Original Article

Check for updates

Available online at
www.heca-analitika.com/malacca_pharmaceutics

Malacca Pharmaceutics

Vol. 3, No. 1, 2025

Appraisal of Antioxidant Potential in Broccoli Microgreens under Different Drying Techniques Utilizing In Vitro and in Silico Methods

Trina Ekawati Tallei [1,2*], Herlina Ineke Surjane Wungouw [3], Billy Johnson Kepel [4], Fatimawali Fatimawali [5], Ismail Celik [6], Nurdjannah Jane Niode [7] and Jayanthi Barasarathi [8]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia; trina_tallei@unsrat.ac.id (T.E.T.)
[2] Department of Biology, Faculty of Medicine, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia
[3] Department of Physiology, Faculty of Medicine, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia; herlinawungouw@unsrat.ac.id (H.I.S.W.)
[4] Department of Chemistry, Faculty of Medicine, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia; billy.kepel@unsrat.ac.id (B.J.K.)
[5] Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, North Sulawesi, Indonesia; fatimawali@unsrat.ac.id (F.F.)
[6] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Erciyes University, Kayseri 38039, Turkey; ismailcelik@erciyes.edu.tr (I.C.)
[7] Department of Dermatology and Venereology, Faculty of Medicine, Sam Ratulangi University, Prof. Dr. R. D. Kandou Central General Hospital, Manado 95115, North Sulawesi, Indonesia; niodejane@unsrat.ac.id (N.J.N.)
[8] Faculty of Health and Life Sciences, INTI International University, Persiaran Perdana BBN, Putra Nilai, 71800 Nilai, Negeri Sembilan, Malaysia; jayanthi.baras@newinti.edu.my (J.B.)

***** Correspondence: trina_tallei@unsrat.ac.id

Article History

Received XX XXX 20XX
Revised XX XXX 20XX
Accepted XX XXX 20XX
Available Online XX XXX 20XX

Keywords:
Bioactive compounds
Protein CYP2C9
Methyl myristate
Molecular docking
Molecular dynamics simulations

Abstract

Broccoli microgreens, rich in bioactive compounds, offer health benefits aligned with SDG 3: "Good Health and Well-Being." Their antioxidants combat oxidative stress tied to chronic diseases, but drying can affect their activity. This study assessed the antioxidant capacities of fresh, microwave-dried, and air-fryer-dried broccoli microgreens using in vitro (DPPH assay) and in silico (molecular docking and dynamics) methods. The microgreens were cultivated under controlled conditions and dried using microwave and air-fryer techniques. Antioxidant activity was evaluated using the DPPH assay using ethanolic extracts. The bioactive compounds of fresh microgreens, detected through GC-MS, were analyzed in silico to evaluate their interactions with the target proteins CYP2C9 and NOX2. The findings revealed that air-fryer-dried microgreens demonstrated the highest DPPH activity, followed by fresh microgreens, while microwave-dried samples exhibited the lowest activity. GC-MS analysis of fresh samples revealed the presence of various compounds, including acids, ketones, sulfides, heterocycles, alcohols, esters, aromatic compounds, phthalate ester, and aldehydes. Molecular docking revealed strong interactions of certain compounds in fresh samples and CYP2C9 and NOX2, suggesting therapeutic potential against oxidative stress. Molecular dynamics simulations (MDS) showed stable binding for the CYP2C9-Methyl myristate complex, while the NOX-(Z)-1,2-Diphenylethene complex displayed weaker stability. In conclusion, broccoli microgreens show potential in mitigating oxidative stress, with air-fryer drying slightly enhancing their antioxidant activity. The antioxidant capacity of fresh microgreens is comparable to that of air-fryer-dried microgreens. In silico analyses demonstrate stable interactions between compounds in fresh microgreens and key proteins implicated in oxidative stress.

1. Introduction

In recent years, there has been a growing interest in investigating the potential health benefits of natural compounds derived from various plant sources due to their potential therapeutic applications and minimal side effects compared to synthetic drugs [1, 2]. Among these, broccoli (*Brassica oleracea*) microgreens have attracted significant attention due to their exceptional nutritional value and potential health-promoting properties [3, 4]. These microgreens, categorized as functional foods, are rich in bioactive components such as glucosinolates, phenolic compounds, vitamins, and essential minerals [3, 5]. Numerous in vitro and in vivo studies have highlighted their biological activities, including antioxidant, anticancer, antimicrobial, anti-inflammatory, anti-obesity, and antidiabetic effects [6, 7]. For instance, broccoli microgreens have demonstrated bio-accessible fractions capable of inhibiting colon cancer cell growth by influencing cellular redox balance, leading to apoptosis via mitochondrial changes and cell cycle arrest [8]. These findings underscore the promising therapeutic potential of broccoli microgreens as a natural intervention for various chronic diseases.

Antioxidants are pivotal in safeguarding cells from oxidative stress, critical in the onset and progression of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions [9, 10]. Oxidative stress arises from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanisms [11]. In cancer, oxidative stress contributes to DNA damage, inflammation, metastasis, and immune dysfunction and disrupts antioxidants [12]. Addressing these challenges has prompted ongoing research into natural antioxidants, particularly those derived from plants, due to their therapeutic efficacy and favorable safety profiles [13, 14]. Broccoli microgreens, with their rich bioactive composition, hold the potential as a natural source of antioxidants to mitigate oxidative stress-related conditions effectively.

Post-harvest handling of broccoli microgreens poses challenges as these nutrient-dense plants are prone to rapid deterioration, resulting in significant nutrient loss. Drying methods often enhance shelf life and retain essential bioactive compounds, particularly antioxidants. However, the drying process can impact the stability of these compounds. Bioactive elements such as vitamin C, polyphenols, flavonoids, glycosides, and volatile compounds are highly susceptible to degradation through oxidation, especially at elevated temperatures. Conversely, higher drying temperatures can reduce drying times, minimizing compound loss [15]. For example, high-temperature drying has been shown to produce darker products, likely due to the formation of phenolic degradation by-products [16]. This underscores the need for optimized drying techniques that balance nutrient preservation with practical shelf-life extension.

Despite advances in understanding the impact of drying methods on bioactive compounds, gaps remain in identifying the most effective techniques for preserving antioxidants in broccoli microgreens. This study aimed to evaluate the antioxidant capacities of fresh broccoli microgreens and those subjected to microwave-drying and air-frying techniques using both in vitro and in silico methods. By integrating these approaches, the study sought to provide comprehensive insights into the potential of broccoli microgreens as functional foods for addressing oxidative stress-related conditions.

2. Materials and Methods

2.1. Seedling and Cultivation of Broccoli Microgreens

The cultivation of broccoli microgreens (Brassica oleracea) followed a standardized protocol [17]. Seeds were soaked in deionized water for 3 hours to enhance germination rates, improve moisture absorption, and reduce germination time. After soaking, seeds were evenly spread on seedling trays lined with plastic mesh to prevent them from slipping through the tray. The trays were kept in a dark environment for three days to promote germination. Afterward, the sprouts were transferred to artificial LED lighting (intensity ~7000 lux) and grown as microgreens under controlled conditions. Humidity levels were maintained at 52–60%, and temperatures were regulated between 26–26.5°C. The microgreens were harvested after 12 days of growth.

2.2. Drying Treatment

After harvesting, the microgreens were subjected to two drying methods: microwave drying and air-fryer drying. For microwave drying, the microgreens were placed in a microwave and processed for 3 minutes until fully dehydrated. In the air-fryer drying method, the microgreens were exposed to a temperature of 160°C for 10 minutes until complete dryness was achieved [17].

