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Research Article

Total Flavonoid Content, Antibacterial Activity and *In Silico* Toxicity Prediction of Ethanolic Extract from *Murraya koenigii* Leaves against *Streptococcus mutans*

Kadar Total Flavonoid, Aktivitas Antibakteri, dan Prediksi Toksisitas *In Silico* dari Ekstrak Etanol Daun Kari (*Murraya koenigii*) terhadap *Streptococcus mutans*

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ABSTRACT

Dental caries is a chronic disease exclusive to humans and remains one of the most prevalent oral health problems worldwide. The increasing emergence of antibiotic resistance and the adverse effects associated with conventional antimicrobials have encouraged the exploration of natural plant-derived compounds for caries prevention. *Murraya koenigii* leaves are widely used as a culinary ingredient and are known to possess various therapeutic properties, including antioxidant, antibacterial, anti-inflammatory, and hepatoprotective activities. This study aimed to evaluate the total flavonoid content, antibacterial activity, and *in silico* toxicity profile of the ethanolic extract of *M. koenigii* leaves against *Streptococcus mutans*, a primary cariogenic pathogen. Total flavonoid content was determined using the aluminum chloride colorimetric method, while antibacterial activity was assessed using the agar disk diffusion assay. The toxicity profile of major flavonoid constituents was further analyzed using in silico ADMET prediction tools. The results showed that the ethanolic extract of *M. koenigii* leaves contained a high total flavonoid content (281.83 ± 10.23 mg QE/g extract) and exhibited strong antibacterial activity against Streptococcus mutans, producing inhibition zones ranging

from 14.4 to 19.4 mm in a concentration-dependent manner. *In silico* analysis indicated that the major flavonoid constituents possessed favorable pharmacokinetic characteristics with low predicted toxicity risks. These findings suggest that the ethanolic extract of *M. koenigii* leaves is rich in flavonoids and demonstrates significant antibacterial potential, supporting its promise as a natural antibacterial agent for oral health applications. Further studies focusing on compound isolation, formulation development, and *in vivo* validation are recommended.

Keywords: Antibacterial, Flavonoids, *Murraya koenigii*, Toxicity Prediction, ADMET

ABSTRAK

Karies gigi merupakan penyakit kronis yang eksklusif pada manusia dan masih menjadi salah satu masalah kesehatan mulut yang paling sering terjadi di seluruh dunia. Meningkatnya resistensi antibiotik serta efek samping dari penggunaan antimikroba konvensional mendorong eksplorasi senyawa alami berbasis tanaman sebagai alternatif pencegahan karies. Daun Murraya koenigii dikenal luas sebagai bahan kuliner dan memiliki berbagai aktivitas terapeutik, antara lain antioksidan, antibakteri, antiinflamasi, dan hepatoprotektif. Penelitian ini bertujuan untuk mengevaluasi kandungan flavonoid total, aktivitas antibakteri, serta profil toksisitas in silico dari ekstrak etanol daun M. koenigii terhadap Streptococcus mutans, bakteri utama penyebab karies gigi. Penetapan kandungan flavonoid total dilakukan menggunakan metode kolorimetri aluminium klorida, sedangkan aktivitas antibakteri diuji menggunakan metode difusi agar cakram. Profil toksisitas senyawa flavonoid utama selanjutnya dianalisis menggunakan alat prediksi ADMET secara in silico. Hasil penelitian menunjukkan bahwa ekstrak etanol daun M. koenigii memiliki kandungan flavonoid total yang tinggi, yaitu 281,83 ± 10,23 mg QE/g ekstrak, serta menunjukkan aktivitas antibakteri yang kuat terhadap Streptococcus mutans dengan diameter zona hambat berkisar antara 14,4–19,4 mm secara bergantung pada konsentrasi. Analisis in silico menunjukkan bahwa senyawa flavonoid utama memiliki karakteristik farmakokinetik yang menguntungkan dengan risiko toksisitas yang rendah. Berdasarkan hasil tersebut, dapat disimpulkan bahwa ekstrak etanol daun M. koenigii kaya akan flavonoid dan memiliki potensi antibakteri yang signifikan, sehingga berpeluang dikembangkan sebagai agen antibakteri alami untuk aplikasi kesehatan gigi. Penelitian lanjutan perlu difokuskan pada isolasi senyawa aktif, pengembangan formulasi, dan uji in vivo.

