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Research Paper

Computational Chemical Interactions Study and Characterization of Rutin-Malic Acid and Rutin-Nicotinamide Binary Mixture by Microwave Irradiation

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Abstract
Rutin is a flavonol glycoside with potent antioxidant activities. However, its low water solubility (122.6–126 μg/mL) limits rutin applications. Binary mixtures of rutin-malic acid (R-MA) and rutin-nicotinamide (R-NIC) are among the strategies used to improve rutin's solubility. This research aimed to evaluate the chemical interactions and physical characteristics of both binary mixtures (in equimolar ratios). The chemical Interactions of R-MA and R-NIC were studied using the molecular docking method (AutoDock4). The results showed that the lowest binding energies of R-MA and R-NIC were -1.18 kcal/mol and -2.77 kcal/mol, respectively, with evidence of hydrogen bonding and π-π stacking interactions. The binary mixture of R-MA (1:1) and R-NIC (1:1) were prepared by microwave irradiation method. R-MA (1:1) and R-NIC (1:1) were prepared by slurrying under continuous stirring at 120 rpm, 70°C for 4 min and the microwave irradiation energy was set to 300 Watt for 5 min of time exposure. The formed binary mixture physical characteristics were evaluated using PXRD, DSC, FTIR, and SEM. The characterization results indicated the presence of binary mixture. Compared to the physical mixture and their parent components (rutin, MA/NIC), diffractograms, thermograms, and IR spectrums respectively showed that the binary mixture has a similar crystallinity profiles, decreased endothermic peaks, and not significantly wavenumber shifts. Morphology analysis depicted that rutin, MA, and NIC are acicular, plate, and columnar shape respectively, while both binary mixture showed an agglomerated fine needle-like, irregular shape, and rough surface particles. This research shows that the binary mixture of R-MA and R-NIC was successfully formed using this method, in accordance with chemical interaction predictions.

Keywords
Rutin, Malid Acid, Nicotinamide, Binary Mixture, Molecular Docking, Microwave Irradiation

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1. INTRODUCTION

Rutin ($C_{27}H_{30}O_{16}$) is a high molecular weight (610.521 gram/-mol) flavonol glycoside consisting of the aglycone quercetin and the disaccharide rutinose, which is widely found in plants such as apples, oranges, buckwheat, sprouts, and tea. The unique structure of quercetin glycoside bound to rutinose at the C-3 position of the C ring makes rutin chemically stable. Rutin exhibits strong antioxidant and immunomodulatory effects, including anti-inflammatory, cardioprotective, neuroprotective, antidiabetic, and so on without showing pro-oxidant and mutagenic activities like its aglycone, quercetin (Ademosun et al., 2016; Ganeshpurkar and Saluja, 2017; Negahdari et al., 2021). However, rutin is classified as a Biopharmaceutical Classification System class II polyhydroxy compound with low water solubility (122.6-126 μg/mL; 0.8 mg/mL), resulting in low bioavailability (C_max = 256.7-269 ng/mL) (Liu et al., 2020; Rahman et al., 2020). Rutin also violates more than one of Lipinski's Rules of Five (RO5), which contributes to low oral absorption and limited intestinal permeability (Wang et al., 2022; Tobar-Delgado et al., 2023). Due to its 10 hydrogen bond donors (HBD) and 16 hydrogen bond acceptors (HBA), rutin can easily interact and form hydrogen bonds with hydrophilic matrices or water (Strugała et al., 2017; Yusuf et al., 2023; National Center for Biotechnology Information, 2025).

Mixtures of active pharmaceutical ingredients (API) with excipients may lead to both physical and chemical interactions. Typically, physical interactions between pharmaceutical ingredients can be classified as eutectic mixtures (conglomerates), cocrystals (molecular compounds), and solid solutions. An eutectic mixture is a specific type of binary system that consists of two pharmaceutical components with a lower melting point than their individual component. Various strategies such as nanocrystals, cocrystallization, and solid dispersions have been utilized to enhance the solubility of rutin (Zhang et al., 2024).

However, these methods often involve complex processes or stability issues. An alternative approach is the formation of binary mixtures, which allow potential physicochemical interactions between rutin and small molecules as complementary components (Winantari et al., 2017). Binary systems are simpler and more predictable than crystal engineering approaches like salt formation or cocrystallization, and may still offer insights into improved dissolution behavior or stability through favorable non-covalent interactions. Earlier research indicated that the interaction between API and excipients in both binary and simple eutectic mixtures could improve the physicochemical properties of API (Zaini et al., 2015). Microwave irradiation has been utilized for various organic due to its advantages, such as more cost-efficient, environmentally friendly, and capable to produce high-purity products in a short time using green solvent (Ahuja et al., 2020; Sakhiya and Borkhataria, 2024).

Previous studies have shown that compounds such as malic acid (MA) and nicotinamide (NIC), both classified as Generally Recognized as Safe based on Food and Drug Administration (GRAS FDA) and have functional groups capable of hydrogen bonding and π-π tacking interactions with bioflavonoid compounds. The presence of a specific hydrogen donor (i.e., carboxylic acid) and hydrogen acceptor (i.e., amine or amide) which capable to form hydrogen bonding interactions is a critical factor for small-molecule component selection (Karagianni et al., 2018). MA and NIC have demonstrated compatibility in crystal engineering systems, but their role in binary mixtures is very limited. A computational chemical interaction study using the molecular docking method was performed using AutoDock4 software to obtain the binding energy values and describe the intermolecular interactions in rutin-malic acid (R-MA) and rutin-nicotinamide (R-NIC) (Lemli et al., 2024). AutoDock4 software can search for several API binding conformations even though the actual binding site is unknown (Che and Zhang, 2025). Although molecular docking is conventionally used for protein-ligand interaction modeling, it still can be applied to predict the possibility of non-covalent interactions such as hydrogen bonding and aromatic stacking in small-molecule binary systems, providing early insight into their intermolecular affinity (Dhibar et al., 2023).

