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RESEARCH ARTICLE

Molecular docking of noni fruit extract (*M. citrifolia L.*) active compound as a radiation protection agent: a bioinformatic approach

Deny Saputra*✉, Sri Wigati Mardi Mulyani*, Nastiti Faradilla Ramadhani*, Jane Evelyn Margareth, Regita Maharani Kharisma Putri**, Farah Tariza Harlens**, Alexander Patera Nugraha*****

*Department of Dentomaxillofacial Radiology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
**Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
***Department of Orthodontics, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
*Jl Prof Dr Moestopo No 47, Surabaya, East Java, Indonesia; ✉ correspondence: deny-s@fkg.unair.ac.id

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ABSTRACT

Noni fruit (*M. citrifolia L.*) is a medicinal plant known for its antioxidant bioactive compounds, which have potential use as radiation protection agents. Despite their traditional use, the specific bioactive compounds and their efficacy as radiation protectants have not been thoroughly investigated. This study aims to address this research gap by evaluating the potential of noni fruit extract as a candidate for radiation protection using in-silico methods. Databases consulted include PubChem, PASS Online, and ProTox-II. The results identified nine bioactive compounds in noni fruit extract: quercetin, kaempferol, morindin, morindone, alizarin, nicotinamide, beta-sitosterol, squalene, and n-hexadecanoic acid. Among these, kaempferol, squalene, and n-hexadecanoic acid were found to be the most potent antioxidants. Kaempferol exhibited a low toxicity level (grade 5) with significant activity as an antioxidant (Pa ≥ 0.7), free radical scavenger (Pa ≥ 0.7), and radioprotector (Pa 0.3 – 0.7). Squalene, a triterpene with low toxicity (class 5), showed antioxidant activity (Pa 0.3 – 0.7), free radical scavenger activity (Pa 0.3 – 0.7), and radioprotection (Pa 0.3 – 0.7). n-Hexadecanoic acid, a metabolite with moderate toxicity (class 4), demonstrated lower antioxidant activity (Pa ≤ 0.3), while morindin exhibited free radical scavenging and radioprotective properties. The findings suggest that kaempferol, squalene, and n-hexadecanoic acid in noni fruit extract hold promise as candidates for radiation protection, as evidenced by in-silico analysis.

Keywords: bioactive compounds; in silico; medicine; Morinda citrifolia; radiation protection

INTRODUCTION

Dental radiography is often used as a diagnostic tool for obtaining real images produced by X-rays. The benefits of radiographic examinations in dentistry include assisting dentists in determining diagnoses, planning treatments, and evaluating the results of previously conducted treatments.[1] However, the potential dangers of the use of radiographic X-rays, such as damage to or death of cells and tissues, remain a significant concern. These X-rays can ionize the cell nucleus directly on DNA or indirectly through the formation of free radicals, causing lipid peroxidation, protein modification, and DNA damage.[2] Damage that is not highly severe can be repaired by the DNA repair system, but if this system has been cut off in various places, the damage cannot be repaired and leads to disruption of cell division and abnormal changes that affect the genes, causing gene mutations and chromosomal aberrations.[3] Chromosomal aberrations lead to chromosomal instability, which damages the tissues, organs, and organisms and can even cause cell death through apoptosis and necrosis.[2] Therefore, radiation protection materials are urgently needed to prevent such oxidative reactions. Plant bioactive compounds with antioxidant properties can capture radical compounds, protecting cellular molecules from damage.[4,5]

Antioxidants are compounds that can prevent and slow damage associated with free radicals by inhibiting oxidative mechanisms.[6] Several natural ingredients have been shown to possess high radiation protection capabilities by reducing oxidative stress in cells caused by radiation exposure. One such material is noni fruit (*M. citrifolia L.*). The noni fruit plant is a medicinal plant that contains antioxidant bioactive compounds that can be used as radiation protection agents.[7] Earlier study identified over 160 bioactive compounds in noni fruit, primarily phenolic compounds, organic acids, and alkaloids. These include anthraquinones (damnacanthal, morindone, morindin), aucubin, asperuloside, and scopoletin, which possess antioxidant properties. The fruit's main organic acid compounds, caproic acid and caprylic acid, have antimicrobial properties. Singh examined noni fruit's bioactive activities, such as antibacterial, antiviral, anti-helminthic, antifungal, antioxidant, liver protection, anti-obesity, hypoglycaemic, analgesic, anti-inflammatory, hypotensive, cardiovascular, immunological, and anti-cancer properties.[9] This study is unique in its approach, as it used silico methods to identify and evaluate the radiation protection potential of noni fruit extract. In-silico testing is advantageous because it reduces the need for the use of excessive tools, materials, and experimental animals, thereby saving experimental costs.[10] By analyzing and comparing the bioactive compounds, this study aims to determine whether noni fruit extract can effectively act as a radiation protector through its antioxidant properties. Thus, this research contributes to the identification of noni fruit (*M. citrifolia L.*) extract as a potential candidate for radiation protection, using in-silico methods to thoroughly evaluate its bioactive compounds.

