizacion Mundial de la Salud., 2003).

Furthermore, LC-MS-based analytical approaches are now the basis for metabolomics studies, which is a comprehensive analysis of all small metabolite compounds in biological systems. Metabolomics in medicinal plants helps to understand the chemical variation, the relationship between structure and bioactivity, and the role of environment on the content of active compounds. LC-MS has been widely used in metabolomics of plants such as Curcuma longa, Andrographis paniculata, and also *Morindacitrifolia*(Gautam et al., 2023).

One important application of compound mapping is in the regulation and registration of herbal products, both in national and global contexts. In many countries, natural ingredient-based products must now include analytical data on active compounds and metabolite profiles to support product safety, effectiveness and stability. Thus, the results of LC-MS-based analysis are instrumental in strengthening the scientific position of natural products from Indonesia in the international market. Indonesia as a country that is very rich in medicinal plants has a great opportunity to contribute to the global phytopharmaceutical industry, provided that it is supported by strong scientific data. With the right approach, Indonesian natural ingredients such as noni can be developed into preparations that are not only safe and effective, but also globally competitive (Silvia et al., 2024).

Based on this background, this study aims to identify chemical compounds in 70% ethanol extract of noni fruit (*Morindacitrifolia*L.) using LC-MS method. With this approach, it is expected to obtain primary data on the phytochemical profile of noni from Indonesia that can be the basis for the development of natural ingredient-based products, quality validation, and further exploration of biological activity.

Methodology

This research is a descriptive laboratory study to profile the phytochemical composition contained in the extract with a non-targeted screening approach. The process of making extracts was carried out at the Pharmaceutical Biology Laboratory of InstitutIlmu Kesehatan Bhakti Wiyata Kediri, while LC-MS analysiswas carried out at the Jakarta Forensic Laboratory Center.

Materials and Tools

The materials used in this study were noni fruit simplisia powder obtained from UPT Laboratorium Herbal Materia Medica Batu (Malang, East Java), ethanol 96% (technical grade, Brataco), methanol (hypergrade for UPLC, Merck), formic acid (ultrapure, Merck), acetonitrile (hypergrade for UPLC, Merck), and water injection 0.05% (for LC-MS use).

Tools used were analytical balance (Mettler Toledo), sonicator (Ultrasonic Cleaner 8890, 40 kHz, Crest Ultrasonics), rotary evaporator (Buchi R-300, Switzerland), water bath (Memmert), LC: ACQUITY UPLC® H-Class system (Waters, USA), MS: Xevo G2-S QTof (Waters, USA), Column: C18 (1.8 µm, 2.1 × 100 mm, ACQUITY UPLC® HSS, Waters, USA), Analysis software: MassLynx v4.1 (Waters Corp.)

Research Procedure

Sample preparation

Ripe noni fruit was washed, cut into small pieces, then dried in an oven at 45°C for 3-5 days until the moisture content was <10%. The dried material was then pulverized into dry powder using a blender, then filtered through a mesh no. 40 sieve.

Extraction

A total of 500 g of simplisia powder was extracted using the Ultrasound-Assisted Extraction (UAE) method with 70% ethanol solvent in a ratio of 1:10 (b/v). The extraction process was carried out in a sonicator with a frequency of 40 kHz for 30 minutes at a temperature of <50°C. The extracted solution was filtered using Whatman No. 1 filter paper. The filtrate was then evaporated using a rotary evaporator at 60°C and 70 rpm until a thick extract was obtained.

LC-MS Analysis

The extract was dried and then redissolved in methanol p.a. for analysis using LC-MS QToF with positive ionization mode (ESI+). The injection volume was 5 µL, the flow rate was 0.3 mL/min, and the column temperature was kept at 40°C. The mobile phase used was solvent A which is a mixture of water with 0.1% formic acid and solvent B which is a mixture of methanol with 0.1% formic acid. Elution was carried out in a gradient for 30 minutes from 5% B to95% B. The compounds were separated

using a C_{18} column with a column length of 100 mm, internal diameter of 2.1 mm and particle size of 1.8 µm.

Data Analysis Methods

Spectral data were analyzed using MassLynx v4.1 software and checked against the internal compound library (UNIFI™ database). Compound identification was performed based on molecular mass, retention time, and fragmentation pattern. Major compounds were determined based on the highest peak intensity in the total ion current (TIC) chromatogram. The identification results were then classified based on chemical groups (alkaloids, glycosides, phenolics, etc.).