A few researchers have focused on the potential of binary mixtures for improving physicochemical characteristics of flavo noids. There have been limited studies concerned on molecular-level interactions between rutin and small-molecule components through computational study and binary systems as solid-state approaches. The formation of binary mixtures is expected to increase the solubility of rutin. One of which method that can produce binary mixtures quickly and environmentally friendly (using water as a non-toxic polar solvent) is microwave irradiation. This method is suitable for compounds with many polar molecules, such as rutin. The polar molecules can absorb microwaves and align themselves with the electric field, resulting in ionic conduction, which generates heat, triggers the atomic vibrations, and chemical interactions (especially hydrogen bonding) between rutin and small-molecule components (Katre, 2024). Ahuja et al. (2020) was prepared sulfamethazine–nicotinamide cocrystal with microwave-assisted slurry conversion technique and drug distribution improvement also efficiency for manufacturing of cocrystal using this method was reported. Chemical reaction is much faster using microwaves due to an influence of the solution as a chemical reaction mediator. Therefore, this research intends to exploring the intermolecular interactions between rutin and two selected small-molecule components (malic acid and nicotinamide) in binary mixtures using molecular docking, produce the binary mixture system, and evaluating their physical characteristics parameters such as crystallinity, thermal analysis, vibrational transition, and morphology through PXRD, DSC, FTIR, and SEM respectively.

2. EXPERIMENTAL SECTION

2.1 Materials

The materials used in this research included 3D conformer structures of the API (rutin) and coformers (nicotinamide and malic acid) obtained from the PubChem database (.sdf format), rutin (pro analysis grade Sigma-Aldrich®, USA), DL-malic acid (pro analysis grade Merck®KGaA, Germany), nicotinamide (pro analysis grade Sigma-Aldrich®, USA), and aqua demineralisata.

2.2 Molecular Docking Method

The chemical interactions occurring between rutin-malic acid (R-MA) and rutin-nicotinamide (R-NIC) were illustrated using molecular docking method. The software used included AutoDock4 (AutoDockTools), Avogadro, Discovery Studio BIOVIA, and Command Prompt. The chemical structures of rutin, malic acid, and nicotinamide were obtained from the PubChem database. Preparation of rutin and MA/NIC was carried out by adding polar hydrogen atoms, calculating Gasteiger charges, and adding Kollman charges. The optimal poses or conformations (with the lowest binding energies) of R-MA and R-NIC were visualized using BIOVIA software to depicted their chemical interactions.

2.3 Sample Preparations

2.3.1 Preparation of R-MA and R-NIC Binary Mixture

Rutin, malic acid, and nicotinamide were carefully weighed in equimolar ratios using an Ohaus analytical balance. Each component was dissolved in aqua demineralisata by slurrying with a Heidolph MR Hei-Tec magnetic stirrer at 120 rpm and 70°C until homogeneous. The R-MA (1:1) and R-NIC (1:1) slurries were put in Sharp model R-753GX (BS) microwave and was irradiationed for 5 minutes, 300 Watt.

2.4 Characterizations

2.4.1 Powder X-ray Diffractometry (PXRD)

Binary mixture R-MA/NIC (1:1), physical mixture R-MA/NIC (1:1), pure MA/NIC, and pure rutin were characterized using Malvern PANalytical Compact XRD Aeris 600 Watt at room temperature 25°C/RH 54%. All samples were flattened in the

Table 1. Binding Energy of Rutin–Malic Acid and Rutin–Nicotinamide (Conformation 1)

Conformation	Binding Energy (kcal/mol)
Rutin–Malic Acid	-1.18
Rutin–Nicotinamide	-2.77

sample holder to prevent particle orientation. Measurement conditions were set as follows: the light beam was targeted at the Cu metal anode X-ray tube, Kα filter, 40 kV and 40 mA voltage. Crystallinity data were obtained by continuous scanning at 5-40° diffraction angle for organic compound, increment rate of 0.2-0.5°/minute and a scanning speed of 10° per minute (Nawatila et al., 2017).

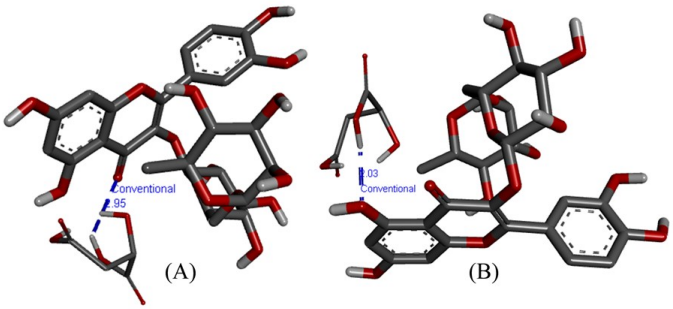

Figure 1. Intermolecular Interaction Visualization of R-MA at (A) 2.95 Å, (B) 2.03 Å

Table 2. Visualization of R–MA Chemical Interactions

Description	Distance (Å)	Interaction Type
	2.95	Hydrogen-bonding
	2.03	Hydrogen-bonding

Table 3. Visualization of R–NIC Chemical Interactions

Description	Distance (Å)	Interaction Type
	2.06	Hydrogen-bonding
	2.07	Hydrogen-bonding
	2.98	Carbon hydrogen-bonding
	4.56	π–π Stacking

2.4.2 Differential Scanning Calorimetry (DSC)

Binary mixture R-MA/NIC (1:1), physical mixture R-MA/NIC (1:1), pure MA/NIC, and pure rutin were analyzed by Mettler Toledo DSC 1 STARe SYSTEM. Approximately 4.0 mg of each sample was weighed using an Ohaus analytical balance, placed in an aluminum pan, and analyzed. The thermograms were obtained to determine the thermal behavior and polymorphism phenomenon in 30-300°C temperature range with a increment rate or heating speed of 10°C/min under nitrogen purging (Winantari et al., 2017).