MATERIALS AND METHODS

This study was conducted using in-silico methods, which use computational and database technology. In-silico testing offers significant advantages, as it reduces the need for the use of excessive tools, materials, and experimental animals, thereby reducing experimental costs. This research did not require ethical clearance, as it did not involve human or animal subjects. This research was conducted using Lenovo IdeaPad 330 2019 hardware, which has an Intel Core i3 7th processor, 4 GB RAM, an ATI Radeon graphics card, and a Windows operating system. The PubChem database, the Prediction of Activity Spectra for Substances (PASS) Online web server, and ProTox-II were the software used in the in-silico research. The material used in this study was the SMILES notation of a collection of bioactive compounds in noni fruit extract (*M. citrifolia L.*).

A literature study was conducted on bioactive compounds in noni fruit extract, then the PubChem Database was used to search for their SMILES structure. Following this, the biological activity (antioxidant, free radical scavenger, and radioprotector) of the SMILES structures of nine bioactive compounds in noni fruit extract was analyzed using the PASS Online web server to obtain the probability to be active (Pa) and probability to be inactive (Pi) values, which were subsequently used to determine the validity of the research. Toxicity tests were conducted on the nine compounds using the ProTox-II web server. Toxicity classification, lethal dose (LD50), hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity were determined.

A Pa value ≥ 0.7 indicates that the compound is potentially active, both computationally and in laboratory tests. A Pa value between 0.3 and 0.7 indicates that the compound is potentially active computationally but has either not been proven to be or has shown low potential in laboratory tests. A Pa value ≤ 0.3 indicates that the compound has low potential, both computationally and in laboratory tests.[11]

A toxicity prediction analysis of bioactive compounds in noni fruit extract (*M. citrifolia L.*) was conducted using the ProTox-II web server (https://tox-new.charite.de/protox_II/) with the SMILES notation. The compounds to be tested for toxicity were obtained from the PubChem web server (https://pubchem.ncbi.nlm.nih.gov/) and analyzed to obtain data on LD50 and the compound toxicity classification based on the Globally Harmonized System (GHS), including hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The LD50 is the amount of compound administered that causes death in 50% of the test animal group.[12]

RESULTS

Table 1 provides information on various compounds found in noni fruit (*M. citrifolia L.*) extract. The columns include the compound name, its role, PubChem compound identifier (CID), and the compound chemical structure in Canonical SMILES notation. Quercetin and kaempferol are classified as flavonoids - compounds that are known for their antioxidant properties. Morindin and beta-sitosterol are categorized as secondary metabolites. These compounds often have various biological activities. Morindone and alizarin belong to the anthraquinone group, which are known for their potential therapeutic effects. Nicotinamide, a pyridine compound, is a form of vitamin B3 and is essential in human nutrition. Squalene is identified as a triterpene and has known antioxidant properties.

Table 2 presents the predicted biological activities of the compounds using PASS Online. The activities evaluated include antioxidant, free radical scavenger, and radioprotector activity and are presented with their corresponding Pa and Pi scores. Most compounds showed high Pa scores, indicating a strong potential for antioxidant activity and, therefore, an ability to protect cells from damage caused by free radicals. Quercetin, kaempferol, and morindin yielded high Pa scores, pointing to their efficacy in neutralizing free radicals. Several compounds, including morindone and alizarin, displayed moderate Pa scores, implying that they may offer protection against radiation damage.

Table 3 presents the analysis of the toxicity of the compounds using the ProTox-II tool. It includes toxicity classification, LD_{50} values, prediction accuracy, and potential toxicity endpoints, such as hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The compounds are categorized into toxicity classes from 1 (most toxic) to 6 (least toxic). For example, quercetin is classified as class 3, indicating higher toxicity compared with compounds in classes 5 and 6, which include morindin and morindone. LD50 values indicate the dose required to cause death in 50% of a test population. Lower LD_{50} values signify higher toxicity. Quercetin had an LD_{50} of 159 mg/kg, indicating a higher toxicity than compounds such as morindone, which had an LD_{50} of 7,000 mg/kg. The accuracy of the toxicity predictions varies, with some compounds showing 100% prediction accuracy. Table 3 highlights various potential toxic effects, such as hepatotoxicity (liver toxicity), carcinogenicity (cancer-causing potential), immunotoxicity (immune system toxicity), mutagenicity (genetic mutation potential), and cytotoxicity (cell toxicity). Beta-sitosterol showed a high cytotoxicity with a score of 0.99, indicating a strong potential for cell damage.