Result and Discussion

The noni plant (*Morindacitrifolia*L.) used in this study was determined at the Unit Pelaksana Teknis (UPT) Materia Medica Batu. Organoleptic examination data from simplisia and noni fruit extract were reviewed from various characteristics such as shape, color, odor and taste. Organoleptic results of simplisia and noni fruit extract are shown in table 1 below

Table 1. Results of Organoleptic Examination of Noni Fruit

Characteristics	Simplisia	Extract
Form	Powder	Viscous
Color	Brownish green	Dark brown
Odor	Distinctive	Distinctive
Taste	Slightly bitter	Tartness

Noni fruit extraction was carried out using the Ultrasound-Assisted Extraction (UAE) method with 70% ethanol solvent. The extraction result obtained was 196 grams derived from 500 grams of simplisia powder, resulting in a yield of 39.2% b/w. This yield value shows a good extraction efficiency and meets the requirements, which is not less than 10.1% (Anonim, 2017).

The extraction process using the Ultrasonic-Assisted Extraction (UAE) method at 40 kHz for 30 min at a temperature of less than 50 ℃in this study has an important role in the successful release of active compounds from the plant matrix. The processes occurring during sonication are able to increase the permeability of the cell membrane, improve the penetration of the solvent into the plant matrix and accelerate the diffusion of the compounds into the solvent. This is why ethanol-soluble compounds, such as glycosides and alkaloids, are successfully extracted in relatively large quantities(Riwanti et al., 2020). In addition, the low temperature during UAE (below 50°C) allows thermolabile compounds such as phenolics and flavonoids that are generally easily degraded at high temperatures to be extracted well [2].

The solvent used in the extraction process is 70% ethanol. This solvent has a polarity balance that allows optimal withdrawal of polar and semi-polar compounds. Polar glycoside compounds and some semipolar alkaloids have a high affinity for 70% ethanol explaining the dominance of these two groups in the profile of detected

compounds. The study before showed that 70% ethanol produced higher total flavonoid levels than 50% or 96% ethanol. This indicates that the solvent concentration has a significant effect on the composition of the extract (Malm et al., 2021; Riwanti et al., 2020)

In this study, the analysis of the compound content of 70% ethanol extract of noni fruit used methanol p.a. Methanol p.a was used as a mobile phase in LC-MS measurements because methanol p.a is universal in dissolving organic compounds both polar and non-polar (Lutfia et al., 2020). The stationary phase used is an octadecylsilane column (ODS or C18). Because the advantage of octadecyl silica as a stationary phase is that it is able to separate compounds ranging from low, medium, to high polarity (Maslakhah et al., 2019).

Furthermore, the samples were analyzed using Liquid Chromatrography-Mass Spectrometry (LC-MS) connected to a Quadrupole Time-of-Flight (Q-ToF) mass spectrometer detector and equipped with a positive mode Electrospray Ionization (ESI) ionization source. The use of QToF LC-MS is due to its ability to provide accurate analysis with high resolution. ESI+ mode is used because it can detect more compounds (Malm et al., 2021)

Based on the chromatogram in Figure 1, the results of phytochemical composition using Liquid Chromatrography-Mass Spectrometry (LC-MS) show that 70% ethanol extract of noni fruit contains 28 compounds, 13 compounds were successfully identified, as shown in Table 2. Identification is done by looking at the height of the chromatogram peak, the peak shows as a component or compound identified at a certain retention time. From the weight and molecular formula obtained, it is continued by looking for compounds that have the same molecular formula found in noni plants using pubchem, chemspider, NIST and massbank websites.