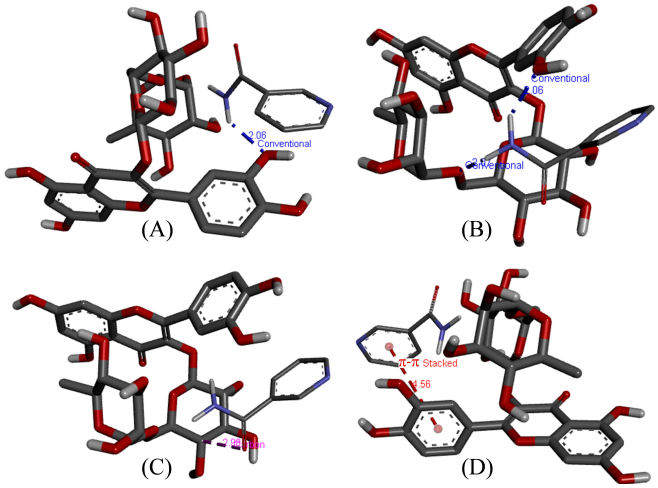

Figure 2. Intermolecular Interaction Visualization of R-NIC at (A) 2.06 Å, (B) 2.07 Å, (C) 2.98 Å, (D) 4.56 Å

2.4.3 Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectrum of binary mixture R-MA/NIC (1:1), physical mixture R-MA/NIC (1:1), pure MA/NIC, and pure rutin were obtained by JASCO 4200 FT-IR. KBr pellet method with 100-200 mg of KBr for initial preparation of solid samples was used. About 1-2 mg of each sample was weighed, mixed, and pressed thoroughly with potassium bromide (KBr) (1:100) to produce a pellet disc. The disc was scanned at 400 to 4000 cm^{-1} wavenumbers (Sulistyowaty et al., 2024b).

2.4.4 Scanning Electron Microscopy (SEM)

An FEI Inspect S50 SEM was used to depicted the morphology of binary mixture R-MA/NIC (1:1), physical mixture R-MA/NIC (1:1), pure MA/NIC, and pure rutin. Approximately 10 mg of each powder sample was placed on an aluminum sample holder with a 10 nm thick gold coating (organic compound). The samples were observed at 2500× and 5000× magnification with an accelerating voltage of 20 kV and 12 mA (Imtihani et al., 2017).

3. RESULTS AND DISCUSSION

3.1 Chemical Interactions Study using Molecular Docking Method

Gibbs free energies (ΔG) or binding energies, types of chemical interactions, and the likelihood of binary system formation between the API and small-molecule components can be identified computationally using molecular docking. This method clarifies the potential chemical interactions between them (Dhibar et al., 2023; Lemli et al., 2024). Rutin, as an API, is a polyhydroxy compound with ≥500 Daltons (610.521 grams/mol) MW, ≥5 HBD (10 hydrogen bond donors), and ≥10 HBA (16 hydrogen bond acceptors). It violates more than

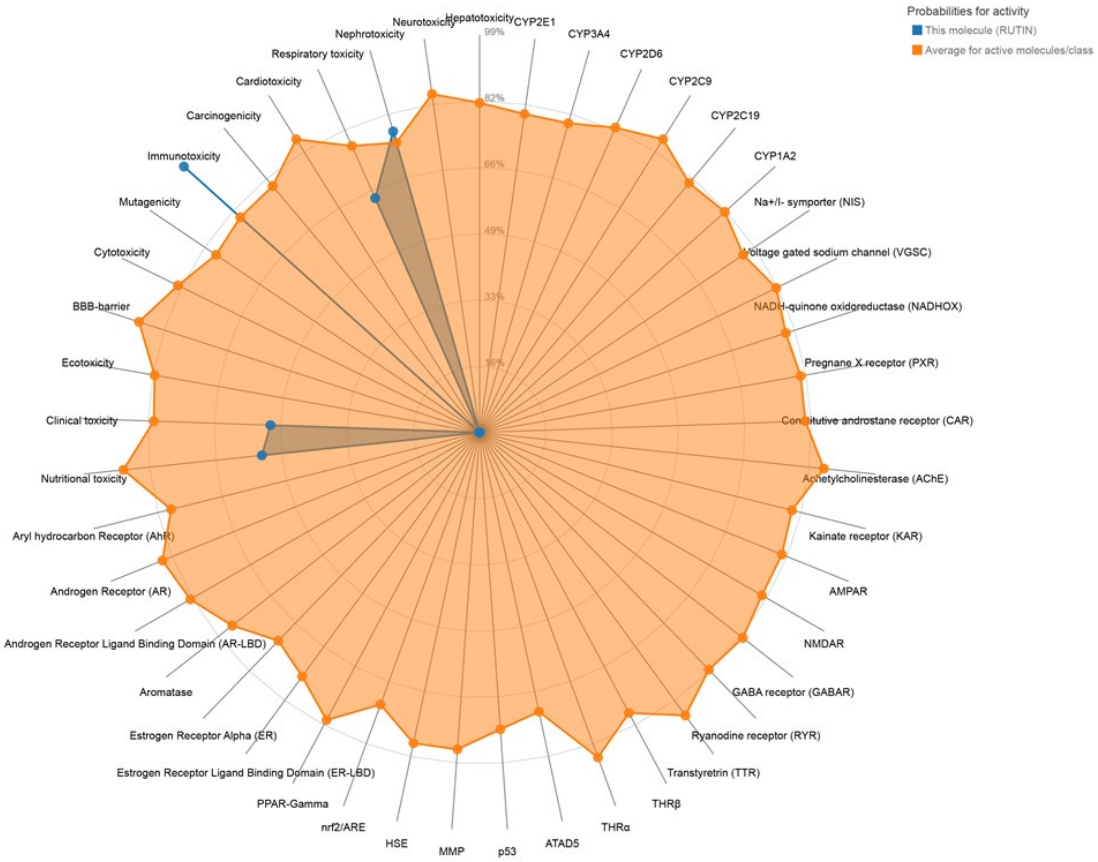

Figure 3. Radar Chart of Rutin's Toxicology Prediction

Table 4. Vibrational Transition of R–MA FTIR Spectrum

Functional Groups (cm^{-1})	Rutin	Malic Acid	R–MA (1:1) Physical Mixture	R–MA (1:1) Binary Mixture
O–H stretch H-bonded	3428.81	3447.14	3433.64	3433.64
C=O stretch	1653.66	1733.69	1733.69	1737.55
C=C stretch aromatic	1598.70	–	1603.52	1604.48
CH_3 bend	1456.96	–	1460.81	1462.74
	1362.46		1367.28	1369.21
CH_2 bend	–	1440.57	–	–
C–O stretch	1295.93	1289.18	1300.75	1302.68
	1203.36		1208.19	1209.15
C–O–C stretch	1063.55	–	1067.41	1068.37
	1014.37		1018.23	1020.16

one Lipinski's Rules of Five, resulting in low oral absorption and limited intestinal permeability.

