UNIVERSITAS NAHDLATUL ULAMA SURABAYA
LEMBAGA PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
Kampus A Wonokromo : Jl. SMEA No.57 Tlp. 031-8291920, 8284508 Fax. 031-8298582 – Surabaya 60243
Kampus B RSIJemursari : Jl. Jemursari NO.51-57 Tlp. 031-8479070 Fax. 031-8433670 – Surabaya 60237
Website : unusa.ac.id Email: info@unusa.ac.id

SURAT KETERANGAN

Nomor: 2022/UNUSA-LPPM/Adm.I/IX/2022

Lembaga Penelitian dan Pengabdin Kepada Masyarakat (LPPM) Universitas Nahdlatul Ulama Surabaya menerangkan telah selesai melakukan pemeriksaan duplikasi dengan membandingkan artikel-artikel lain menggunakan perangkat lunak **Turnitin** pada tanggal 28 September 2022.

Judul	: Phytochemical screening, antioxidant activity and cytotoxicity assay from noni juice and fermented noni (Morinda citrifolia L.)
Penulis	: Handayani, Devyana Dyah Wulandari, Devyani Diah Wulansari
No. Pemeriksaan	: 2022.09.26.820

Dengan Hasil sebagai Berikut:

Tingkat Kesamaan diseluruh artikel (*Similarity Index*) yaitu 11%

Demikian surat keterangan ini dibuat untuk digunakan sebagaimana mestinya.

Surabaya, 30 September 2022

Ketua LPPM

Achmad Syafiuddin, Ph.D
NPP: 20071300

Phytochemical screening, antioxidant activity and cytotoxicity assay from noni juice and fermented noni (Morinda citrifolia L.)

by HandayaniHandayani

Submission date: 28-Sep-2022 03:36PM (UTC+0700)
Submission ID: 1911125305
File name: 025_-_3534_-_Handayani_-_Galley.pdf (250.61K)
Word count: 2659
Character count: 14717

ORIGINAL ARTICLE

Bali Medical Journal (*Bali MedJ*) 2022, Volume 11, Number 3: 1168-1171
P-ISSN.2089-1180, E-ISSN: 2302-2914

Phytochemical screening, antioxidant activity and cytotoxicity assay from noni juice and fermented noni (*Morinda citrifolia L.*)

Handayani[1*], Devyana Dyah Wulandari[2], Devyani Diah Wulansari[3]

[1]Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia;
[2]Medical Laboratory Technologyst, Faculty of Health, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia;
[3]Faculty of Pharmacy Universitas Surabaya, Surabaya, Indonesia;

*Corresponding author:
Handayani;
Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Surabaya, Indonesia;
dr.handayani@unusa.ac.id

Received: 2022-07-16
Accepted: 2022-08-11
Published: 2022-09-13

ABSTRACT

Introduction: Noni fruit is one of the plants that is often used in traditional medicine as a medicine for high blood pressure, beriberi, smooth urination, inflammation of the gallbladder, colitis, dysentery, constipation, spleen pain, liver disease, diabetes and back pain. This study aimed to analyze the content of bioactive compounds, antioxidant properties and cytotoxic activity of fermented noni juice and noni juice.
Method: Identification of the content of bioactive compounds was carried out using Gas Chromatography (GC), antioxidant activity testing using the DPPH method (2,2-Diphenyl-1 picrylhydrazine) and cytotoxic testing using the Brine Shrimp Lethality Test (BSLT) method.
Results: The results showed that the content of phenolic compounds contained in noni juice and fermented noni juice had strong antioxidant properties with the IC50 value for noni juice of 22.23 ppm and for fermented noni juice of 13.95 ppm. Meanwhile, the results of the cytotoxic test of noni juice and fermented noni showed that the LC50 values of 33.26 ppm and 2.25 ppm respectively.
Conclusion: It can be concluded that both of these materials have high antioxidant activity and potential as anticancer.

Keywords: Noni, Antioxidant, Sitotoksik, Phenolic, Fermented.
Cite This Article: Handayani., Wulandari, D.D., Wulansari, D.D. 2022. Phytochemical screening, antioxidant activity and cytotoxicity assay from noni juice and fermented noni (*Morinda citrifolia L.*). *Bali Medical Journal* 11(3): 1168-1171. DOI: 10.15562/bmj.v11i3.3534

INTRODUCTION

Morinda citrifolia, a small to medium-sized (3–10 m tall) tree with a pantropical distribution, is known by the popular name "noni." Numerous substances, including as flavonoids, glycosides, vitamins, anthraquinones, and polyunsaturated fatty acids, are among noni's active ingredients. The active ingredient is a polysaccharide-rich material that has an in vitro anticancer activity, according to in vitro studies and animal models. Noni, however, has not been demonstrated to have anticancer efficacy in clinical trials. Since 1996, noni juice has been sold in the US, and in 2003, the European Commission certified it as a "new food." Despite being generally regarded as harmless, there have been a number of case reports of adverse outcomes linked to the use of noni, despite frequent criticism for the absence of chemical examination of the actual product to rule out misidentification and contamination.[1]