Kata Kunci: Antibakteri, Flavonoid, Murraya koenigii, Prediksi Toksisitas, ADMET

I. INTRODUCTION

Dental caries is a prevalent oral health problem resulting from the interaction between tooth surfaces, microbial biofilms, and dietary carbohydrates that are metabolized into organic acids (Chen et al., 2020). One of the primary contributors to dental caries is dental plaque, a structured multispecies biofilm dominated by acidogenic and aciduric bacteria capable of adhering to and colonizing tooth surfaces, particularly Streptococcus mutans (Mosaddad et al., 2019). This bacterium plays a central role

in the initiation and progression of dental caries through its ability to form biofilms, produce organic acids, and promote enamel demineralization.

In Indonesia, the prevalence of dental caries reaches 57.6%, as reported by the National Basic Health Research (RISKESDAS) (Badan Litbangkes, Kementerian Kesehatan RI, 2018). Globally, dental caries affects approximately 80–90% of children under 18 years of age in European, American, and Asian countries, including Indonesia (Maharani et al., 2019). Conventional management of dental caries includes restorative procedures and the use of antibiotics such as amoxicillin. However, the excessive and prolonged use of antibiotics has contributed to the emergence of antimicrobial resistance, leading to reduced therapeutic efficacy, increased morbidity, and higher healthcare costs (Khasanah et al., 2019).

In response to the growing concern over antibiotic resistance and adverse effects associated with conventional antimicrobials, natural plant-derived compounds have attracted increasing interest as alternative agents for caries prevention. These compounds offer advantages such as chemical diversity, relatively low toxicity, wide availability, and a reduced likelihood of resistance development (Kamal et al., 2021). Among cariogenic microorganisms, Streptococcus mutans is widely recognized as the primary etiological agent of dental caries, making it a key target for antibacterial strategies in oral health management (Priya et al., 2023; Diogo et al., 2023). Accordingly, Streptococcus mutans was selected as the microorganism test in this study.

Murraya koenigii leaves are widely used in Asian cuisine as a culinary spice and are also known for their medicinal properties. These leaves have been reported to possess various therapeutic activities, including antidiabetic, antioxidant, antibacterial, antifungal, anti-inflammatory, anticarcinogenic, and hepatoprotective effects (Sanandia et al., 2024; Ansari & Mahapatra, 2021). Previous studies have demonstrated the antibacterial and antifungal activity of *M. koenigii* leaf extracts obtained using ethanol and other organic solvents. Rajendran et al. (2014) reported that ethanolic extracts of *M. koenigii* leaves exhibited antibacterial activity against Escherichia coli with an inhibition zone of 15 ± 3 mm, while Disegha & Izionworu (2014) observed antifungal activity against Aspergillus niger with an inhibition zone of 14 ± 1 mm. Nevertheless, these studies did not evaluate the antibacterial potential of *M. koenigii* leaves against Streptococcus mutans, the primary pathogen associated with dental caries. This gap highlights the

need for further investigation into the potential application of *M. koenigii* as a natural antibacterial agent for oral health.

In the development of new pharmacological agents, toxicity evaluation is a critical step to ensure compound safety and efficacy prior to therapeutic application. Traditional toxicity assessments based on in vitro and in vivo models, although informative, are often time-consuming, costly, and ethically challenging, and may provide limited insight into molecular-level interactions. Advances in computational biology have enabled the use of in silico toxicity prediction as a complementary approach to assess the toxicological profile of bioactive compounds based on their chemical structure and biological interactions (Agamah et al., 2020; Pérez Santín et al., 2021).