Rutin's solubility can be enhanced by malic acid and nicotinamide. The phenolic hydroxyl groups of rutin can form hydrogen-bond interactions with the carboxyl group of malic acid (3 HBD; 5 HBA) and the amide group of nicotinamide (1 HBD; 2 HBA). The carboxyl and amide groups can act as hydrogen bond donors and acceptors, respectively, to the phenolic hydroxyl group of rutin, and vice versa. The pyridine ring ($N_{aromatic}$) of nicotinamide can also form π-π stacking interactions with the aromatic ring of rutin, thereby shielding its hydrophobic part of rutin from water (Vasisht et al., 2016, 2017).

The grid box-based method in AutoDock4 software allows rapid calculation of binding energies and the search for available conformational space for coformers to bind around the

Table 5. Vibrational Transition of R-NIC FTIR Spectrum

Functional Groups (cm^{-1})	Rutin	Nicotinamide	R-NIC (1:1) Physical Mixture	R-NIC (1:1) Binary Mixture
O–H stretch H-bonded	3428.81	–	3426.89	3425.92
NH_2 stretch	–	3368.07 3159.79	3367.10	3369.03
C=O amide stretch	–	1682.59	–	1679.70
C=O stretch	1653.66	–	1654.63	1655.59
C=C stretch aromatic	1598.70 1485.88	–	1599.66	1597.74
CH_3 bend	1456.96 1362.46	–	1456.96	1455.99 1361.50
C–N primary aliphatic amide stretch	–	1423.21 1396.21	1423.21	1423.21
C–O–C stretch	1203.36 1063.55 1014.37	–	1203.36 1062.59 1014.37	1203.36 1061.62 1013.41

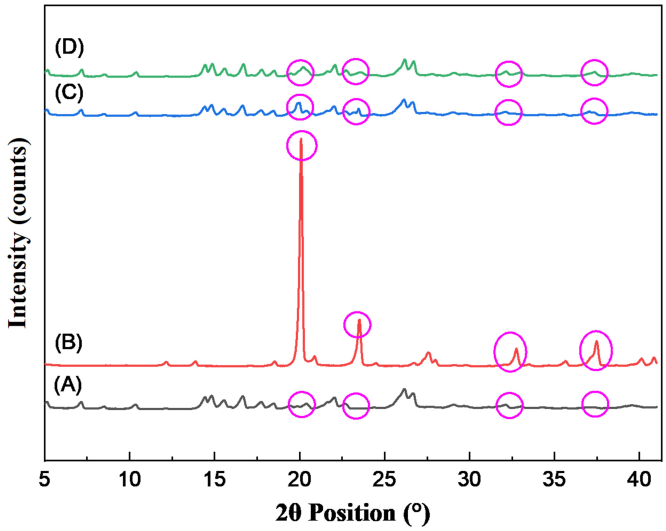

Figure 4. Diffractograms of (A) Rutin, (B) Malic Acid, (C) Physical Mixture of R-MA, (D) Binary Mixture of R-MA

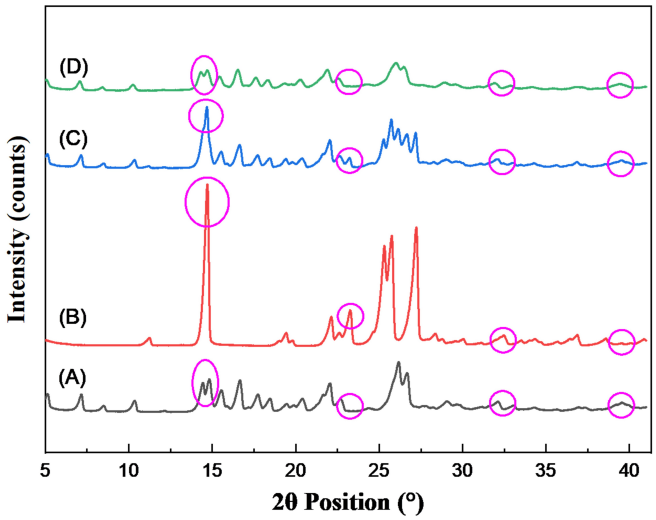

Figure 5. Diffractograms of (A) Rutin, (B) Nicotinamide, (C) Physical Mixture of R-NIC, (D) Binary Mixture of R-NIC

API. The grid box area should be focused on the naturally active rutin-small molecules binding site (grid box center 0.000). The optimal pose/conformation search from multiple populations uses a Lamarckian genetic algorithm (GA), an algorithm that allows each population to search for its minimum conformational space locally and globally (Morris et al., 2009). Gibbs free energy (ΔG) contributes to determining the direction of a chemical reaction (Jiménez and Benítez, 2024).

The molecular docking method used in this study is blind docking. This means that researchers must set a search space (grid box) large enough to cover the entire API and coformer structure. This allows AutoDock4 software to search for multiple API-coformer binding conformations even though the actual binding site is unknown. AutoDock4 is capable of depicting the chemical interactions occurring in rutin-malic acid (R-MA) and rutin-nicotinamide (R-NIC). The map types (XxYxZ) were set to a grid box 60Å× 60Å× 60Å, with 250 GA runs for 150 populations. The lower binding energy (ΔG), the more stable the intermolecular interactions and the stronger the binding affinity of R-MA and R-NIC (Dhibar et al., 2023).