One of the products of Islamic boarding school X is noni juice. This product has been claimed to have several properties including being able to treat diabetes, lower cholesterol, improve the digestive, respiratory and other systems. Therefore, this study wanted to prove that this noni juice has efficacy as an antioxidant and to determine its toxic potential through a cytotoxic test to obtain information that this product is safe to use. The cytotoxic test was performed using the BSLT (Brine Shrimp Lethality Test) method, whereas the antioxidant activity test was performed using the DPPH method (2,2-diphenyl-1-picrylhydrazil). It is required to conduct phytochemical screening to identify the active chemical components present in noni juice prior to testing utilizing these two techniques.[2]

This study aimed to analyze the content of bioactive compounds, antioxidant properties and cytotoxic activity of fermented noni juice and noni juice.

METHOD

Phytochemical Screening

Tannin Test

To noni ethanol extract samples, 0.1 percent FeCl3 and up to 2-3 drops were added. After homogenizing the mixture, the color changed to a greenish yellow or greenish brown color, which was deemed positive (+) for tannin components.

Saponin Test

Ten milliliters of noni fruit ethanol extract were used to test for the presence of saponin compounds. Five milliliters of distilled water were added, and the mixture was agitated until foam emerged. Three to five drops of olive oil were then added, and the mixture was again shaken.

Flavonoid Test

Homogenize after adding 5 ml of the ethanol extract from noni fruit and 5 ml of the diluted ammonia.

As a result, the color will slowly turn

yellow before being classified as positive (+) flavonoid chemicals.

Terpenoid Test

A drip plate was used to hold samples weighing 50–100 mg, and acetic acid was poured until all samples were completely covered. After 15 minutes, 6 drops of the solution were transferred into a test tube, and 2–3 drops of strong sulfuric acid were added. The resulting color's intensity is employed as a comparative indicator of the sample's triterpenoid and steroid content when the color variations are detected. An appearance of a red, orange, or purple tint indicates the existence of triterpenoids.

Quantitative Determination of Secondary Metabolites using Gas Chromatography (GC)

Shimadzu LC-10 HPLC apparatus with UV detector positioned at 283 nm was utilized. Eclipse DB-C18 (4.6 mm 150 mm; 5 m ID) was the column that was utilized. The mobile phase had a flow rate of 1.0 ml/min and was composed of acetonitrile and 0.1 percent phosphoric acid. After injecting the sample solution (20 l at 35 1 °C), chromatograms were taken. Each sample's peak area is measured, and the peak of the sample solution is compared to the reference peak.[3]

Antioxidant Activity Test

In accordance with the procedure outlined in the prior literature, the antioxidant activity of noni juice was assessed in vitro using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. 500 l of DPPH (0.005%) methanol solution and 3 ml of methanol extract at various concentrations ranging from 100 g/ml to 900 g/ml were combined. The absorbance (A) of the control and sample was measured spectrophotometrically at 517 nm after 30 min of incubation at room temperature and in the dark. The positive control used was ascorbic acid. Free radical scavenging activity is calculated in percentage as follows:

$$\text{Free radical scavenging activity (\%)} = \frac{\text{(A) control} - \text{(A) sample}}{\text{(A) control}} \times 100\%$$

From the graph of the percentage inhibition graphing against the extract, IC50 was graphically computed.[4]

Cytotoxicity Test with Brine Shrimp Lethality Test (BSLT) Method

The noni juice cytotoxicity test was observed using the BSLT method. In a conical container with 300 mL of fake seawater made from commercial sea salt (40g/L), Artemia salina eggs were incubated. The flask was adequately aerated with the aid of an air pump and kept at a temperature of 29–30 oC in a water bath. The powerful light source remains on. After 48 hours, the hatchery tank's light chamber was empty of active nauplii, which were taken out and used for testing. Dimethylsulfoxide (DMSO) was used to dissolve the sample, and the final DMSO content was kept under 0.05 percent by diluting it with synthetic seawater. The stock sample (1 mg/mL) was serially diluted to create various sample concentrations. 10 mL of aerated seawater were added to 1 mL of each concentration before being transferred to a sterile, clean universal vial. Around 10 nauplii were present in each vial, and they were cultivated for 24 hours. DMSO (negative control) and podophyllotoin were employed as controls (positive control). Finally, a count and record of the surviving at each dose of extract and control were made. Using Ecel software's linear regression analysis, the lethal concentration of each extract that caused 50% of brine shrimp to perish (LC50) was determined.[5]

RESULT

Phytochemical Screening

Alkaloids, flavonoids, tannins, saponins, and terpenoids were among the secondary metabolites screened by phytochemical analysis in noni juice and fermented noni juice. The results of the phytochemical screening test can be seen in table 1.