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Therefore, this study aims to quantify the total flavonoid content of *M. koenigii* leaves ethanolic extract, evaluate its antibacterial activity against Streptococcus mutans, and assess the safety profile of its major flavonoid constituents using in silico toxicity prediction. This integrated pharmacological and toxicological evaluation is expected to support the potential application of *M. koenigii* as a natural antibacterial agent for oral health therapy. To the best of our knowledge, this study represents the first integrated evaluation combining quantitative total flavonoid profiling, in vitro antibacterial activity against Streptococcus mutans, and in silico ADMET–toxicity prediction of major flavonoid constituents of *M. koenigii*. Unlike previous studies that primarily focused on antimicrobial screening of *M. koenigii* leaves, this study integrates quantitative flavonoid profiling, concentration-dependent antibacterial evaluation against Streptococcus mutans, and predictive ADMET–toxicity

assessment to provide a translational safety–efficacy framework relevant for oral therapeutic development.

II. METHOD

A. Materials and Apparatus

Fresh *M. koenigii* leaves were collected from Medan Amplas District, Medan City, North Sumatra, Indonesia (approximately 3.548° N, 98.676° E). Ethanol (96%) was used as the extraction solvent. Quercetin was employed as the reference standard for total flavonoid determination. Streptococcus mutans was used as the test microorganism for antibacterial evaluation. Mueller–Hinton agar (MHA) was used as the growth medium. All chemicals and reagents were of analytical grade.

B. Sample Preparation and Characterization

The collected *M. koenigii* leaves were cleaned to remove adhering impurities and washed with distilled water. The samples were air-dried at ambient temperature in the absence of direct sunlight and subsequently dried in an oven at 40 °C until a constant weight was achieved. The dried leaves were pulverized using a blender and sieved through a No. 80 mesh to obtain a uniform powder. The powdered samples were stored in airtight containers at room temperature and protected from light (Nasution et al., 2022).

Characterization of the dried leaf powder included determination of moisture content, water-soluble extractive value, ethanol-soluble extractive value, total ash content, and acid-insoluble ash content, following standard pharmacognostic procedures (Pulungan et al., 2022).

C. Phytochemical Screening

Qualitative phytochemical screening of the dried leaf powder was performed to identify the presence of secondary metabolites, including alkaloids, flavonoids, saponins, tannins, glycosides, and steroids, using standard phytochemical methods (Nurmazela et al., 2022).

D. Preparation of Ethanolic Extract

A total of 500 g of dried *M. koenigii* leaf powder was macerated with 3750 mL of 96% ethanol in a closed container and kept at room temperature for five days with occasional stirring. The mixture was filtered, and the residue was re-macerated with 1250 mL of ethanol. The combined filtrates were allowed to stand for 48 h and subsequently concentrated under reduced pressure using a rotary evaporator at 50 °C to obtain the ethanolic extract (Satria,

Harahap, Dalimunthe, Hertiani, et al., 2023; Syahputra et al., 2023).

E. Determination of Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride colorimetric method with quercetin as the reference standard. A quercetin stock solution (1000 µg/mL) was prepared and serially diluted to obtain working solutions ranging from 20–100 µg/mL. Each aliquot was mixed with ethanol, 10% aluminum chloride solution, 1 M sodium acetate, and distilled water, followed by incubation at room temperature for 30 min.

Absorbance was measured at a wavelength of 437 nm using a UV–Vis spectrophotometer. Total flavonoid content was calculated from the quercetin calibration curve and expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract) (Triyasmono et al., 2020; Yuza et al., 2023).

F. Antibacterial Activity Assay

Antibacterial activity of the ethanolic extract was evaluated using the disk diffusion method against Streptococcus mutans. A standardized bacterial suspension equivalent to 0.5 McFarland was uniformly spread onto Mueller–Hinton agar plates. Sterile paper disks were impregnated with extract concentrations of 10%, 30%, and 50%, while DMSO served as the negative control and amoxicillin (25 µg) as the positive control.