2.3. Extraction of Bioactive Compounds

Ethanolic extracts were prepared from 10 grams of fresh, microwave-dried, and air-fryer-dried microgreens. Each sample was combined with 100 mL of ethanol in separate flasks. The flasks were sealed and subjected to mechanical shaking at room temperature for 24 hours to facilitate compound extraction. Following this, the mixtures were filtered to separate the crude extracts, which were then concentrated using a rotary evaporator under reduced pressure and controlled temperature. The

concentrated extracts were stored at 4°C for subsequent analyses.

2.4. In Vitro Antioxidant Analysis

The antioxidant activity of the microgreens was assessed using the DPPH assay. A 0.1 mM DPPH solution was prepared in ethanol. For the assay, 100 µL of each extract (at concentrations of 25, 50, 100, 200, and 400 µg/mL) was mixed with 2 mL of the DPPH solution. The mixture was incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A solvent control (without extract) was included. A decrease in absorbance indicated an increased scavenging of DPPH radicals by antioxidants present in the extract. The percentage inhibition of DPPH radicals was calculated using Equation 1:

$$\%Inhibition = \left[\frac{A_c - A_s}{A_c}\right] \times 100 \quad (1)$$

where A_c is the absorbance of the control, and A_s is the absorbance of the sample.

The IC_{50} values, representing the concentration required to inhibit 50% of DPPH radicals, were calculated by plotting the percentage inhibition against the extract concentrations. As reported in previous studies, a lower IC_{50} value denotes higher antioxidant activity [18, 19]. All experiments were performed in triplicate to ensure reproducibility. Experiments were conducted in triplicate, and data were analyzed using one-way ANOVA with post-hoc tests. A p-value of <0.05 was considered statistically significant.

2.5. GC-MS Analysis of Chemical Composition in Microgreens Extract

The chemical composition of the microgreens' ethanolic extract was analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis was conducted with a Thermo Scientific TRACE™ 1310 gas chromatograph coupled to a Thermo Scientific ISQ LT Single Quadrupole mass spectrometer, following an established protocol [20].

2.6. Molecular Docking Analysis

The chemicals identified through GC-MS analysis were used as ligands for molecular docking in this study. The docking simulations targeted Cytochrome P450 CYP2C9 and NADPH oxidase (NOX2) proteins, to identify compounds capable of inhibiting their activity or scavenging reactive oxygen species (ROS). The protein structures for CYP2C9 (PDB ID: 1OG5) and NOX2 (PDB ID: 7U8G) were retrieved from the Protein Data Bank. Molecular docking simulations were conducted using the Gnina software [21], accessed via its repository (https://github.com/gnina/gnina), and executed on Google Colab using validated command templates [22].

2.7. Molecular Dynamics Simulations

Molecular Dynamics (MD) simulations were performed using GROMACS version 2021.2, following established methodologies [23, 24], to evaluate the computational stability of protein-ligand complexes involving CYP2C9 with methyl myristate and NOX2 with (Z)-1,2-diphenylethene. Input files for the simulations were generated via the CHARMM-GUI server, with parameterization based on the AMBER99SB force field. Simulations were carried out for 200 nanoseconds, producing 2,000 frames. Key simulation parameters included the use of the "md" integrator with a time step of 0.002 ps and a total of 100,000,000 steps. Energy and trajectory data were saved every 50,000 steps, while energy and log files were recorded at intervals of 100 and 1,000 steps, respectively.

The Verlet cut-off scheme was applied with a cut-off distance of 0.9 nm for van der Waals interactions and short-range electrostatics. No alterations were made to the van der Waals cut-off, and dispersion corrections were implemented for energy and pressure adjustments. Long-range electrostatic interactions were computed using the Particle Mesh Ewald (PME) method. Temperature coupling was achieved via the Nose-Hoover thermostat with a coupling time of 1.0 ps and a reference temperature of 303.15 K. Pressure coupling was performed isotropically using the Parrinello-Rahman algorithm [25, 26] with a coupling time of 5.0 ps, a compressibility of 4.5×10^{-5} bar^{-1}, and a reference pressure of 1.0 bar. The LINCS algorithm was used to constrain hydrogen bonds. Visualization and graphical analysis of MD simulation data were conducted using QtGrace version 0.2.0, while MD animation videos were created using UCSF Chimera version 1.15.

3. Results and Discussion

3.1. In Vitro Antioxidant

The DPPH activity of microgreens under different drying treatments, compared to fresh samples, is shown in Figure 1. This activity was evaluated across three sample treatments: fresh, air-fried, and microwaved preparations. Higher scavenging activity percentages indicate greater antioxidant potency [18]. Statistical analysis using one-way ANOVA followed by Tukey's post-hoc test revealed significant differences among the treatments ($p < 0.05$). The microwaved samples exhibited significantly lower DPPH scavenging activity compared to both fresh ($p = 0.001$) and air-fried samples ($p < 0.001$).

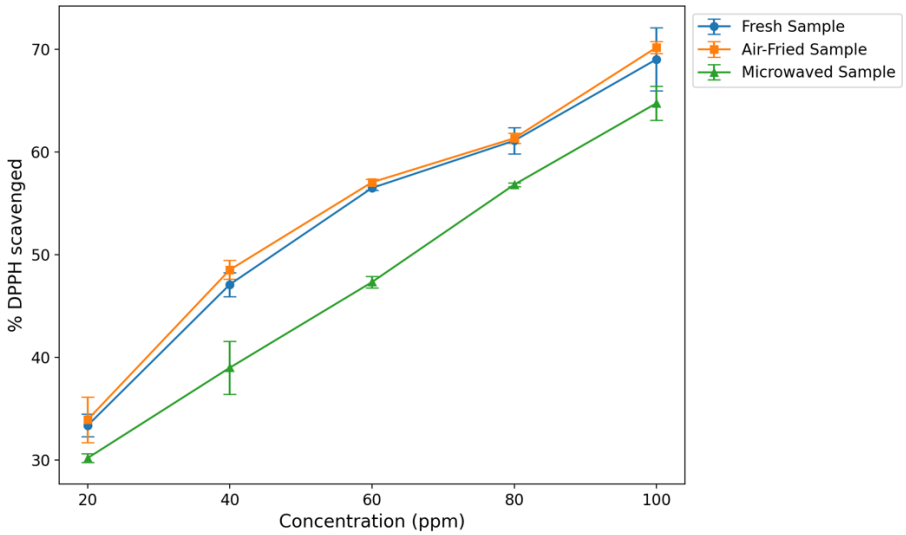


Figure 1. The percentage of scavenging activity on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was evaluated for ethanol extracts obtained from fresh, air-fried, and microwaved broccoli microgreens.

Notably, no significant difference was observed between the fresh and air-fried samples ($p = 0.647$), suggesting that air-frying treatment maintained an antioxidant capacity comparable to that of fresh samples. These findings indicate that while air-frying preserves the antioxidant properties, microwave treatment may significantly compromise the DPPH radical scavenging capacity of the samples. The concentration-dependent increase in DPPH scavenging activity was observed across all treatments, with the fresh and air-fried samples consistently demonstrated higher scavenging activity than their microwaved counterparts throughout the concentration range of 20-100 ppm.