Table 6. Comparison of Microwave Irradiation with Other Researchers Methods in Binary Mixture/Cocrystal Formation

API-small molecule (Systems)	Method	Condition	Conclusion	Reference
Rutin-Malic Acid and Rutin-Nicotinamide	Microwave irradiation with slurrying preparation	Irradiationing 300 W, 5 min using solvent (water), slurrying 120 rpm, 70°C, 4 min	Successful formation of R-MA and R-NIC binary mixture confirmed by molecular docking (hydrogen bonds and π- π stacking interactions), PXRD, DSC, FTIR, and SEM analysis	This research
Acetylsalicylic Acid-Theophylline (ASA-THE)	Slurry Method Under Microwave Irradiation	ASA-THE 1:1 ratio, irradiationing 600 W, 6 min (solvent-free)	Successful formation of ASA-THE cocrystal (1:1) confirmed by TG-DTG (Thermogravimetric -Derivative Thermogravimetric) and UATR-FTIR (Universal Attenuated Total Reflectance-FTIR)) analysis	(Fuliăş et al., 2014)
Sulfamethazine–Nicotinamide (SMT-NIC)	Microwave assisted slurry conversion	SMT-NIC 1:1 ratio, irradiationing 500 W using solvent (1 mL acetonitrile), slurrying 120 rpm, 70°C, 4 min	Successful formation of SMT-NIC cocrystal (1:1) confirmed by PXRD, DSC, TGA, and FTIR analysis	(Ahuja et al., 2020)
p-Methoxycinnamic Acid-Succinic Acid (PMCA-SA)	Microwave Irradiation	PMCA-SA 1:1 ratio, irradiationing 540 W, 20 min using solvent (10% w/w methanol)	Successful formation of PMCA-SA cocrystal (1:1) confirmed by PXRD, DSC, FTIR, and SEM analysis	(Sulistyowaty et al., 2024b)
p-Methoxycinnamic Acid-Caffeine (PMCA-CAF)	Microwave Irradiation	PMCA-CAF 1:1 ratio, irradiationing 450 W, 15 min using solvent (water)	Successful formation of PMCA-CAF cocrystal (1:1) confirmed by PXRD, DSC, FTIR, and SEM analysis	(Sulistyowaty et al., 2024a)
Moringa oleifera leaves extract-MCC PH102-Poloxamer 188	Microwave irradiation	1:2:0.5 and 1:4:0.5 ratio, irradiationing 300 W, 4 min, solvent-free	Successful surface solid dispersion of Moringa oleifera leaves extract-MCC PH102-Poloxamer 188 (1:2:0.5 and 1:4:0.5) confirmed by PXRD, DSC, FTIR, and SEM analysis	(Rani et al., 2024)

The best conformations of R-MA and R-NIC had the lowest binding energies of -1.18 kcal/mol and -2.77 kcal/mol, respectively as shown in Table 1. This indicates that 1 mol requires 1.18 kcal and 2.77 kcal of energy to break the bond-interaction. A negative value indicates that the chemical reaction is exothermic and proceeds spontaneously in the specified direction. When rutin, malic acid, and nicotinamide atoms interact intramolecularly, the molecules of each compound release energy to the environment to break chemical bonds, allowing intermolecular chemical interactions between the two different compounds. All interactions occur favorably, meaning that the non-covalent intermolecular interactions occurring in the donor-acceptor groups of R-MA and R-NIC are more stable and do not repel each other.

Hydrogen bonding is the strongest type of non-covalent interaction, occurring between an electronegative atom bonded to a partially positive hydrogen atom and another atom with high electronegativity and a lone pair of electrons. Figure 1 and Table 2 shows the hydrogen bonding interaction (OH···O) that occurs in rutin-malic acid (R-MA), (A) the OH···O interaction between the OH group at C1 of malic acid and the C=O group at the C4 position of the C ring of rutin (2.95 Å) and (B) the OH···O interaction between the OH group at C1 of malic acid and the 5−OH group of the A ring of rutin (2.03 Å). Figure 2 and Table 3 shows several chemical interactions that occur in rutin-nicotinamide (R-NIC), (A) hydrogen-bond interaction NH···O between the NH group of nicotinamide and the 3'−OH of routine ring B (2.06 Å), (B) hydrogen-bond interaction NH···O between the NH group of nicotinamide and the O of the β-glycosidic bond of rutin (2.07 Å), (C) carbon hydrogen-bond interaction CH···O between the CH group at C4 of rutin glucose and the C=O amide group on nicotinamide (2.98 Å), (D) π-π stacking interaction between the C=C group of the aromatic benzene ring B of rutin and

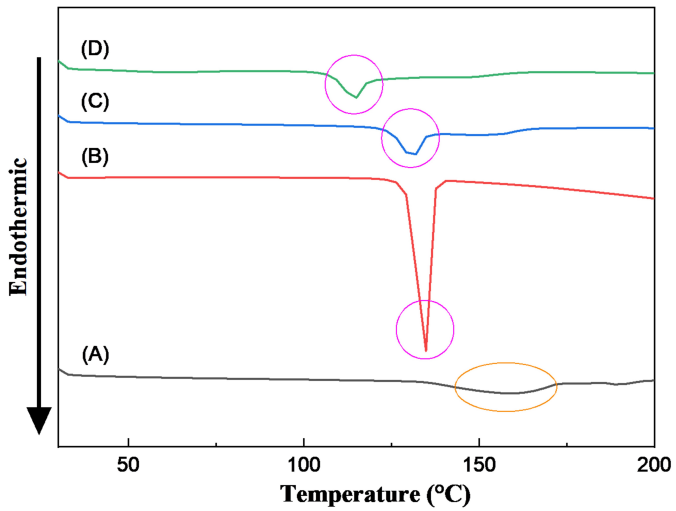

Figure 6. Thermograms of (A) Rutin, (B) Malic Acid, (C) Physical Mixture of R-MA, (D) Binary Mixture of R-MA

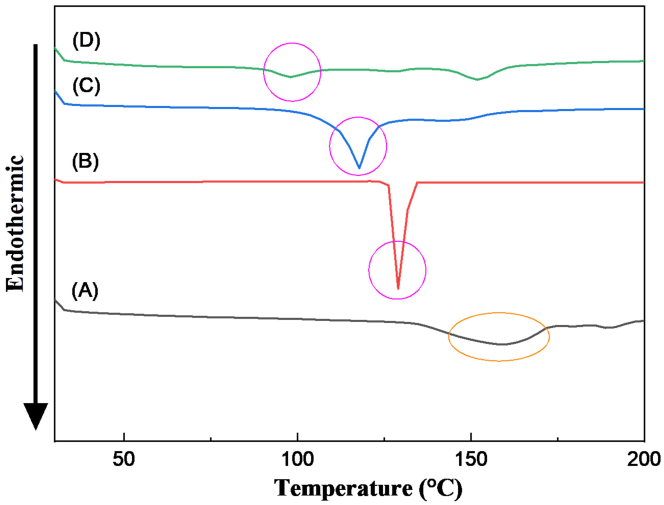

Figure 7. Thermograms of (A) Rutin, (B) Nicotinamide, (C) Physical Mixture of R-NIC, (D) Binary Mixture of R-NIC

the aromatic pyridine ring of nicotinamide (4.56 Å) which can be confirmed by the FTIR spectrum.