Table 1. Results of compound analysis of noni fruit extract (*M. citrifolia L*) with PubChem

CompoundName	Role	CID	Canonical SMILES
Quercetin	Flavonoid	5280343	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
Kaempherol	Flavone	5280863	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
Morindin	Sec. Metab	151621	CC1=C(C2=C(C=C1)C(=O)C3=C(C2=O)C=CC(=C3O)OC4C(C(C(C(O4)COC5C(C(C(CO5)O)O)O)O)O)O)O
Morindone	anthraquinone	442756	CC1=C(C2=C(C=C1)C(=O)C3=C(C2=O)C=CC(=C3O)O)O
Alizarin	anthraquinone	6293	C1=CC=C2C(=C1)C(=O)C3=C(C2=O)C(=C(C=C3)O)O
Nicotinamide	pyridine	936	C1=CC(=CN=C1)C(=O)N
beta-Sitosterol	Sec. Metab	222284	CCC(CCC(C)C1CCC2C1(CCC3C2CC=C4C3(CCC(C4)O)C)C)C(C)C
Squalene	triterpene	638072	CC(=CCCC(=CCCC(=CCCC=C(C)CCC=C(C)CCC=C(C)C)C)C)C
n-hexadecanoicacid	Sec. Metab	985	CCCCCCCCCCCCCCCC(=O)O

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Table 2. Results of biological activity of noni fruit compounds (*M. citrifolia L*) with PASS Online

Compound Name	Role	Pubchem CID	Pa Score	Pi Score	Activity
Quercetin	Flavonoid	5280343	0.872	0.003	Antioxidant
			0.811	0.003	Free radical scavenger
			0.541	0.021	Radioprotector
Kaempherol	Flavone	5280863	0.856	0.003	Antioxidant
			0.771	0.003	Free radical scavenger
			0.538	0.022	Radioprotector
Morindin	Sec. Metab	151621	0.727	0.004	Antioxidant
			0.846	0.002	Free radical scavenger
			0.522	0.023	Radioprotector
Morindone	anthraquinone	442756	0.423	0.010	Antioxidant
			0.401	0.017	Free radical scavenger
			0.348	0.064	Radioprotector
Alizarin	anthraquinone	6293	0.421	0.010	Antioxidant
			0.357	0.022	Free radical scavenger
			0.366	0.056	Radioprotector
Nicotinamide	pyridine	936	-	-	Antioxidant
			0.184	0.090	Free radical scavenger
			0.229	0.123	Radioprotector
beta-Sitosterol	Sec. Metab	222284	0.178	0.072	Antioxidant
			-	-	Free radical scavenger
			0.418	0.039	Radioprotector
Squalene	triterpene	638072	0.657	0.004	Antioxidant
			0.456	0.013	Free radical scavenger
			0.649	0.013	Radioprotector
n-hexadecanoicacid	Sec. Metab	985	0.222	0.045	Antioxidant
			0.315	0.027	Free radical scavenger
			0.628	0.014	Radioprotector

DISCUSSION

Radiation protection has the potential to protect patients and medical personnel and reduce the damaging effects of radiation exposure. Several theories hold that noni fruit (*M. citrifolia L*) contains bioactive compounds that have potential as antioxidants that are included in the category of very strong antioxidants.[13] However, research related to the in-silico exploration of noni fruit extract (*M. citrifolia L*) as a candidate for radiation protection is lacking; therefore, further research on the potential of noni fruit extract as a candidate for in-silico radiation protection is needed.

This research was conducted using a computational or in-silico approach to determine the bioactive compounds in noni fruit extracts that may be radiation protection agents. In doing so, this research explored the compounds' ability to reduce free radical reactions. The activity of noni fruit extract (*M. citrifolia L*) in relation to the inhibition of free radicals is due to its nine bioactive compounds, which are quercetin, kaempferol

Table 3. Results of toxicity analysis of compounds of noni (*M. citrifolia L*) with ProTox-II

Compound Name	Toxicity Class	LD50 (mg/kg)	Prediction accuracy	Hepatotoxicity	Carcinogenecity	Immunotoxicity	Mutagenicity	Cytotoxicity
Quercetin	3	159 mg/kg	100%	0.69	0.68	0.87	0.51	0.99
Kaempherol	5	3919 mg/kg	70.97%	0.68	0.72	0.96	0.52	0.98
Morindin	5	3000 mg/kg	69.26%	0.90	0.84	0.98	0.61	0.60
Morindone	6	7000 mg/kg	70.97%	0.70	0.54	0.78	0.88	0.93
Alizarin	6	7000 mg/kg	72.9%	0.71	0.58	0.86	0.98	0.93
Nicotinamide	5	2500 mg/kg	100%	0.54	0.92	0.98	0.94	0.85
beta- Sitosterol	4	890 mg/kg	70.97%	0.87	0.60	0.99	0.98	0.94
Squalene	5	5000 mg/kg	100%	0.79	0.76	0.99	0.98	0.81
n- hexadecanoicacid	4	900 mg/kg	100%	0.52	0.63	0.99	1.0	0.74

morindin, morindone, alizarin, nicotinamide, beta-sitosterol, squalene, and n-hexadecanoic acid.