Thermal processing methods have been widely studied for their effects on the antioxidant properties of *Brassica* vegetables. Among these, air-frying has consistently emerged as a promising technique for preserving or enhancing antioxidants. For example, air-frying at 160°C for 10 minutes significantly increased the levels of phenolic compounds and flavonoids in kale and broccoli sprouts, thereby improving their antioxidant potential [27]. Similarly, other studies have demonstrated that air-frying not only enhances phenolic content in kale and carrots but also reduces antinutritional factors such as oxalates [28]. Furthermore, air-frying has been highlighted as a sustainable pre-treatment method for seed roasting, effectively increasing the antioxidant activity of canola meal by generating sinapic acid derivatives [29]. This method is particularly advantageous when performed at higher temperatures (>140°C) for shorter durations with continuous hot air circulation, as it preserves nutrients and phenolic compounds while minimizing nutrient loss through leaching [30].

In contrast, the relatively lower percentage of DPPH scavenging activity observed in the microwave-dried sample suggests that this drying method may substantially reduce the antioxidant activity of broccoli microgreens. While microwave cooking has been shown to enhance the antioxidant properties of carrots, its effects on *Brassica* vegetables appear less favorable compared to air-frying [28]. This discrepancy underscores the importance of selecting appropriate thermal processing methods to optimize the retention of antioxidants and other bioactive compounds in vegetables.

3.2. Chemical Constituents of Ethanolic Extract of Fresh Broccoli Microgreens Analyzed through GC-MS

The gas chromatograms of the ethanolic extract from fresh broccoli microgreens, as shown in Figure 2, revealed the presence of several notable compounds with distinct retention times. GC-MS analysis identified a total of 11 compounds, as detailed in Table 1. The analysis highlighted the compositional diversity of the sample, with 2,5-cyclohexadiene-1,4-dione emerging as the predominant compound, accounting for approximately 47.22% of the total retention area. Other significant compounds included 5-Hexyn-1-ol (7.66%), Pivalic acid (7.28%), and Acetic acid, decyl ester (6.08%). Compounds present in smaller proportions, each contributing less than 5% of the total retention area, included Acetic acid (3.10%), dimethyl trisulfide, Benzene, 1,1'-(1,2-ethenediyl)bis-, (Z)-, and Myristic acid, methyl ester, which were notable for their distinct profiles.

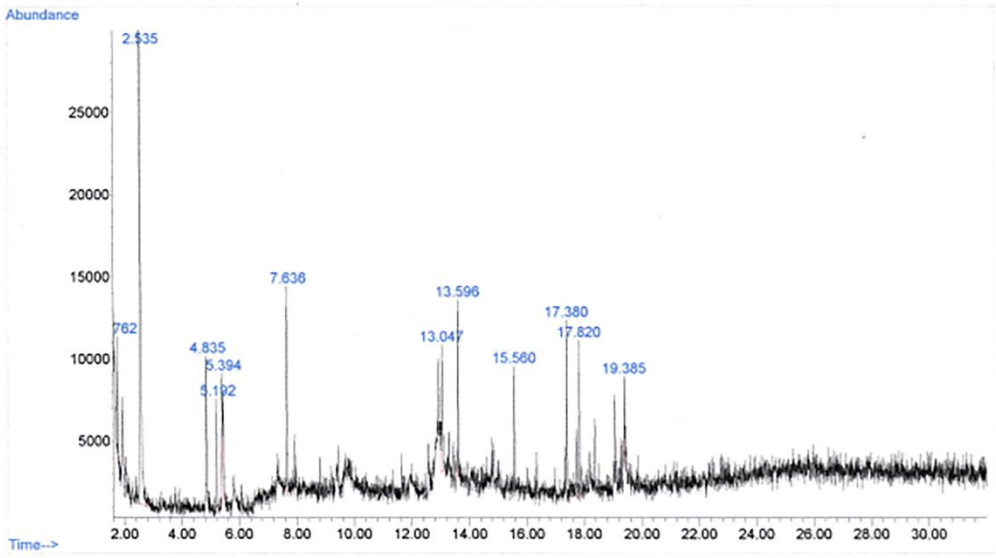


Figure 2. GC-MS chromatogram of the ethanolic extract of fresh broccoli microgreens, illustrating the presence of multiple compounds with distinct retention times.

Table 1. Compounds identified in the ethanolic extract of fresh broccoli microgreens through GC-MS analysis.

Retention Time (min)	Retention Area (%)	Compound IUPAC Name	Compound Group
1.762	3.10	Acetic Acid	Acids
2.535	47.22	2,5-cyclohexadiene-1,4-dione	Ketones
4.835	7.28	Pivalic acid	Acids
5.192	4. 96	Trisulfide, dimethyl	Sulfides
5.394	2. 28	Thiazole, 2,4-dimethyl-	Heterocycles
7.636	7.66	5-Hexyn-1-ol	Alcohols
13.047	6.08	Acetic acid, decyl ester	Esters
13. 596	5.85	Benzene, 1, 1'-(1, 2-ethenediyl)bis-, (Z)-	Aromatic compounds
15.564	3.51	Naphthalene, 2-(1-methylethyl)-	Aromatic compounds
17.38	5.06	Myristic acid, methyl ester	Esters
17.828	4.89	Dibutyl phthalate	Phthalate ester
19.38	2.11	Nonanal	Aldehyde

Additionally, Nonanal was identified at a relatively lower concentration of approximately 2.11%.

The identified compounds were classified into the following categories: acids, including acetic acid and pivalic acid; ketones, represented by 2,5-cyclohexadiene-1,4-dione; sulfides, such as trisulfide, dimethyl; heterocycles, including thiazole, 2,4-dimethyl-; alcohols, exemplified by 5-hexyn-1-ol; esters, such as acetic acid, decyl ester and myristic acid, methyl ester; aromatic compounds, including benzene, 1,1'-(1,2-ethenediyl)bis-(Z-isomer) and naphthalene, 2-(1-methylethyl)-; phthalate esters, represented by dibutyl phthalate; and aldehydes, with nonanal as a representative.

Microgreens are recognized as a rich source of nutrients, including carotenoids, chlorophylls, and various organic acids such as L-ascorbic acid, phytic acid, oxalic acid, citric acid, malic acid, and quinic acid [31]. However, there are no reports documenting the presence of acetic acid in microgreens or sprouts. 2,5-Cyclohexadiene-1,4-dione (commonly known as 1,4-benzoquinone) is a naturally occurring compound identified in organisms such as *Blaps lethifera*, *Uloma tenebrionoides*, and *Thevetia peruviana* [32]. This compound exhibits a range of bioactivities, including cytotoxicity against human tumor cell lines, as well as hypocholesterolemic, antimicrobial, immunosuppressive, antioxidant, nematicidal, and antiproliferative properties [32, 33]. Pivalic acid (synonym: 2,2-dimethylpropionic acid), a branched short-chain fatty acid, has been detected in Nicotiana tabacum and can exist in free form or as derivatives and homologues [34].

Benzene, 1,1'-(1,2-ethenediyl)bis-, (Z)- is a (Z)-stilbene compound, belonging to the class of phenolic compounds [35]. Resveratrol, the most common stilbene, predominantly occurs in its trans-isomeric form within plant tissues [36]. Studies have demonstrated that resveratrol, a naturally occurring food component, possesses strong antioxidant activity [37]. The robust antioxidant capacity of phenolic compounds plays a critical role in preventing cardiovascular diseases by

Table 2. Binding-free energy (BFE) values (in kcal/mol) of various compounds with the target molecules CYP2C9 and NOX2.