3.2 ADMET Analysis and Drug-Likeness Study

Pharmacodynamic evaluation including ADMET prediction and drug-likeness study. ProTox 3.0 was developed as a web-server with advanced algorithms and experimental data for multispectral toxicity prediction. ProTox 3.0 can accurately predict the toxicity of chemical compounds, saving costs and time, and reducing the use of animal models. Toxicity prediction using computational methods like this is particularly important for facilitating early toxicity assessments and minimizing the use of laboratory and animal testing in early biochemical research, also reducing the risk to humans and the environment (Banerjee et al., 2024). Toxicology radar chart on Figure 3 shows high probability that rutin is neurotoxic and nephrotoxic, hence overall this bioflavonoid compound is classified in category 5 (safe) with an LD50 of 5000mg/kg, also have a high number of violation on drug-likeness study with molsoft website.

3.3 Crystallinity Using PXRD

Crystallinity was characterized using PXRD within the 2θ angle of 5-40°. The diffractograms showed decrease intensity for the R-MA and R-NIC binary mixtures compared to their parent components (rutin, MA/NIC). At the same 2θ angle, the diffractograms of R-MA (20.0891°; 23.5229°; 32.1182°; 37.3884°) and R-NIC (14.6884°; 23.2621°; 31.9769°; 39.3661°) indicated that each solid phase has a diffraction pattern based on its atomic structure. The decrease in the degree of crystallinity (intensity) of the diffraction peaks at the same 2θ angle and superposition state in R-MA and R-NIC diffraction patterns compared to their parent components (rutin, MA/NIC) indicates that a solid phase binary mixture of R-MA and R-NIC was formed as shown in Figure 4 and Figure 5. These changes

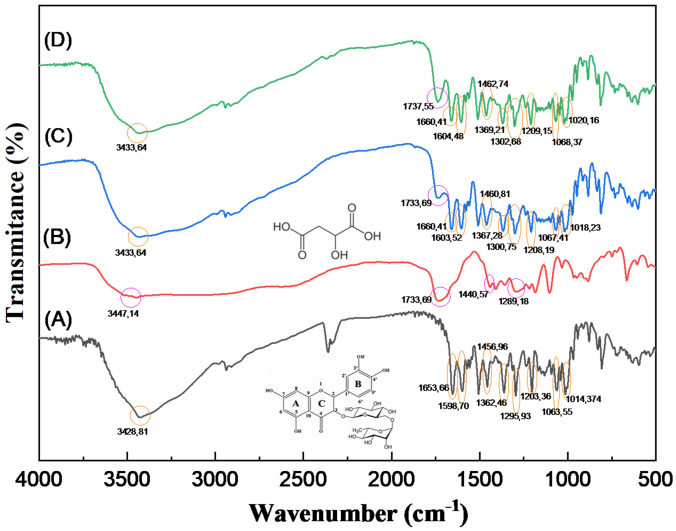

Figure 8. IR Spectrum of (A) Rutin, (B) Malic Acid, (C) Physical Mixture of R-MA, (D) Binary Mixture of R-MA

may also indicate the presence of polymorphs, which were further confirmed using DSC.

3.4 Thermal Analysis Using DSC

DSC can be used to observe the presence of polymorphism phenomenon and changes in thermal physical characteristics. Polymorphism is the ability of a compound to form crystals with different internal structures (atomic or molecular arrangements) despite the same chemical composition. The thermograms of R-MA and R-NIC binary mixture showed that there is rutin polymorphism in decomposed forms at melting points or endothermic peaks of 147.14°C and 151.46°C, respectively, caused by the melting process in microwave irradiation method.

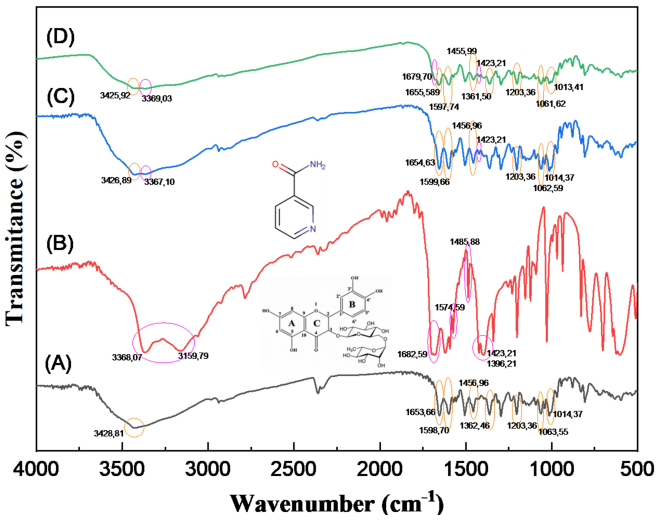

Figure 9. IR Spectrum of (A) Rutin, (B) Nicotinamide, (C) Physical Mixture of R-NIC, (D) Binary Mixture of R-NIC

Meanwhile, the first endothermic peak with a melting point of 97.68°C (ΔH = 16.43 Jg^{-1}) in the R-NIC binary mixture thermogram can be referred as a pseudopolymorph (hydrate). It is assumed that this polymorph-like hydrate appears due to the addition of water during slurrying process. Melting temperature shows the change in solid to liquid phase, which is characterized by appearance of an endothermic peak (heat energy absorption) when a solid substance melts.