The plates were incubated at 37 °C for 18–24 h. Antibacterial activity was assessed by measuring the diameter of the inhibition zones using a digital caliper. All assays were performed in triplicate (Nasri et al., 2024; Rani et al., 2023).

G. *In Silico* Toxicity Prediction

In silico toxicity prediction was conducted to evaluate the pharmacokinetic and toxicological profiles of major flavonoid compounds present in *M. koenigii* leaves. Computational analysis was performed using widely accepted online prediction platforms, including PubChem, pkCSM, and ProTox-II. These tools were selected due to their extensive validation, ability to predict absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, and frequent application in early-stage drug safety assessment.

All computational analyses were carried out using a personal computer operating on a Windows-based system. The in silico approach was employed as a complementary method to experimental studies, aiming to provide preliminary safety information while minimizing the

need for extensive in vitro and in vivo toxicity testing (Nugraha et al., 2024; Wangsasaputra et al., 2023).

H. Preparation of Compounds for In Silico Toxicity Analysis

Major flavonoid compounds reported to be present in *M. koenigii* leaves, including quercetin, apigenin, kaempferol, rutin, catechin, and myricetin, were selected for in silico toxicity evaluation. The chemical structures of these compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

For each compound, Canonical SMILES representations were retrieved from PubChem and used as molecular input for subsequent in silico analyses. The use of Canonical SMILES ensures standardized and reproducible molecular descriptions, allowing consistent prediction of pharmacokinetic and toxicological properties across different computational platforms (Kim et al., 2016).

I. Toxicity Prediction Using pkCSM Tools

The pkCSM online platform (http://biosig.unimelb.edu.au/pkcsm/prediction) was used to predict the ADMET properties of the selected flavonoid compounds. Canonical SMILES of each compound were submitted to the pkCSM interface, and the ADMET prediction option was selected to obtain pharmacokinetic and toxicity-related parameters.

The predicted absorption and distribution parameters included water solubility, volume of distribution at steady state (VDss), fraction unbound, blood–brain barrier (BBB) permeability, and central nervous system (CNS) permeability. Metabolic properties were evaluated based on interactions with key cytochrome P450 enzymes, while excretion was assessed through total clearance predictions. In addition, toxicity-related endpoints such as AMES toxicity, hepatotoxicity, hERG channel inhibition, acute oral toxicity, and chronic toxicity parameters were analyzed.

The pkCSM platform employs graph-based signatures to model the relationship between chemical structure and biological behavior, enabling reliable prediction of ADMET characteristics from molecular input alone. The results obtained from pkCSM were used to provide an initial safety profile of the major flavonoid constituents of *M. koenigii,* supporting their potential suitability for further pharmacological development (Abhishek et al., 2023; Nugraha et al., 2024).

III. RESULTS AND DISCUSSION

A. Characterization and Phytochemical Screening of *M. koenigii* Leaves

The maceration method was employed for the extraction of *M. koenigii* leaves due to its simplicity and ability to preserve thermolabile bioactive compounds. The quality of the plant material was evaluated through physicochemical characterization of the simplicia in accordance with the standards of the Indonesian Materia Medika. The results of simplicia characterization are presented in Table I.

The moisture content of the simplicia was 7%, which is below the maximum limit of 10%, indicating good storage stability. The water-soluble extractive value (28%) and ethanol-soluble extractive value (15.3%) exceeded the minimum required standards, suggesting the presence of a considerable amount of polar and semi-polar bioactive constituents. The total ash content (8.3%) and acid-insoluble ash content (1%) were within acceptable limits, reflecting low inorganic contamination. Overall, these results confirm that the *M. koenigii* leaves simplicia met the quality requirements for further pharmacognostic evaluation.

Phytochemical screening results (Table II) revealed the presence of flavonoids, alkaloids, saponins, tannins, steroids, and glycosides in both the dried leaf powder and ethanolic extract. The detection of these secondary metabolites supports the pharmacological potential of *M. koenigii*, particularly its antibacterial activity, which is often associated with flavonoids and phenolic compounds.