Compounds	Binding-Free Energy (kcal/mol)	
	CYP2C9	NOX2
Native ligand	-13.70	-
Native ligand	-	-11.08
Benzene, 1, 1'-(1, 2-ethenediyl)bis-, (Z)-	-4.48	-7.02
Dibutyl phthalate	-5.57	-6.75
Naphthalene, 2-(1-methylethyl)-	-4.92	-6.71
Myristic acid, methyl ester	-5.04	-5.62
2,5-cyclohexadiene-1,4-dione	-4.39	-5.05
Nonanal	-4.06	-4.83
Acetic acid, decyl ester	-5.39	-4.81
Pivalic acid	-3.58	-4.32
Thiazole, 2,4-dimethyl-	-3.74	-3.96
Trisulfide, dimethyl	-3.08	-3.25
5-Hexyn-1-ol	-3.00	-3.76
Acetic Acid	-3.15	-2.28

neutralizing free radicals, protecting nitric oxide (NO), and supporting vascular health [38].

Myristic acid, methyl ester (synonym: methyl tetradecanoate), is a methyl ester derivative of myristic acid. It functions as a plant metabolite, flavoring agent, and fragrance compound associated with tetradecanoic acid. Fatty acid methyl esters (FAMEs), derived from vegetable oils such as soybean, corn, sunflower, and microalgae, exhibit notable antifungal and antioxidant properties [39, 40]. Decyl acetate (acetic acid, decyl ester) is a natural compound identified in *Ophrys sphegodes* and *Houttuynia cordata*. It belongs to the class of fatty alcohol esters, closely related to FAMEs, and has demonstrated antibacterial activity. Fatty alcohol esters, depending on their chemical structure and functional groups, can also exhibit antioxidant properties [41]. The characteristics of these detected compounds suggest that fresh broccoli microgreens have significant potential as a natural source of antioxidants, contributing to their health-promoting properties.

3.3. Molecular Docking Results

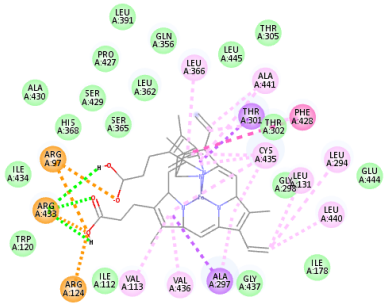
The compounds identified in the ethanolic extract of fresh samples were analyzed computationally to evaluate their binding interactions with two target receptors: cytochrome P450 CYP2C9 and NOX2. Table 2 presents the binding-free energy (BFE) values of the compounds with these targets, expressed in kcal/mol. BFE is a critical parameter for assessing the strength of interactions between a compound and its target, where lower (more negative) BFE values indicate stronger binding affinity and tighter interaction [42].

The analysis revealed variability in the binding affinities of the compounds to CYP2C9. Some compounds demonstrated weaker binding affinities, reflected by less negative BFE values, while others exhibited moderate to strong binding. Specifically, compounds such as dibutyl phthalate, myristic acid methyl ester, acetic acid decyl ester, and naphthalene, 2-(1-methylethyl)- showed stronger binding affinities to CYP2C9. In contrast, acetic acid, pivalic acid, and 5-hexyn-1-ol exhibited weaker interactions with this target. Notably, the native ligand provided a strong binding reference, highlighting its high affinity for CYP2C9 compared to other compounds in the analysis.

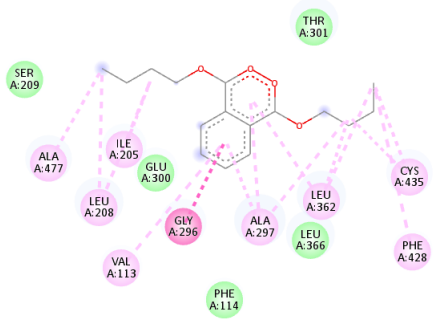
Cytochrome P450 (CYP450) enzymes play a critical role in the metabolism of a wide range of endogenous and exogenous substances, including drugs and hormones [43]. Among these, CYP2C9 is notably associated with the production of reactive oxygen species (ROS) during metabolic processes. Targeting CYP2C9 presents a potential strategy for developing antioxidant therapies to combat oxidative stress-related diseases. Identifying molecules that interact with CYP2C9 could aid in inhibiting ROS production or enhancing its antioxidant capacity.

NADPH oxidase 2 (NOX2), a member of the NOX superfamily, is another key contributor to ROS production, particularly the superoxide anion. NOX2 plays an essential role in immune responses and is predominantly expressed in neutrophils and macrophages. Its primary function is generating ROS to destroy pathogens during respiratory infections [44]. However, excessive ROS production can trigger chronic inflammation, contributing to the development of diseases such as diabetes and cancer [45]. These findings suggest that targeted modulation of NOX2 activity may provide therapeutic benefits for conditions characterized by oxidative stress and inflammation.

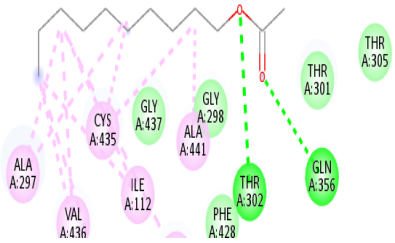
Figure 3 illustrates the results of molecular docking, highlighting how selected ligands interact with CYP450 and NOX2 receptors. The ligands were selected based on



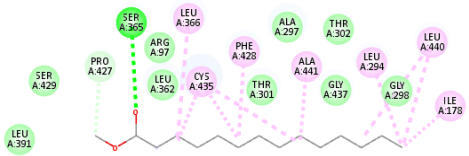
Native ligand (BFE -13,70 kcal/mol)



Dibutyl phthalate (BFE -5,57 kcal/mol)

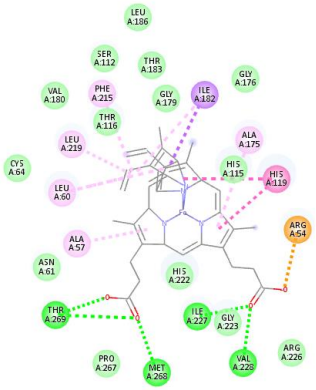


Acetic acid, decyl ester (BFE -5,39 kcal/mol)

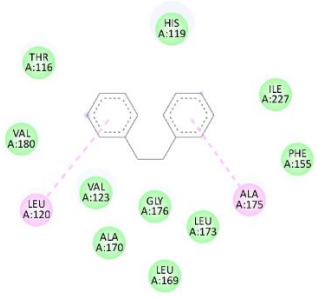


Myristic acid, methyl ester (BFE -5,04 kcal/mol)

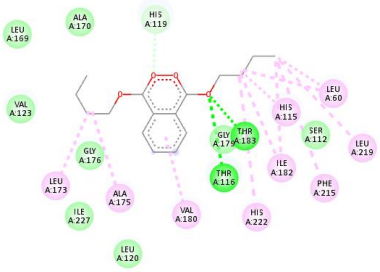
Receptor CYP2C9 (PDB ID: 1OG5)



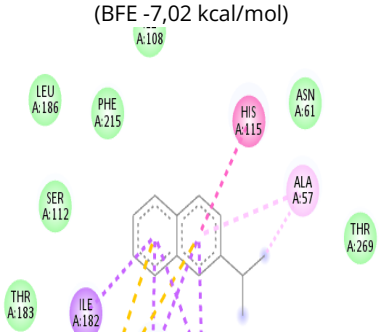
Native ligand (BFE -11,08 kcal/mol)



Benzene, 1, 1'-(1, 2-ethenediyl)bis-, (Z)-
(BFE -7,02 kcal/mol)