Based on the results in table 2, it shows that the noni juice sample contains volatile compounds such as methanol, ethanol, 1,2,3-propanetriyl ester, Glycerol tricaprylate, Glycerol tricaprylate and 14-Beta-H-Pregna. However, fermented noni does not contain Glycerol tricaprylate and 14-Beta-H-Pregna compounds.

Based on the data in table 3 shows that both samples of noni juice and fermented noni juice have strong antioxidant properties, indicated by the IC50 value for noni juice of 22.23 ppm and for fermented noni juice of 13.95 ppm.

Meanwhile, based on the results of the cytotoxic test using the Brine Shrimp Lethality Test (BSLT) method, it showed that samples of fermented noni juice and noni juice were very toxic, indicated by the LC50 values of 33.26 ppm and 2.25 ppm respectively, but fermented noni juice showed properties that are more toxic than noni juice.

DISCUSSION

Based on a qualitative phytochemical screening test, it showed that both noni juice and fermented noni juice indicated the presence of flavonoid compounds, tannins, saponins, and terpenoids which were indicated by a distinctive color change with each reagent. Meanwhile, the content of volatile compounds identified using Gas Chromatography-Mass Spectrometry (GC-MS) shows that there are several volatile compounds as shown in table 2. The presence of 1,2,3-propanetriyl ester compounds is an indication of the occurrence of an esterification process during the fruit ripening process. This is the case with other fruits.[6] The DPPH (2,2-diphenyl-1-picrylhydraza) method, one of the most efficient, practical, and accurate in vitro techniques with the capacity to scavenge free radicals, is one of the techniques used to investigate the antioxidant activity of substances. The highest absorption of DPPH, a persistent purple organic nitrogen radical, occurs between 515 and 520 nm, and the lower the IC50 value, the greater the material's antioxidant activity.[7] According on the data in table 3 shows that samples of noni juice and fermented noni juice have strong antioxidant properties. Almost all flavonoid groups have antioxidant

Table 1. Phytochemical Screening.

Phytochemical Screening	Noni Juice		Fermented Noni	
	Change	Result	Change	Result
Flavonoid	Reaction with ammonia turns yellowish	+	Reaction with ammonia turns yellowish	+
Tanin	Reaction with FeCl3 produces a dark green color	+	Reaction with FeCl3 produces a dark green color	+
Saponin	Permanent foam appears for 3 minutes after shaking	+	Permanent foam appears for 3 minutes after shaking	+
Terpenoid	Reaction with acetic acid and sulfuric acid shows a reddish color	+	Reaction with acetic acid and sulfuric acid shows a reddish color	+

Note:
+ : Contained compound
- : Contained no compound

Table 2. Characterization with GC-MS.

No.	Noni Juice	Fermented Noni
1.	Methanol	Oxygen
2.	Nitrogen oxide	Nitrogen Oxide
3.	Ethanol	Methanol
4.	Decanoic Acid	Ethanol
5.	1,2,3-propanetriyl ester	1-O-Decanoyl-D-Xylitol
6.	Glycerol tricaprylate	Decanoic Acid
7.	8-Pentadecanol	1,2,3-propanetriyl ester
8.	14-Beta-H-Pregna	

Table 3. Antioxidant Activity with DPPH method.

No.	Sample	IC_{50} (ppm)	Antioxidant Activity
1.	Noni Juice	22.23	Strong
2.	Fermented Noni	13.95	Strong

Table 4. Citotoxic with BSLT.

No	Sample	LC_{50} (ppm)	Information
1.	Noni Juice	33.26	Very toxic
2.	Fermented Noni	2.25	Very toxic

properties. It has been reported that several types of flavonoids are the most powerful for protecting the body against reactive oxygen species. Cells and tissues of the body are constantly threatened by damage caused by free radicals and reactive oxygen species, generated during metabolic processes or as a result of induced by exogenous damage. In addition to enzymes like glutathione peroxidase, catalase, and superoxide dismutase, the body's antioxidant defense systems also contain non-enzyme components including glutathione, ascorbic acid, and -tocopherol. Ingestion and depletion of endogenous scavenging chemicals occur as a result of increased generation of reactive oxygen species after damage. Endogenous scavenging substances are neutralized by flavonoids.[8]