Previous studies have established quercetin as a potent antioxidant with significant health benefits, including cancer prevention.[14] Quercetin's ability to scavenge free radicals and inhibit oxidative stress is well documented. In this study, quercetin showed high antioxidant (Pa = 0.872) and free radical scavenging activity (Pa = 0.811), which is consistent with previous findings. However, our toxicity analysis revealed a high toxicity class (class 3) and potential carcinogenic and mutagenic effects, which have not been emphasized in earlier studies. This highlights the need for cautious use and further investigation into safe dosage levels.[13]

Kaempferol, a flavone, exhibited strong antioxidant activity (Pa = 0.856) and free radical scavenging capabilities (Pa = 0.771). It functions both directly, by donating hydrogen ions to neutralize free radicals, and indirectly, by enhancing the expression of endogenous antioxidant genes. Kaempferol's low toxicity (class 5) and inactivity in several toxicity tests make it a safer option for use as a radiation protection agent.[15] In previous, kaempferol treatment increased levels of anti-mullerian hormone (AMH), estradiol (E2), antral follicle count (AFC), superoxide dismutase, and catalase and decreased the levels of follicle-stimulating hormone, reactive oxygen species (ROS), and malonaldehyde, indicating that kaempferol can be used as a protective agent against ionizing radiation.[16]

Morindin, beta-sitosterol, and n-hexadecanoic acid act as secondary metabolites with varied bioactivity. In this study, morindin showed a high free radical scavenging activity (Pa = 0.846) and presented low toxicity but posed potential risks for immune system disorders and gene mutations. Beta-sitosterol, despite a moderate antioxidant and radioprotector activity, showed a high immunotoxicity risk, necessitating further examination. n-Hexadecanoic acid showed moderate toxicity but significant radioprotector activity (Pa = 0.628), making it a potential radiation protection agent.[17]

Morindone and alizarin, classified as anthraquinones, are effective in cancer treatment due to their antioxidant properties.[18] Morindone has moderate biological activity but a high likelihood of causing immune disorders and gene mutations, disqualifying it as a radiation protection agent. Similarly, alizarin, despite its antioxidant and radioprotector activities, has high carcinogenic and mutagenic potential, rendering it unsuitable for radiation protection. Nicotinamide, a pyridine compound, lacks significant antioxidant activity but serves as a free radical scavenger and radioprotector. However, its potential hepatotoxic effects require careful consideration. Squalene, a triterpene, shows excellent antioxidant (Pa = 0.657) and radioprotector activities (Pa = 0.649) with low toxicity, making it a promising candidate for radiation protection. The compounds in noni fruit extract exhibit three primary biological activities: antioxidant, free radical scavenger, and radioprotector. Antioxidants inhibit oxidative mechanisms, protecting the body from ROS and other free radicals. Free radical scavengers neutralize these harmful molecules, while radioprotectors mitigate the side effects of radiation by eliminating ROS and enhancing DNA repair.[18] Based on this in-silico study, the compounds in noni fruit extract that can be used as radiation protection agents are kaempferol, squalene, and n-hexadecanoic acid. These compounds showed biological activity as antioxidants, free radical scavengers, and radioprotectors and have low toxicity. Therefore, they are potential candidates for use as radiation protection agents.

This study has several limitations. It relies solely on in-silico methods, which, while powerful, cannot fully replicate in vitro and in vivo conditions. The toxicity predictions require validation through experimental studies to confirm the safety and efficacy of the compounds. In future studies, in vitro and in vivo research should be conducted to validate these findings and assess the practical applicability of the identified compounds as radiation protectors. In addition, investigating the optimal dosages and delivery methods for these compounds can help maximize their protective effects while minimizing potential toxicity. Exploring the synergistic effects of combining these bioactive compounds with known antioxidants, such as vitamin C, may further enhance their radiation protection capabilities.[19]

CONCLUSION

Based on the research results, it can be concluded that noni fruit (*M. citrifolia L*) extract has potential as an in-silico radiation protection agent.

CONFLICT OF INTEREST

The authors declare no competing interests.

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