According to Figure 6 and Figure 7, Rutin, malic acid, R-MA physical mixture (1:1), nicotinamide, and R-NIC physical mixture (1:1) respectively showed endothermic peaks with negative enthalpy at 158.37°C (ΔH = 94.73 Jg^{-1}), 132.80°C (ΔH = 256.34 Jg^{-1}), 130.38°C (ΔH = 60.17 Jg^{-1}), 128.76°C (ΔH = 210.62 Jg^{-1}), and 117.58°C (ΔH = 90.70 Jg^{-1}). The endothermic peaks in DSC thermograms of R-MA and R-NIC binary mixture are 113.96°C (ΔH = 59.50 Jg^{-1}) and 127.69°C (ΔH = 4.03 Jg^{-1}), respectively. The R-MA and R-NIC binary mixture endothermic peak appears at a lower melting temperature and enthalpy than the parent components and the physical mixture. Although it is not significantly different from their parent components, the weak strength of hydrogen bonds and the limited number of interactions in R-MA and R-NIC can reduce the thermal stability of the compound, resulting in a lower melting point. The DSC thermogram results appear to indicates the formation of R-MA and R-NIC solid phase binary mixture.

3.5 Vibrational Transition Using FTIR

The functional groups of the binary mixture constituents (rutin, malic acid, and nicotinamide) which capable to form intermolecular hydrogen-bond interactions will produce molecular vibrations and absorbance peaks at specific wavenumbers. This can be identified through a shift in wavenumber and corresponding changes in peak intensity in the FTIR spectrum. The higher the peak at a given wavenumber, the stronger the molecular bonding interaction (Wicaksono et al., 2020). The FTIR spectrum of R−MA binary mixture showed a wavenumber shift in the O−H stretch peak of rutin from 3428.81 cm^{-1} to 3433.64 cm^{-1}, along with the disappearance of the O-H stretch peak of malic acid. The C=O peak of the rutin ketone stretch shifted from 1653.66 cm^{-1} to 1660.41 cm^{-1}, similar to the physical mixture, while the C=O peak of the malic acid ketone stretch shifted from 1733.69 cm^{-1} to 1737.55 cm^{-1}. The O−H peaks appearing in the FTIR spectrum indicate the presence of all free O−H groups in the rutin and malic acid structures. These wavenumber shifts confirm the occurrence of intermolecular hydrogen bonding (OH···O) interactions between the hydroxyl O-H groups of malic acid (donors) and the C=O and O−H groups of rutin (acceptors).

Meanwhile, the FTIR spectrum of the R-NIC binary mixture shows a wavenumber shift in the O-H stretch peak of rutin from 3428.81 cm^{-1} to 3425.92 cm^{-1} and the NH_2 peak of nicotinamide from 3368.07 cm^{-1} to 3369.03 cm^{-1}. This wavenumber shift confirms the occurrence of intermolecular hydrogen bonding (NH···O) interactions between the N−H functional groups of nicotinamide as donors and the O−H of rutin as acceptors. The primary amide C−N peak (1423.21 cm^{-1}) successively does not show a shift from nicotinamide, meaning that the nicotinamide C−N group is not involved in the formation of any interaction. The shift of the C=C aromatic rutin peak from 1598.70 cm^{-1} to 1597.74 cm^{-1} in R-NIC binary mixture indicates the occurrence of a π-π stacking interaction that overlaps the pi orbital between the pi orbital cloud on the rutin B ring and the nicotinamide pyridine ring. According to Table 4 and Table 5, the similar wave number shifts and intensity changes at certain wave numbers in all typical functional groups in the IR spectrum of R-MA and R−NIC binary mixture indicates that there is no hydrogen bond interaction between the free O−H of malic acid and N−H of nicotinamide with O−H or C=O of rutin as further shown in Figure 8 and Figure 9.

3.6 Morphology Analysis Using SEM

The morphology was observed using SEM at 500×, 1000×, and 2500× magnifications. SEM photomicrographs showed that rutin exhibited an irregular, acicular, cylindrical morphology with aggregated rough surface. Malic acid crystals habit were plate-shaped with flat, thick morphology, similar in size, and a rough surfaces, while nicotinamide crystals habit were columnar with long, wide, and thicker morphology than acicular, flat, and sharp surfaces resembling a knife. The presence of malic acid and nicotinamide as small-molecule components will affect the size and shape of the R−MA and R−NIC binary mixture. Both binary mixtures were agglomerated, irregular needle-like, and a rough surface stacking particles joined together, resembling the morphology of their constituent compounds (rutin, malic acid, and nicotinamide). The similar R−MA and R−NIC morphology compared to the constituent compounds individually showed that R-MA and R-NIC binary mixture were successfully formed as depicted in Figure 10 and