The role of flavonoid chemicals, which prevent larvae from feeding, is thought to be connected to the mechanism of larval mortality. In addition, there are numerous theories regarding how flavonoids work as an anti-cancer agent, according to Woo et al.[9] Flavonoids work as antioxidants, namely via activating the apoptotic pathway in cancer cells. According to this view, DNA fragmentation causes the cell apoptosis process. Fragmentation the proximal DNA chain is first released in this process by reactive oxygen substances like hydroxyl radicals. The inhibition of protein kinase activity by flavonoids as tumor/cancer growth inhibitors also blocks signal transduction pathways from membrane to nucleus cells. Due to tyrosine kinase receptor activity's increased function in the formation of cancerous cancer cells, flavonoids suppress the activity of tyrosine receptors kinase. Additionally, flavonoids work to lessen tumor resistance to chemotherapy drugs.[4]

CONCLUSIONS

Based on the results showed that the content of phenolic compounds contained in noni juice and fermented noni juice had strong antioxidant properties with the IC50 value for noni juice of 22.23 ppm and for fermented noni juice of 13.95 ppm. Meanwhile, the results of the cytotoxic test of noni juice and fermented noni showed that the LC50 values of 33.26 ppm and 2.25 ppm respectively. It can be concluded that both of these materials have high antioxidant activity and potential as anticancer becaused of their phenolic compound content such as flavonoid, terpenoid, tanin, and saponin. Further research with different study designs and larger samples is needed to find out more about other factors that influence the effects of content of bioactive compounds, antioxidant properties and cytotoxic activity of fermented noni juice and noni juice.

ACKNOWLEDGEMENTS

We appreciate the financing and simple access to research facilities provided by the Universitas Nahdlatul Ulama Surabaya. We also appreciate the support for data collection provided by the Central Drugs Evaluation Analysis Laboratory Universitas Surabaya.

AUTHOR CONTRIBUTION

All authors contributed to this study's conception and design, data analysis and interpretation, article drafting, critical revision of the article, final approval of the article, and data collection.

FUNDING

This research was funded by financial support from Universitas Nahdlatul Ulama Surabaya.

CONFLICT OF INTEREST

The author declared that no competing interests.

ETHICAL CONSIDERATION

This study has been declared ethical by the Ethical Commission for Health Research of the Universitas Nahdlatul Ulama Surabaya.

REFERENSI

1. Greaves I, Porter K, Garner J. Trauma Care Manual. 3rd ed. CRC Press; 2021.
2. Gupta RK, Patel AK. Do the health claims made for *Morinda citrifolia* (Noni) harmonize with current scientific knowledge and evaluation of its biological effects. Asian Pac J Cancer Prev. 2013;14(8):4495–9.
3. Ketkomol P, PUNJANON T. Comparative Studies on Physicochemical Properties Of *Morinda citrifolia* Gel And Ointment Formulations. Asian J Pharm Clin Res. 2019;12(11):208–12.
4. Rahimah S, Ba FM, Limbong BA. Uji Toksisitas Ekstrak Etanol Daun Belimbing Wuluh (Averrhoa bilimbi L .) Menggunakan Metode Brine Shrimp Lethality Test. J Pharm Med Sci. 2019;4(1):10–4.
5. Asnaashari S, Delazar A, Asgharian P, Lotfipour F, Moghaddam SB, Afshar FH. In-vitro bioactivity and phytochemical screening of extracts from rhizomes of eremostachys azerbaijanica rech. F. Growing in Iran. Iran J Pharm Res. 2017;16(1):306–14.
6. Abou Assi R, Darwis Y, Abdulbaqi IM, khan AA, Vuanghao L, Laghari MH. *Morinda citrifolia* (Noni): A comprehensive review on its industrial uses, pharmacological activities, and clinical trials. Arab J Chem. 2017;10(5):691–707. Available from: http://dx.doi.org/10.1016/j.arabjc.2015.06.018
7. Souza BL, Leitão Gindri A, De Andrade Fortes T, Felli Kubiça T, Enderle J, Roehrs R, et al. Phytochemical Analysis, Antioxidant Activity, Antimicrobial Activity, and Cytotoxicity of Chaptalia nutans Leaves. Adv Pharmacol Pharm Sci. 2020;2020.
8. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci. 2016;5:1–15.
9. Woo HD, Kim J. Dietary flavonoid intake and risk of stomach and colorectal cancer. World J Gastroenterol. 2013;19(7):1011–9.

Phytochemical screening, antioxidant activity and cytotoxicity assay from noni juice and fermented noni (Morinda citrifolia L.)

ORIGINALITY REPORT

11%	11%	3%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

MATCH ALL SOURCES (ONLY SELECTED SOURCE PRINTED)

4%

★ www.hindawi.com

Internet Source

Exclude quotes	On	Exclude matches	< 1%
Exclude bibliography	On		