Dibutyl phthalate (BFE -6,75 kcal/mol)



Naphthalene, 2-(1-methylethyl)-
(BFE -6.71 kcal/mol)

Receptor NOX2 (PDB ID: 7U8G)

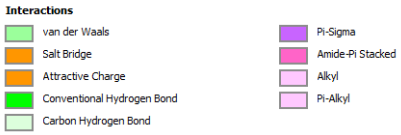


Figure 3. A visual representation of the molecular docking results, highlighting the binding interactions between the selected ligands and the CYP450 and NOX2 receptors.

their strong binding affinities to these receptors and their ability to form diverse interactions. Among these interactions, hydrogen bonds (H-bonds) play a pivotal role in stabilizing receptor-ligand complexes. H-bonds are formed between hydrogen atoms and electronegative atoms, such as oxygen or nitrogen, and are essential for maintaining the structure and function of biological systems [46–48]. The findings of this study confirm the presence of H-bonds within the receptor-ligand complexes, underscoring their significance in molecular recognition processes. These bonds not only enhance the stability of the complexes but also contribute to the specificity and efficacy of the ligand-receptor interaction [48, 49]. This underscores the potential of the selected ligands to modulate CYP450 and NOX2 activities effectively through stable and specific binding.

Salt bridges are frequently formed through interactions involving aspartic acid residues, contributing to the stability of protein-ligand complexes [50]. In contrast, van der Waals interactions are relatively weak electrostatic attractions arising from permanent or induced molecular polarity. Although weaker than hydrogen bonds, van der Waals interactions significantly influence ligand-receptor inhibition due to their prevalence [47]. A thorough understanding of ligand-receptor interactions is essential for advancing therapeutic strategies targeting these receptors. The number of hydrogen bonds formed with amino acid residues plays a critical role in determining bond strength and energy stability within the complex [51]. Additionally, hydrophobic interactions between ligands and receptors reduce amino acid-water interactions, often involving Pi-Sigma and Alkyl/Pi-Alkyl bonds. These interactions typically occur when nonpolar amino acid residues cluster within the protein's core, minimizing contact with water molecules [52]. Binding-free energy (BFE) values for ligands derived from broccoli microgreens were higher (less negative) compared to control compounds, indicating relatively lower receptor affinity. However, these findings still suggest that the investigated ligands possess the potential for selective modulation of CYP450 and NOX2 receptors, offering a foundation for future optimization and therapeutic development.

3.4. Molecular Dynamics Simulations

Throughout the 200 ns molecular dynamics simulation, the root-mean-square deviation (RMSD) of both the protein and ligand in the CYP2C9-Methyl myristate complex remained below 0.25 nm, reflecting stable dynamic behavior (Figure 4A). The RMSD consistently staying below 5 Å further suggests that structural deviations were minimal throughout the simulation [53]. Initially, the protein's RMSD was approximately 0.15 nm, while the ligand's RMSD was around 0.2 nm. By the 150–200 ns mark, both values stabilized near 0.2 nm, suggesting an initial phase of mutual adjustment followed by a period of more stable interactions. The extended equilibrium-like behavior observed during the latter phase of the simulation highlights a stable configuration characterized by balanced forces and minimal thermal fluctuations. The convergence of RMSD fluctuations toward the end of the simulation further supports the formation of a stable binding interaction between the ligand and the protein. The initial discrepancies in RMSD values indicate that the ligand may not have been strongly bound or optimally oriented at the start of the simulation. However, the subsequent alignment and stabilization of RMSD values suggest a successful binding event, reflecting the establishment of a stable protein-ligand complex.

In comparison, the molecular dynamics simulation of the NOX and (Z)-1,2-Diphenylethene complex reveals notable dynamics, as evidenced by the analysis of RMSD fluctuations (Figure 4B). Over the 200 ns simulation, a clear discrepancy is observed between the RMSD behaviors of the protein and the ligand. The protein exhibits relatively high RMSD fluctuations, ranging between 0.3 and 0.4 nm, indicating significant structural flexibility and potential conformational changes. In contrast, the ligand maintains consistently low RMSD values, remaining below 0.2 nm throughout the simulation, suggesting a stable conformation with minimal perturbations. This divergence in RMSD patterns suggests that the ligand may not be strongly or stably bound to the protein. The ligand's consistent and minimized RMSD values imply that it is not forming robust interactions with the protein's binding site, indicating weak or transient binding. Moreover, the pronounced RMSD fluctuations of the protein indicate a lack of stabilization by the ligand, further supporting the notion of an unstable or ineffective interaction within the complex.

The molecular dynamics simulations revealed distinct differences in binding stability and interaction dynamics between the two complexes. The CYP2C9-Methyl myristate complex exhibited stable binding interactions, characterized by consistent and low RMSD values for both the protein and ligand, indicating strong mutual adaptation and robust interactions. In contrast, the NOX-(Z)-1,2-Diphenylethene complex displayed weak and transient binding, with significant protein flexibility and a lack of effective stabilization by the ligand. These findings highlight the superior binding performance of the CYP2C9-Methyl myristate complex, suggesting its

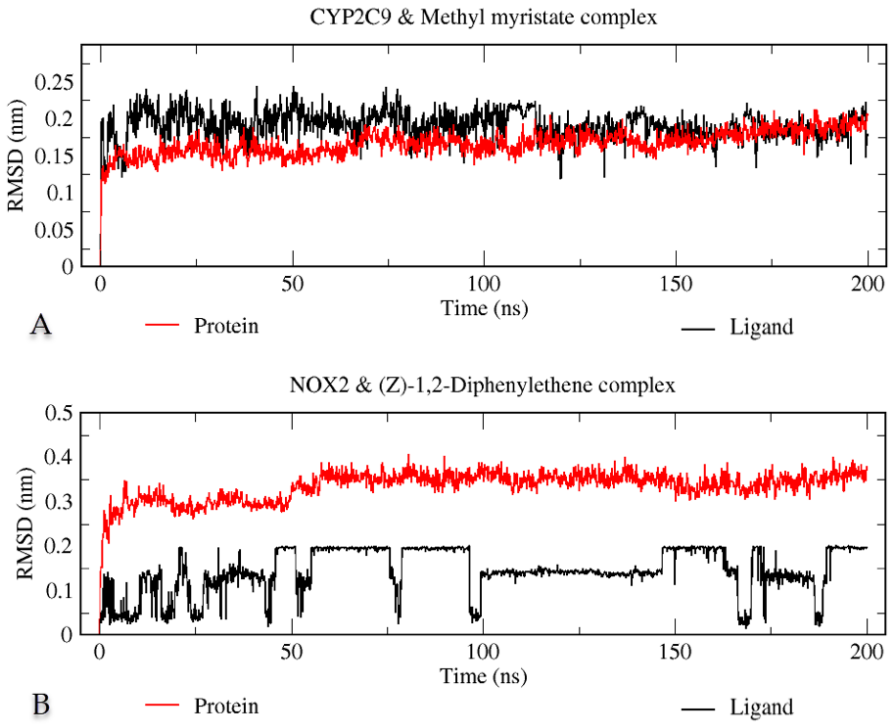


Figure 4. Results of molecular dynamics (MD) simulations for the CYP2C9-Methyl myristate complex (A) and the NOX2-(Z)-1,2-Diphenylethene complex (B), illustrating the dynamic interactions and stability of the respective protein-ligand systems.

potential for therapeutic targeting, while the NOX-(Z)-1,2-Diphenylethene complex requires further optimization to enhance its binding affinity and stability.