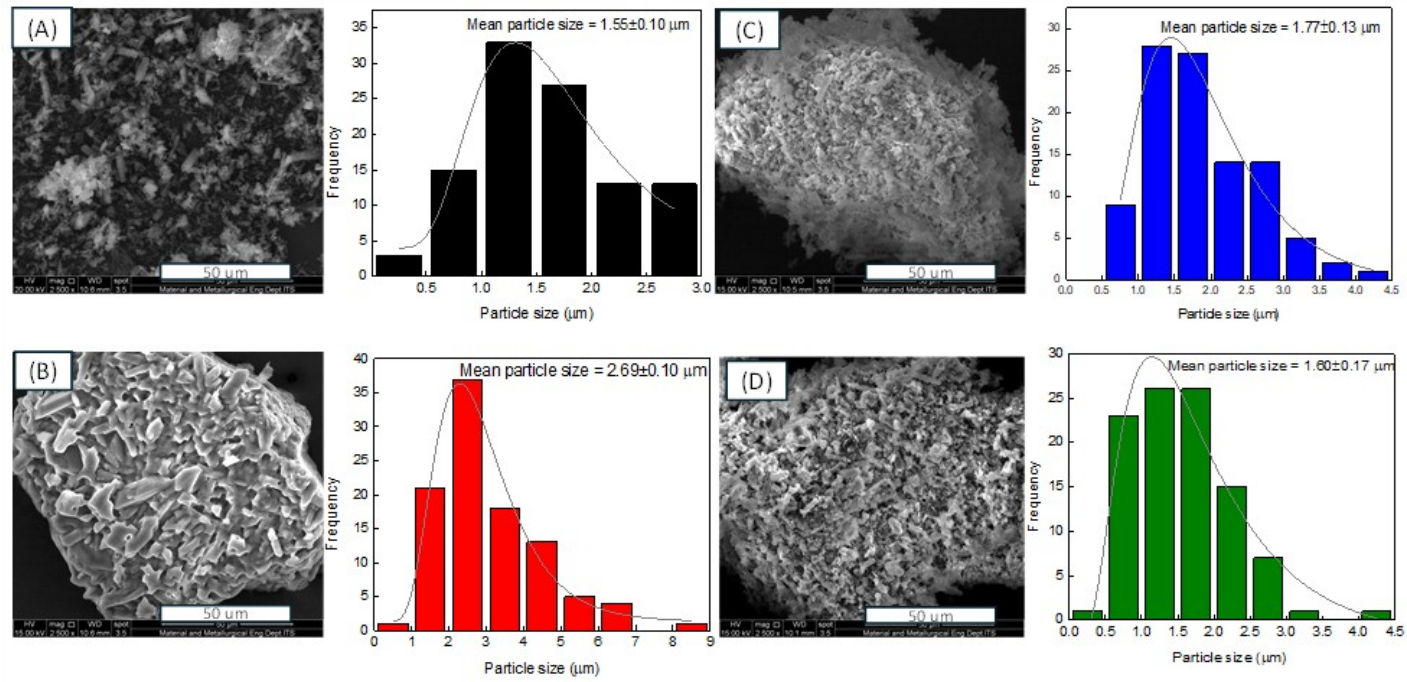

Figure 10. Particle Size Distribution of (A) Rutin, (B) Malic Acid, (C) Physical Mixture of R-MA, (D) Binary Mixture of R-MA

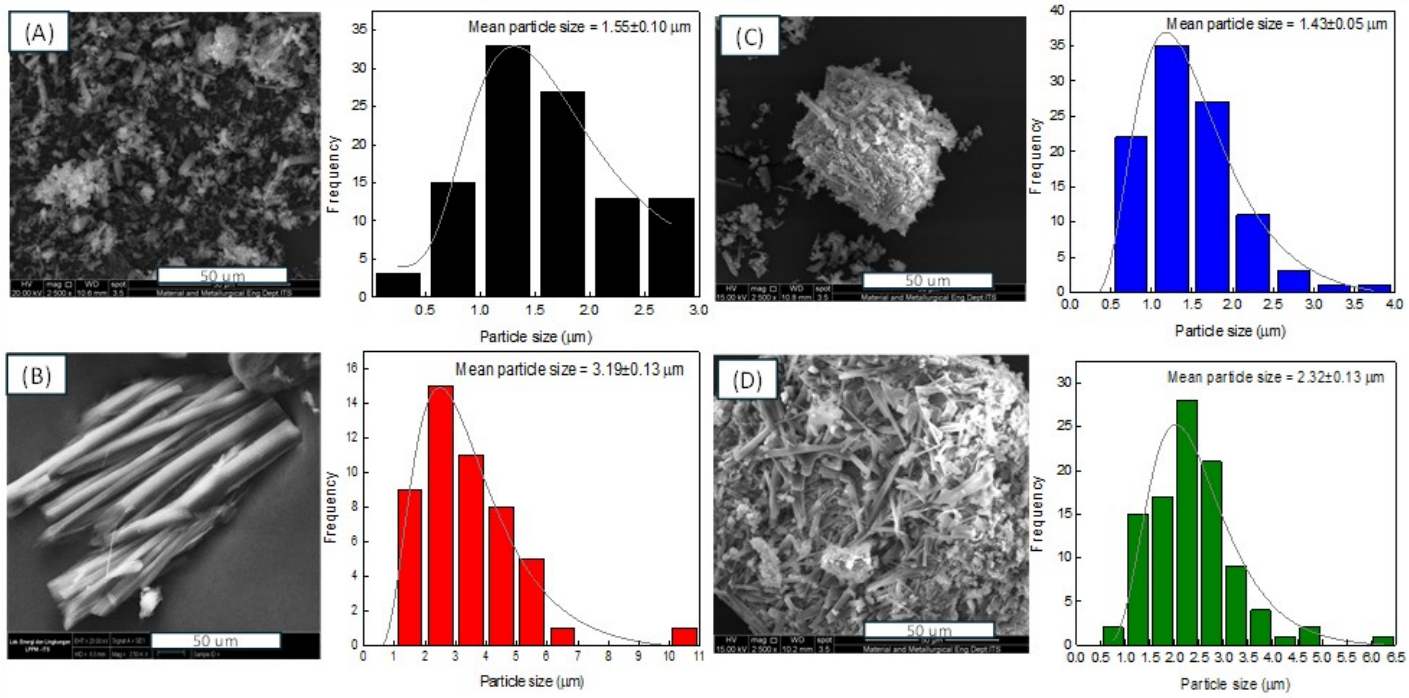

Figure 11. Particle Size Distribution of (A) Rutin, (B) Nicotinamide, (C) Physical Mixture of R-NIC, (D) Binary Mixture of R-NIC

Figure 11.

3.7 Microwave Irradiation Efficiency

Compared to the other studies, Table 6 shows that microwave irradiation is an rapid, efficient, and environmentally friendly

method for preparation of R−MA (1:1) and R−NIC (1:1) binary mixtures. The irradiation process under rapid time-exposure (5 min) proved the efficiency of microwave irradiation method. Although ASA-THE and SMT-NIC cocrystallization also utilized microwave irradiation, they required higher energies, 600 W and 500 W, respectively with longer time-exposure. This indicates that rutin (the API in this research) has higher microwave absorptivity due to its multiple hydroxyl groups and polarity, leading to rapid interactions and binary mixture formation.

4. CONCLUSIONS

R−MA and R−NIC binary mixture through microwave irradiation method was successfully formed hydrogen-bond interactions and a solid phase according to molecular docking, PXRD, DSC, FTIR, and SEM analysis. Although no significant differences were observed compared with the physical mixtures and parent components (rutin, MA/NIC), respectively, nevertheless, the molecular docking and microwave irradiation method is a good choice for chemical interactions study and green synthesis experimental. We believe that this method will suitable for future applications in active pharmaceutical material engineering and pharmaceutical science.

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