Table I. Result of examination of characterization of *M. koenigii* leaves simplicia

Parameter	Result (%)	MMI 1989 requirements (%)
Water content	7	≤ 10
Water soluble essence content	28	≥ 25.5
Solubility of essence in ethanol	15.3	≥ 12
Ash content	8.3	≤ 15
Acid insoluble ash content	1	≤ 1.5

Table II. Results of examination of phytochemical screening of *M. koenigii* leaves simplicia

Class of chemical compounds	Simplicia	ethanolic extract
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Steroids	+	+
Glycoside	+	+

Note: (+) = contains the substance under investigation, and (–) = does not contain the substance under investigation

B. Determination of Total Flavonoid Content

Quantification of total flavonoid content was performed using the aluminum chloride colorimetric method with quercetin as the reference standard. A linear calibration curve was obtained over the concentration range of 20–100 µg/mL, yielding the regression equation y = 0.0079x + 0.0421 with a correlation coefficient (r^2) of 0.993, indicating excellent linearity (Figure 1).

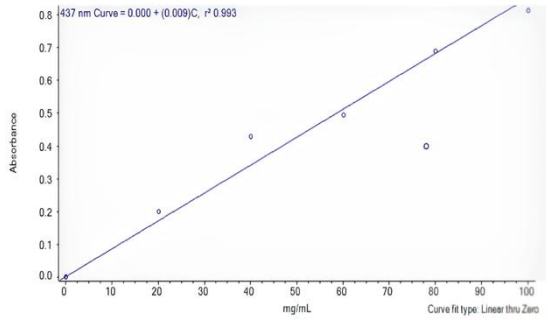

Figure 1. Quercetin calibration curve

The total flavonoid content of the ethanolic extract of *M. koenigii* leaves was determined based on six independent measurements. As shown in Table III, the extract exhibited a high total flavonoid content of 281.83 ± 10.23 mg QE/g extract, with a relative standard deviation (RSD) below 5%, demonstrating good precision and reliability of the analytical method.

Flavonoids are well-recognized secondary metabolites with diverse pharmacological activities, including antioxidant, anti-inflammatory, and antibacterial effects. The high flavonoid content observed in this study suggests that *M. koenigii* leaves ethanolic extract possesses a strong biochemical basis for its biological activity, particularly in inhibiting microbial growth (Ayipo et al., 2023; Mutha et al., 2021). The findings of measuring the *M. koenigii* leaves extract's total flavonoid concentration are displayed in Table III.

Table III. Results of examination of phytochemical screening of *M. koenigii* leaves simplicia

No	Sample Weight (g)	Absorbance	Concentration (µg/ml)	Total flavonoid content of ethanolic extract (mgQE/g)	Actual rate (mgQE/g Ethanolic Extract)
1	0.025	0.282	30.3670	303.670	281.834 ± 10.234 mg QE/g extract
2	0.025	0.282	30.3670	303.670	
3	0.025	0.271	28.9746	289.746	
4	0.025	0.255	26.9493	269.493	
5	0.025	0.265	28.2151	282.151	
6	0.025	0.268	28.5949	285.949	

*The relative standard deviation (RSD) of the total flavonoid content measurement was calculated to evaluate the precision of the analytical method and was found to be within acceptable limits (<5%)

C. Antibacterial Activity against Streptococcus mutans

The antibacterial activity of the ethanolic extract of *M. koenigii* leaves against the Gram-positive bacterium Streptococcus mutans was evaluated using the disk diffusion method. The results are summarized in Table IV. No inhibition zone was observed for the DMSO negative control, confirming that the solvent had no antibacterial effect.

The extract exhibited clear inhibition zones at all tested concentrations (10%, 30%, and 50%). The average inhibition zone diameters increased with extract concentration, measuring 14.4 mm, 16.5 mm, and 19.4 mm, respectively, indicating a concentration-dependent antibacterial effect. According to the Indonesian Pharmacopoeia V classification, inhibition zone diameters within the range of 10–20 mm are categorized as strong antibacterial activity.