3.5. Study Limitations and Outlook for Further Research

This study identified several important findings while acknowledging its limitations. The analysis focused on the antioxidant activity and molecular interactions of broccoli microgreens subjected to specific drying methods, with results derived from in vitro and in silico approaches that cannot fully replicate in vivo conditions. The study was limited to a single variety of broccoli microgreens, and the impact of drying on other potentially bioactive compounds was not explored in detail. Despite these limitations, the findings highlight the potential of broccoli microgreens as functional foods, with air-frying demonstrating promise for preserving antioxidant activity. The identification of specific bioactive compounds from fresh broccoli microgreens interacting strongly with CYP2C9 and NOX2 underscores their therapeutic potential in managing oxidative stress-related conditions, contributing valuable insights into plant-based dietary interventions for chronic disease prevention. This work uniquely integrates in vitro and in silico approaches to evaluate the effects of drying methods, providing novel insights into the preservation of bioactive compounds and their applications. Future research should explore a wider range of drying techniques, microgreen varieties, and in vivo validation to optimize post-harvest processing and maximize health benefits.

4. Conclusions

This study explores the antioxidant capacities of broccoli microgreens under different drying methods, highlighting their potential health contributions and implications for oxidative stress management. The results demonstrate that both fresh and air-fried broccoli microgreens possess significant antioxidant activity, with air-frying effectively preserving this property. In contrast, microwave drying led to a marked reduction in antioxidant capacity, underscoring the critical influence of drying methods on the retention of bioactive compounds. These findings emphasize the role of broccoli microgreens, particularly in fresh and air-fried forms, in mitigating oxidative stress, a major factor in the development of chronic diseases. Molecular docking and dynamics simulations provide valuable insights into the therapeutic potential of compounds in fresh microgreens, with methyl myristate showing stable interactions with receptors involved in regulating oxidative stress. These findings underscore the importance of selecting the optimal preparation method to maximize the therapeutic and nutritional benefits of broccoli microgreens. Beyond their nutritional value, the study suggests potential applications in developing

antioxidant-based therapies for managing oxidative stress-related conditions and preventing chronic diseases. The integration of experimental analyses with computational modeling has provided a deeper understanding of the antioxidant potential of broccoli microgreens and the impact of preparation methods on their bioactive compounds. Further research should focus on exploring alternative drying methods and investigating other varieties of microgreens to enhance our knowledge of their bioactive profiles and health-promoting properties. Such studies could pave the way for innovative strategies in functional food development and therapeutic interventions.

Author Contributions: Conceptualization, T.E.T. and H.I.S.W.; methodology, T.E.T. and I.C.; software, T.E.T. and I.C..; validation, T.E.T., B.J.K. and F.F; formal analysis, N.J.N. and J.B.; investigation, T.E.T., H.I.S.W, B.J.K. and I.C.; resources, T.E.T. and F.F.; data curation, N.J.N. and I.C..; writing—original draft preparation, T.E.T., I.C., N.J.N., F.F. and J.B.; writing—review and editing, T.E.T. and H.I.S.W.; visualization, T.E.T. and I.C.; supervision, T.E.T.; project administration, T.E.T.; funding acquisition, T.E.T. All authors have read and agreed to the published version of the manuscript.

Funding: The authors gratefully acknowledge the financial support provided by Sam Ratulangi University through the R_MAPALUS research program, under Contract Number 825/UN12/LL/2023 and Letter of Assignment Number 784/UN12/LL/2023.

Ethical Clearance: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting this study are available from the corresponding author upon reasonable request.

Acknowledgments: The molecular dynamics simulations and numerical calculations presented in this article were partially conducted at TÜBİTAK ULAKBİM (The Scientific and Technological Research Council of Turkey's Academic Network and Information Center), utilizing the computational resources of the High Performance and Grid Computing Center, TRUBA.

Conflicts of Interest: All the authors declare that there are no conflicts of interest.

References

1. Singarimbun, E., Elfrida, E., and Indriaty, I. (2024). Indigenous Knowledge and Herbal Medicine: Exploring the Ethnobotany of the Karo Tiganderket Tribe in Indonesia, *Heca Journal of Applied Sciences*, Vol. 2, No. 2, 74–86. doi:10.60084/hjas.v2i2.208.
2. Ratte, T. A., Fatimawali, F., Tallei, T. E., Suoth, E. J., Antasionasti, I., and Yamlean, P. (2023). Evaluation of Antibacterial Properties from Endophytic Fungi of Chrysanthemum indicum (L.) Flowers against Escherichia coli and Staphylococcus aureus, *Grimsa Journal of Science Engineering and Technology*, Vol. 1, No. 2, 71–77. doi:10.61975/gjset.v1i2.15.
3. Le, T. N., Chiu, C.-H., and Hsieh, P.-C. (2020). Bioactive Compounds and Bioactivities of Brassica oleracea L. var. Italica Sprouts and Microgreens: An Updated Overview from a Nutraceutical Perspective., *Plants (Basel, Switzerland)*, Vol. 9, No. 8. doi:10.3390/plants9080946.
4. Dereje, B., Jacquier, J.-C., Elliott-Kingston, C., Harty, M., and Harbourne, N. (2023). Brassicaceae Microgreens: Phytochemical Compositions, Influences of Growing Practices, Postharvest Technology, Health, and Food Applications, *ACS Food Science & Technology*, Vol. 3, No. 6, 981–998. doi:10.1021/acsfoodscitech.3c00040.
5. Alloggia, F. P., Bafumo, R. F., Ramirez, D. A., Maza, M. A., and Camargo, A. B. (2023). Brassicaceae Microgreens: A Novel and Promissory Source of Sustainable Bioactive Compounds, *Current Research in Food Science*, Vol. 6, 100480. doi:https://doi.org/10.1016/j.crfs.2023.100480.
6. Ramirez, D., Abellán-Victorio, A., Beretta, V., Camargo, A., and Moreno, D. A. (2020). Functional Ingredients From Brassicaceae Species: Overview and Perspectives, *International Journal of Molecular Sciences*, Vol. 21, No. 6. doi:10.3390/ijms21061998.
7. Ebert, A. W. (2022). Sprouts and Microgreens-Novel Food Sources for Healthy Diets., *Plants (Basel, Switzerland)*, Vol. 11, No. 4. doi:10.3390/plants11040571.
8. Fuente, B. de la, López-García, G., Máñez, V., Alegría, A., Barberá, R., and Cilla, A. (2020). Antiproliferative Effect of Bioaccessible Fractions of Four Brassicaceae Microgreens on Human Colon Cancer Cells Linked to Their Phytochemical Composition., *Antioxidants (Basel, Switzerland)*, Vol. 9, No. 5. doi:10.3390/antiox9050368.
9. Rudrapal, M., Khairnar, S. J., Khan, J., Dukhyil, A. Bin, Ansari, M. A., Alomary, M. N., Alshabrmi, F. M., Palai, S., Deb, P. K., and Devi, R. (2022). Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant Potentials and Mechanism(s) of Action, *Frontiers in Pharmacology*, Vol. 13. doi:10.3389/fphar.2022.806470.
10. Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., Setzer, W. N., Calina, D., Cho, W. C., and Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases, *Frontiers in Physiology*, Vol. 11. doi:10.3389/fphys.2020.00694.
11. Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health., *Oxidative Medicine and Cellular Longevity*, Vol. 2017, 8416763. doi:10.1155/2017/8416763.
12. Arfin, S., Jha, N. K., Jha, S. K., Kesari, K. K., Ruokolainen, J., Roychoudhury, S., Rathi, B., and Kumar, D. (2021). Oxidative Stress in Cancer Cell Metabolism., *Antioxidants (Basel, Switzerland)*, Vol. 10, No. 5. doi:10.3390/antiox10050642.
13. Shahid, M., Fazry, S., Azfaralariff, A., Najm, A. A. K., Law, D., and Mackeen, M. M. (2023). Bioactive Compound Identification and In Vitro Evaluation of Antidiabetic and Cytotoxic Potential of Garcinia atroviridis Fruit Extract, *Food Bioscience*, Vol. 51, 102285. doi:https://doi.org/10.1016/j.fbio.2022.102285.
14. Shahid, M., Law, D., Azfaralariff, A., Mackeen, M. M., Chong, T. F., and Fazry, S. (2022). Phytochemicals and Biological Activities of Garcinia atroviridis: A Critical Review, *Toxics*, Vol. 10, No. 11. doi:10.3390/toxics10110656.
15. ElGamal, R., Song, C., Rayan, A. M., Liu, C., Al-Rejaie, S., and ElMasry, G. (2023). Thermal Degradation of Bioactive Compounds during Drying Process of Horticultural and Agronomic Products: A Comprehensive Overview, *Agronomy*, Vol. 13, No. 6. doi:10.3390/agronomy13061580.
16. Dhara, J., Saha, S. K., Saha, M., and Chakraborty, R. (2023). Study on Drying Kinetics, Antioxidant Activity, Total Bioactive Compounds, Physicochemical Properties and Microstructural Characteristics of Dehydrated Star Fruits (Averrhoa carambola) by Different Drying Methods, *Sustainable Food Technology*, Vol. 2023.
17. Tallei, T. E., Savitri, M., Lee, D., Rampengan, DerrenD. C. H., Park, M. N., Syahputra, R. A., Taslim, N. A., Moon, S., Nurkolis, F., and Kim, B.