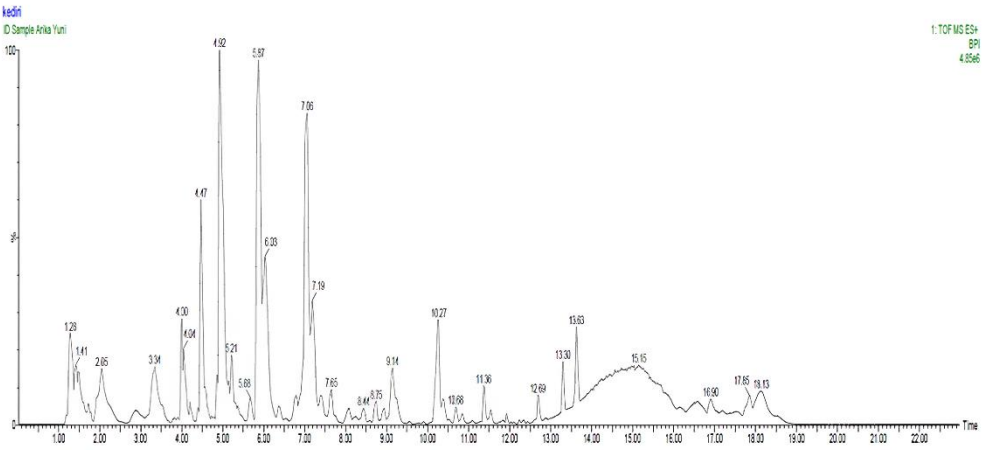

Figure 1. LC-MS Chromatogram of 70% Ethanol Extract of Noni Fruit

Major compounds are compounds whose content is higher than the content of other compounds contained in the extract (Ma'arif et al., 2019). The major compound in this study was taken from the highest peak height of the chromatogram, namely at a retention time of 4.923 but the retention time with the highest spectra of the

compound was not detected. Similarly, the retention time of 5.872 with the highest spectra was not detected, this is likely because the compound is not easily ionized so it is not detected by the LC-MS instrument and is not registered in the database. But at retention time 7.061 with the highest spectra obtained molecular weight 485.2485 g/mol and molecular formula C20H39NO12 detected the compound is Methyl (5R)-2,3,4-tris-O-(methoxymethyl)5{2[(2R,3R,4R,5R,6R)3,4,5trihydroxy6(hydroxymethyl)-2-piperidinyl]ethyl}-α-D-xylopyranoside, including the glycoside group. Almost most of the glycoside compounds appeared in this study, this is because glycosides are polar compounds and generally the solvents used will attract more compounds with the same polarity.

Table 2. Phytochemical Composition Results Using LC-MS Method

No	Rt	Molecular Weight	Formula	Name of Compound	Group
1	1.282	159.0899	$C_3H_9N_7O$	2-(N-Carbamimidoylcarbamimidoyl) hydrazine carboxamide	Alkaloids
2	1.282	197.046	$C_4H_{11}N_3O_4S$	1-Cyclopropyl guanidine sulfate (1:1)	Alkaloids
3	1.407	143.0951	$C_3H_9N_7$	N-Carbamimidoylimidodicarbonimidic diamide	Alkaloids
4	2.048	275.1381	$C_8H_{17}N_7O_4$	2-{[(Dimethylcarbamoyl)amino]acetyl}-N-(2-hydrazino-2-oxoethyl)hydrazinecarboxamide	Peptide
5	2.048	293.1506	$C_{12}H_{23}NO_7$	1,2-di-O-methyl-4-[(2R)-2,4-dihydro butyramido]-4,6-dideoxy-α-D-mannopyranoside	Glycosides
6	3.341	152.1317	C9H16N2	1,8-Diazabicyclo [5.4.0]undec-7-ene	Alkaloids
7	4.002	449.1533	$C_{18}H_{27}NO_{12}$	Ethyl [({[(2S,3R,4R,5R,6R)-4,5-diacetoxy-6-(acetoxymethyl)-3-hydroxytetrahydro-2H-pyran-2-	Glikosida

				yl]oxy}acetyl)amino]acetat e	
8	5.211	192.0437	$C_{10}H_8O_4$	6-Methoxy-7-hydroxycoumarin	Phenolic
9	7.061	485.2485	$C_{20}H_{39}NO_{12}$	Methyl (5R)-2,3,4-tris-O-(methoxy methyl)-5-{2-[(2R,3R,4R, 5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-piperidinyl]ethyl}-α-D-xylopyranoside	Glycosides
10	8.438	170.1317	$C_6H_{14}N_6$	1,4-Piperazine dicarboximidamide	Alkaloids
11	8.438	229.206	$C_9H_{23}N_7$	2-[3-({4-[(Diamino methylene)amino]butyl}a mino)propyl]guanidine	Alkaloids
12	10.681	354.2524	$C_{15}H_{30}N_8O_2$	4-(6-Amino-2-{[3-(dimethylammonio)propyl] amino}-5-nitro-4-pyrimidinyl)-1-ethylpiperazin-1-ium	Alkaloids
13	11.363	611.3522	$C_{24}H_{49}N_7O_{11}$	(2S)-4-Amino-N-{(1R,2S,3R,4R,5S)-5-amino-3-[(4-amino-4,6-dideoxy-β-D-glucopyranosyl) oxy]-2-(2-amino ethoxy)-4-[(2,6-diamino-2,6-dideoxy-α-D-glucopyranosyl)oxy]cycloh exyl}-2-hydroxybutanamide	Aminoglyco sides

The distribution of compound groups is presented in Figure 2. It can be seen that alkaloids are the dominant group in the extract, which supports the initial hypothesis that nitrogen compounds such as alkaloids are abundant in noni fruit. These results support the research objective to comprehensively explore the chemical profile of noni fruit, while providing a basis for further bioactivity evaluation. The fact that the major compounds were glycosides and important phenolic compounds such as scopoletin were also successfully detected, indicating that the LC-MS method has the capability to detect major and minor compounds well.