The positive control, amoxicillin (25 µg), produced a larger inhibition zone with an average diameter of 22.0 mm, classified as very strong antibacterial activity. Although the inhibitory effect of the extract was lower than that of the antibiotic control, the consistent increase in antibacterial activity with rising extract concentration demonstrates the effectiveness of the bioactive compounds present in the extract.

The antibacterial activity of *M. koenigii* leaf ethanolic extract is likely attributable to its high flavonoid content. Flavonoids are known to exert antibacterial effects through multiple mechanisms, including disruption of bacterial cell membrane integrity, complex formation with extracellular proteins, inhibition of nucleic acid synthesis, and interference with bacterial energy metabolism. These mechanisms collectively contribute to the inhibition of bacterial growth and biofilm formation by Streptococcus mutans (Donadio et al., 2021).

Table IV. *M. koenigii* leaves ethanolic extract antibacterial activity test.

Sample	Inhibition zone (mm) Replication			Average zone of inhibition (mm)	Categorize
	1	2	3		
Negative control	-	-	-	-	-
10% extract	16.6	13.4	13.3	14.4	Strong
30% extract	17.6	15.7	16.3	16.5	Strong
50% extract	20.5	18.7	19.1	19.4	Strong
Positive control	21.0	23.0	22.0	22.0	Very strong

Negative control: DMSO; positive control: Amoxicillin 25 µg

According to the Indonesian Pharmacopoeia V, inhibition zone diameters of 10–20 mm are classified as strong antibacterial activity, while diameters equal to or greater than 20 mm are considered very strong. Based on this classification, the ethanolic extract of *M. koenigii* leaves at a concentration of 50% demonstrated strong antibacterial activity against Streptococcus mutans, supporting its potential as a natural antibacterial agent for oral health applications (Haro et al., 2020; Satria, Harahap, Dalimunthe, Septama, et al., 2023).

D. Toxicity Prediction of Compounds

Several flavonoid compounds, including quercetin, apigenin, kaempferol, rutin, catechin, and myricetin, have been reported as bioactive constituents of *M. koenigii* leaves based on previous phytochemical and GC–MS analyses (Rajendran et al., 2014; Ansari & Mahapatra, 2021). This study employed silico toxicity prediction to evaluate the safety profile of these major flavonoid constituents. Utilizing the pkCSM and ProTox-II online platforms, toxicity and ADME properties of the selected compounds were assessed. Utilizing the HP Pavilion Gaming Laptop 15-ec2xxx 64-bit laptop equipment, the pKCSM online tool application (http://biosig.unimelb.edu.au/pkcsm/), and the Protox application online tool (https://tox-new.charite.de/protox_II/), we conducted toxicity profile testing and ADME analysis. The findings from ADME and toxicity profiling are shown in Tables V-VII.

The evaluation of pharmacokinetic activity and toxicity may be made more accessible using computational tools, which include the assessment, simulation, and prediction of chemical toxicity. Previous research has shown that the PK-CSM model can accurately predict the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) features of a chemical (Sulistyowaty et al., 2023). The results shown in Table V demonstrate that apigenin has a water solubility of -3.329 log mol/L, which is a relatively low value compared to the solubility of many other components. Given this data, it seems that the lower solubility of apigenin may cause difficulties in the process of intraoral absorption of the compound. There is a maximum value of -3.117 log mol/L for catechin's water solubility, which indicates that catechin has outstanding solubility in water overall. As an additional point of interest, the water solubility of the remaining components is as follows: -3.04 for kaempferol, -2.925 for quercetin, -2.915 for Myricetin, and -2.892 for rutin.