(2024). A Comparative Analysis on Impact of Drying Methods on Metabolite Composition in Broccoli Microgreens, *LWT*, Vol. 210, 116866. doi:https://doi.org/10.1016/j.lwt.2024.116866.

18. Olugbami, J. O., Gbadegesin, M. A., and Odunola, O. A. (2014). In Vitro Evaluation of the Antioxidant Potential, Phenolic and Flavonoid Contents of the Stem Bark Ethanol Extract of Anogeissus Leiocarpus., *African Journal of Medicine and Medical Sciences*, Vol. 43, No. Suppl 1, 101–109.
19. Goutzourelas, N., Kevrekidis, D. P., Barda, S., Malea, P., Trachana, V., Savvidi, S., Kevrekidou, A., Assimopoulou, A. N., Goutas, A., Liu, M., Lin, X., Kollatos, N., Amoutzias, G. D., and Stagos, D. (2023). Antioxidant Activity and Inhibition of Liver Cancer Cells’ Growth of Extracts from 14 Marine Macroalgae Species of the Mediterranean Sea, *Foods*, Vol. 12, No. 6. doi:10.3390/foods12061310.
20. Tumilaar, S. G., Fatimawali, F., Niode, N. J., Effendi, Y., Idroes, R., Adam, A. A., Rakib, A., Emran, T. Bin, and Tallei, T. E. (2020). The Potential of Leaf Extract of Pangium edule Reinw As HIV-1 Protease Inhibitor: A Computational Biology Approach, *Journal of Applied Pharmaceutical Science*. doi:10.7324/JAPS.2021.110112.
21. McNutt, A. T., Francoeur, P., Aggarwal, R., Masuda, T., Meli, R., Ragoza, M., Sunseri, J., and Koes, D. R. (2021). GNINA 1.0: Molecular Docking with Deep Learning., *Journal of Cheminformatics*, Vol. 13, No. 1, 43. doi:10.1186/s13321-021-00522-2.
22. Tallei, T. E., Fatimawali, Adam, A. A., Ekatanti, D., Celik, I., Fatriani, R., Nainu, F., Kusuma, W. A., Rabaan, A. A., and Idroes, R. (2023). Molecular Insights into the Anti-Inflammatory Activity of Fermented Pineapple Juice Using Multimodal Computational Studies, *Archiv Der Pharmazie*, No. August. doi:10.1002/ardp.202300422.
23. Celik, I., Khan, A., Dwivany, F. M., Fatimawali, Wei, D.-Q., and Tallei, T. E. (2022). Computational Prediction of the Effect of Mutations in the Receptor-Binding Domain on the Interaction between SARS-CoV-2 and Human ACE2, *Molecular Diversity*, Vol. 26, No. 6, 3309–3324. doi:10.1007/s11030-022-10392-x.
24. Celik, I., Yadav, R., Duzgun, Z., Albogami, S., El-Shehawi, A. M., Fatimawali, Idroes, R., Tallei, T. E., and Emran, T. Bin. (2021). Interactions of the Receptor Binding Domain of SARS-CoV-2 Variants with hACE2: Insights from Molecular Docking Analysis and Molecular Dynamic Simulation, *Biology*, Vol. 10, No. 9. doi:10.3390/biology10090880.
25. Parrinello, M., and Rahman, A. (1981). Polymorphic Transitions in Single Crystals: A New Molecular Dynamics Method, *Journal of Applied Physics*, Vol. 52(12), No. 12, 7182–7190. doi:10.1063/1.328693.
26. Nosé, S., and Klein, M. L. (1983). Constant Pressure Molecular Dynamics for Molecular Systems, *Molecular Physics*, Vol. 50, No. 5, 1055–1076. doi:10.1080/00268978300102851.
27. Nandasiri, R., Semenko, B., Wijekoon, C., and Suh, M. (2023). Air-Frying Is a Better Thermal Processing Choice for Improving Antioxidant Properties of Brassica Vegetables, *Antioxidants*, Vol. 12, No. 2. doi:10.3390/antiox12020490.
28. Maqbool, N., Sofi, S. A., Makroo, H. A., Mir, S. A., Majid, D., and Dar, B. N. (2021). Cooking Methods Affect Eating Quality, Bio-Functional Components, Antinutritional Compounds and Sensory Attributes of Selected Vegetables, *Italian Journal of Food Science*, Vol. 3, No. SP1, 150–162.
29. Fadairo, O. S., Nandasiri, R., Nguyen, T., Eskin, N. A. M., Aluko, R. E., and Scanlon, M. G. (2022). Improved Extraction Efficiency and Antioxidant Activity of Defatted Canola Meal Extract Phenolic Compounds Obtained from Air-Fried Seeds, *Antioxidants*, Vol. 11, No. 12. doi:10.3390/antiox11122411.
30. Fadairo, O., Nandasiri, R., Alashi, A. M., Eskin, N. A. M., and Thiyam-Höllander, U. (2021). Air Frying Pretreatment and the Recovery of Lipophilic Sinapates from the Oil Fraction of Mustard Samples., *Journal of Food Science*, Vol. 86, No. 9, 3810–3823. doi:10.1111/1750-3841.15861.
31. Wojdyło, A., Nowicka, P., Tkacz, K., and Turkiewicz, I. P. (2020). Sprouts vs. Microgreens as Novel Functional Foods: Variation of Nutritional and Phytochemical Profiles and Their In Vitro Bioactive Properties., *Molecules (Basel, Switzerland)*, Vol. 25, No. 20. doi:10.3390/molecules25204648.
32. Edo, G. I. (2022). Antibacterial, Phytochemical and GC-MS Analysis of Thevetia peruviana Extracts: An Approach in Drug Formulation, *Natural Resources for Human Health*, Vol. 2, No. 4, 418–426. doi:10.53365/nrfhh/146543.
33. Petronzi, C., Festa, M., Peduto, A., Castellano, M., Marinello, J., Massa, A., Capasso, A., Capranico, G., La Gatta, A., De Rosa, M., Caraglia, M., and Filosa, R. (2013). Cyclohexa-2,5-Diene-1,4-Dione-Based Antiproliferative Agents: Design, Synthesis, and Cytotoxic Evaluation, *Journal of Experimental & Clinical Cancer Research*, Vol. 32, No. 1, 24. doi:10.1186/1756-9966-32-24.
34. Rezanka, T., Kolouchová, I., Cejková, A., and Sigler, K. (2012). Biosynthesis and Metabolic Pathways of Pivalic Acid., *Applied Microbiology and Biotechnology*, Vol. 95, No. 6, 1371–1376. doi:10.1007/s00253-012-4267-x.
35. Cheynier, V. (2012). Phenolic Compounds: From Plants to Foods, *Phytochemistry Reviews*, Vol. 11, No. 2, 153–177. doi:10.1007/s11101-012-9242-8.
36. Crozier, A., Jaganath, I. B., and Clifford, M. N. (2006). Phenols, Polyphenols and Tannins: An Overview, *Plant Secondary Metabolites*, John Wiley & Sons, Ltd, 1–24. doi:https://doi.org/10.1002/9780470988558.ch1.
37. Salehi, B., Mishra, A. P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., Fokou, P. V. T., Martins, N., and Sharifi-Rad, J. (2018). Resveratrol: A Double-Edged Sword in Health Benefits., *Biomedicines*, Vol. 6, No. 3. doi:10.3390/biomedicines6030091.
38. Frombaum, M., Le Clanche, S., Bonnefont-Rousselot, D., and Borderie, D. (2012). Antioxidant Effects of Resveratrol and Other Stilbene Derivatives on Oxidative Stress and *NO Bioavailability: Potential Benefits to Cardiovascular Diseases., *Biochimie*, Vol. 94, No. 2, 269–276. doi:10.1016/j.biochi.2011.11.001.
39. Pinto, M. E. A., Araújo, S. G., Morais, M. I., Sá, N. P., Lima, C. M., Rosa, C. A., Siqueira, E. P., Johann, S., and Lima, L. A. R. S. (2017). Antifungal and Antioxidant Activity of Fatty Acid Methyl Esters from Vegetable Oils., *Anais Da Academia Brasileira de Ciencias*, Vol. 89, No. 3, 1671–1681. doi:10.1590/0001-3765201720160908.
40. Davoodbasha, M., Edachery, B., Nooruddin, T., Lee, S.-Y., and Kim, J.-W. (2018). An Evidence of C16 Fatty Acid Methyl Esters Extracted from Microalga for Effective Antimicrobial and Antioxidant Property., *Microbial Pathogenesis*, Vol. 115, 233–238. doi:10.1016/j.micpath.2017.12.049.
41. Morris, S. G., Kraekel, L. A., Hammer, D., Myers, J. S., and Riemenschneider, R. W. (1947). Antioxidant Properties of the Fatty Alcohol Esters of Gallic Acid, *Journal of the American Oil Chemists Society*, Vol. 24, No. 9, 309–311. doi:10.1007/BF02641952.
42. Du, X., Li, Y., Xia, Y.-L., Ai, S.-M., Liang, J., Sang, P., Ji, X.-L., and Liu, S.-Q. (2016). Insights into Protein-Ligand Interactions: Mechanisms, Models, and Methods., *International Journal of Molecular Sciences*, Vol. 17, No. 2. doi:10.3390/ijms17020144.
43. Mittal, B., Tulsyan, S., Kumar, S., Mittal, R. D., and Agarwal, G. (2015). Chapter Four - Cytochrome P450 in Cancer Susceptibility and Treatment, *Advances in Clinical Chemistry*, Vol. 71, 77–139. doi:10.1016/bs.acc.2015.06.003.
44. Vignais, P. V. (2002). The Superoxide-Generating NADPH Oxidase: Structural Aspects and Activation Mechanism., *Cellular and Molecular Life Sciences : CMLS*, Vol. 59, No. 9, 1428–1459. doi:10.1007/s00018-002-8520-9.
45. Vermot, A., Petit-Härtlein, I., Smith, S. M. E., and Fieschi, F. (2021). NADPH Oxidases (NOX): An Overview from Discovery, Molecular Mechanisms to Physiology and Pathology., *Antioxidants (Basel, Switzerland)*, Vol. 10, No. 6. doi:10.3390/antiox10060890.
46. Herschlag, D., and Pinney, M. M. (2018). Hydrogen Bonds: Simple After All?, *Biochemistry*, Vol. 57, No. 24, 3338–3352. doi:10.1021/acs.biochem.8b00217.
47. Tallei, T. E., Tumilaar, S. G., Niode, N. J., Fatimawali, Kepel, B. J., Idroes, R., Effendi, Y., Sakib, S. A., and Emran, T. Bin. (2020). Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (Mpro)