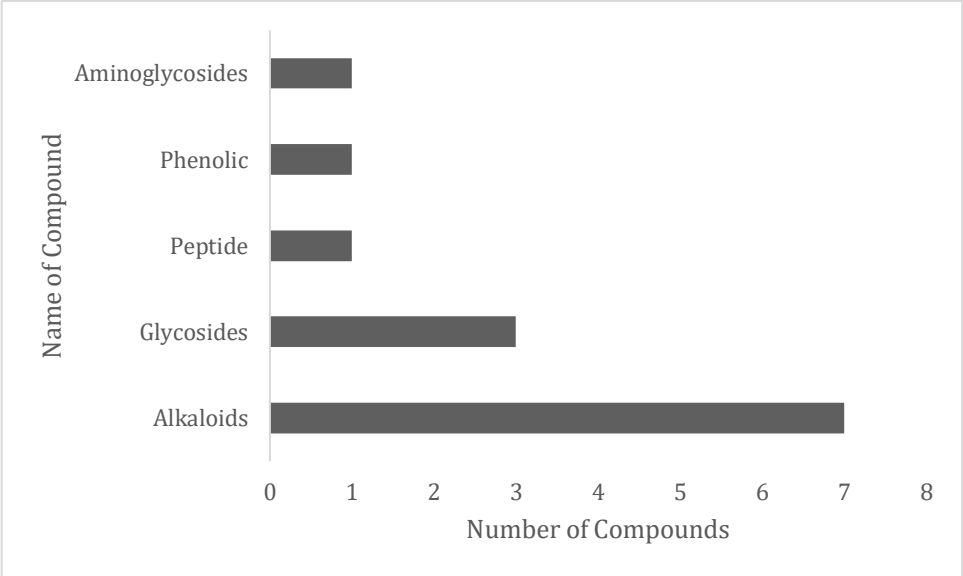

Figure 2. Distribution of identified compound groups

The presence of large amounts of alkaloids correlates with the typical pharmacological activities of Morindacitrifolia. Alkaloids are known to have antihypertensive, antimicrobial, and central nervous system stimulant effects(Bhambhani et al., 2021). Meanwhile, glycosides are known for their role in anti-inflammatory and antioxidant activities. The identification of compounds such as scopoletin, although minor has the potential to act as a drugcandidate in the treatment of cancer, liver disease, diabetes, neurodegenerativedisease, and mental disorders(Gao et al., 2024). This suggests that it is not only the dominant compounds that need attention, but also the minor components that are potentially synergistic in biological activity.

This finding is in line with the study of Nuengchamnong et al., (2023)who also used LC-MS/MS on noni fermentation products and identified glycoside and iridoid compounds as the main groups. However, this study adds an important contribution in the form of primary data based on non-fermented 70% ethanol extracts extracted through UAE, thus providing a different picture of the metabolite profile compared to previous approaches.

The results of this study provide a strong scientific basis for the development of natural medicine with noni extract as the active ingredient. The compound profile information obtained can be used for standardization of raw materials, quality testing, and selection of target compounds in product development. This is in line with the direction of regulations in Indonesia and globally that have begun to require chemical profile data of herbal materials for registration and quality control purposes (BPOM RI, 2021) In the long run, the use of LC-MS methods as part of quality control not only increases scientific confidence in herbal preparations, but also opens up opportunities for exports of products based on local Indonesian natural ingredients.

Conclusion

This study successfully identified 13 major compounds from a total of 28 LC-MS peaks in 70% ethanol extract of noni fruit using Ultrasound-Assisted Extraction and LC-MS Q-ToF methods. The dominant compound group was alkaloids, with the major compound detected at m/z 485.25, namely Methyl (5R)-2,3,4-tris-O-(methoxymethyl)-5-{2-[(2R,3R,4R,5R,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)-2-piperidinyl]ethyl}-α-D-xylopyranoside. The main limitation of this research is the absence of quantitative validation and full identification of all detected peaks. Nevertheless, LC-MS Q-ToF provides high mass accuracy (typically <2 ppm) and sensitivity, making it reliable for complex phytochemical analysis (Allen & McWhinney, 2019).These findings provide important primary data related to the phytochemical profile of *Morindacitrifolia*from Indonesia, which can be used to support the development of phytopharmaceutical preparations and standardization of natural raw materials.

Declaration of Competing Interest

We have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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