The steady-state volume of distribution is the volume that is necessary in theory for the uniform distribution of the complete dosage of the medication, which ultimately results in the same concentration as the systemic circulation (Paliwal et al, 2024). A greater quantity of the drug is distributed to the plasma tissue in proportion to the value of the volume of distribution of the drug (VDss), which rises (Gaohua et al., 2023). For the volume of distribution to be considered low, the Log VDss value needs to be lower than -0.15. Therefore, given that the Log VDss value is more than 0.45, the distribution volume is considered good quality. The VDss value of rutin is the highest among the six components, measuring 1.663. This is followed by the VDss value of 1.559 for quercetin, 1.317 for myricetin, 1.274 for kaempferol, 1.027 for catechin, and 0.822 for apigenin. To predict the distribution of the substance, it is necessary to evaluate parameters associated with the steady-state volume of distribution (VDss) (Poulin et al., 2024). Because the compound in question has a VDss value lower than 0.7, the results of the distribution prediction project showed that the compound in question has poor distribution. Because this chemical does not display any metabolic activity via the key liver enzyme CYP2D6, the likelihood of adverse drug interactions occurring through this particular metabolic route is significantly reduced (Eid et al., 2024; Nofziger et al., 2020). It is essential to emphasize this fact. Apigenin has the most efficiency in its removal from the human body compared to the other five components, as shown by the total clearance rate of 0.566 log ml/min/kg that was measured for it. This may be deduced from the observation that it has the highest clearance rate.

Table V. Profile ADME

Property	Model Name	Predicted Value						Unit
		Quercetin	Apigenin	Kaempferol	Rutin	Catechin	Myricetin	
Absorption	Water Solubility	-2.925	-3.329	-3.04	-2.892	-3.117	-2.915	Numeric (log mol/L)
Distribution	VDss (human)	1.559	0.822	1.274	1.663	1.027	1.317	Numeric (log L/kg)
Metabolism	CYP2D6 Substrate	No	No	No	No	No	No	Categorical (Yes/No)
Excretion	Total Clearance	0.407	0.566	0.477	-0.369	0.183	0.422	Numeric (log ml/min/kg)

Table VI. Prediction toxicity (pKCSM)

Property	Model Name	Predicted Value						Unit
		Quercetin	Apigenin	Kaempferol	Rutin	Chatecin	Myricetin	
Toxicity	AMES toxicity	No	No	No	No	No	No	Numeric (log ml/min/kg)
Toxicity	Max. tolerated dose (human)	0.499	0.328	0.531	0.452	0.438	0.51	Categorical (Yes/No)
Toxicity	hERG I inhibitor	No	No	No	No	No	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	No	No	No	Yes	No	No	Numeric (log mg/kg/day)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.471	2.45	2.449	2.491	2.428	2.497	Categorical (Yes/No)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	2.612	2.298	2.505	3.673	2.5	2.718	Categorical (Yes/No)
Toxicity	Hepatotoxicity	No	No	No	No	No	No	Numeric (mol/kg)
Toxicity	Skin Sensitisation	No	No	No	No	No	No	Numeric (log mg/kg_bw/day)
Toxicity	T. Pyriformis toxicity	0.288	0.38	0.312	0.285	0.347	0.286	Categorical (Yes/No)
Toxicity	Minnow toxicity	3.721	2.432	2.885	7.677	3.585	5.023	Categorical (Yes/No)

Table VII. Toxicity class (protox online)

Parameters	Quercetin	Apigenin	Kaempferol	Rutin	Catechin	Myricetin
Predicted LD 50 (mg/kg)	159	2500	3919	5000	10000	159
Predicted toxicity class	3	5	5	5	6	3
Average similarity (%)	100	81.24	82.46	100	100	100
Prediction accuracy (%)	100	70.97	70.97	100	100	100

The ability of Pro-Tox II to predict the amount of oral toxicity, organ toxicity (particularly hepatotoxicity), toxicological endpoints (including mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity), toxicity pathways, and target toxicity is one of the many benefits of this particular test (Ji et al., 2024; Frydrych & Jurowski, 2024; Nugraha et al., 2024). The data make it easier to identify molecular pathways that could be involved in the development of toxicity. There are numerical numbers that are used to symbolize the replies. The categorizing method known as the Globally Harmonized method (GHS) breaks down the toxicity of compounds into six distinct groups (Allen et al., 2019; Arowolo & Suvorov, 2024; Zwickl et al., 2022). Class I compounds have a lethal dose (LD50) value that is five or lower. Compounds that have LD50 values that are more than five but lower than fifty are classified as Class II. Classes III are comprised of substances