and Spike (S) Glycoprotein Inhibitors: A Molecular Docking Study, *Scientifica*, Vol. 2020, 1–18. doi:10.1155/2020/6307457.

48. Tallei, T. E., Fatimawali, Yelnetty, A., Idroes, R., Kusumawaty, D., Emran, T. Bin, Yesiloglu, T. Z., Sippl, W., Mahmud, S., Alqahtani, T., Alqahtani, A. M., Asiri, S., Rahmatullah, M., Jahan, R., Khan, M. A., and Celik, I. (2021). An Analysis Based on Molecular Docking and Molecular Dynamics Simulation Study of Bromelain as Anti-SARS-CoV-2 Variants, *Frontiers in Pharmacology*, Vol. 12, No. August, 1–18. doi:10.3389/fphar.2021.717757.
49. Hubbard, R. E., and Kamran Haider, M. (2010). Hydrogen Bonds in Proteins: Role and Strength, *Encyclopedia of Life Sciences*. doi:10.1002/9780470015902.a0003011.pub2.
50. Celik, I., Abdellattif, M. H., and Tallei, T. E. (2022). An Insight Based on Computational Analysis of the Interaction between the Receptor-Binding Domain of the Omicron Variants and Human Angiotensin-Converting Enzyme 2., *Biology*, Vol. 11, No. 5. doi:10.3390/biology11050797.
51. Shah R, T., and Misra, A. (2011). Proteomics. Challenges in Delivery of Therapeutic Genomics and Proteomics, Gujarat, 686.
52. Arwansyah, A., Ambarsari, L., and Sumaryada, T. I. (2014). Simulasi *Docking* Senyawa Kurkumin dan Analognya Sebagai Inhibitor Reseptor Androgen pada Kanker Prostat, *Current Biochemistry*, Vol. 1, No. 1, 11–19. doi:10.29244/cb.1.1.11-19.
53. Rudnev, V. R., Nikolsky, K. S., Petrovsky, D. V, Kulikova, L. I., Kargatov, A. M., Malsagova, K. A., Stepanov, A. A., Kopylov, A. T., Kaysheva, A. L., and Efimov, A. V. (2022). 3β-Corner Stability by Comparative Molecular Dynamics Simulations, *International Journal of Molecular Sciences*, Vol. 23, No. 19. doi:10.3390/ijms231911674.