that have LD50 values that are higher than 50 but lower than 300. Some chemicals fall under Class IV and have LD50 values ranging from 300 to 2000. Compounds with LD50 values that are more than 2000 but do not exceed 5000 are classified as belonging to Class V. LD50 values that are more than 5000 are seen in substances classed as Class VI. There is a correlation between higher LD50 values and decreased toxicity (Banerjee et al., 2018).

The LD50 values of all of the chemicals included in Table VI range from 159 mg/kg to 10000 mg/kg, which highlights that these drugs are categorized into distinct groups. With a value of 10,000 mg/kg, catechin has the highest LD50 value, which indicates that it is highly safe and non-toxic. This is because catechin is included in the categorization of class 6, which indicates that it is included in the category. Following that is rutin (5000 mg/kg), Kaempferol (3919 mg/kg), and Apigenin (2500 mg/kg), all of which are included in the categorization class 5 with the same meaning, which is to say that they are safe and are included in the category of non-toxic substances. Therefore, Quercetin and Myricetin, which have a dosage of 159 mg/kg, are classified as category 3 and continue to be in the safe, non-toxic category. With class 4 showing mild toxicity and class 3 indicating moderate toxicity, the toxicity classification system suggests that even while there is a certain amount of risk, it is not too high; still, it is exceptionally vital to exercise care (McCarty et al., 2020; Ridwanto et al., 2023; Sigurnjak Bureš et al., 2021).

An examination of the hepatotoxic characteristics reveals that none of the components are toxic to the liver, which means they are suitable for usage over an extended period. In the course of the safety assessment, it is essential to consider that the AMES test reveals that none of the components exhibit any signs of genetic toxicity. On the other hand, these substances do not produce toxicity to hERG I inhibitors; nevertheless, they can cause toxicity to hERG II (only rutin demonstrates toxicity to this mechanism), which may affect the heart's function. In the most crucial aspect, the likelihood of skin sensitization is low, which indicates that this chemical may be applied directly to the skin without causing any adverse effects (Audebert et al., 2023; Saponara et al., 2021; Kodavanti & Mehendale., 2020).

The high total flavonoid content observed in the ethanolic extract of *M. koenigii* leaves is closely associated with its strong antibacterial activity against Streptococcus mutans. Flavonoids are known to exert antibacterial effects through multiple mechanisms, including disruption of bacterial cell membranes,

inhibition of nucleic acid synthesis, and interference with bacterial energy metabolism. Furthermore, the in silico toxicity prediction results demonstrated that the major flavonoid constituents possess favorable ADMET profiles and low toxicity risks. Taken together, these findings indicate that the antibacterial efficacy of the extract is supported by both its phytochemical composition and its predicted safety profile, highlighting its potential as a natural and safe antibacterial agent for oral health applications.

IV. CONCLUSION

The ethanolic extract of *Murraya koenigii* (L.) Spreng leaves contains various bioactive secondary metabolites, including alkaloids, flavonoids, saponins, tannins, steroids/triterpenoids, and glycosides. The extract exhibited strong antibacterial activity against Streptococcus mutans at concentrations of 10%, 30%, and 50%, along with a high total flavonoid content of 281.83 ± 10.23 mg QE/g extract, supporting its antibacterial potential.

In silico toxicity prediction of six major flavonoid constituents indicated generally low toxicity profiles, with predicted LD_{50} values ranging from 159 to 10,000 mg/kg, absence of hepatotoxicity, and favorable ADMET characteristics. Collectively, these findings suggest that *M. koenigii* leaf ethanolic extract possesses a balanced efficacy–safety profile and holds promise as a natural flavonoid-rich antibacterial candidate for oral health applications, warranting further formulation and in vivo studies.

CONFLICT OF INTEREST

All authors declare that there is no conflict of interest in this research.

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