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Phytochemical Composition, Antioxidant Activity, and In Silico Reactome-Based Evaluation of the Potential Effects of *Nonea pulla* Methanol Extract on the SOD1 Pathway: A Pioneer Study

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ABSTRACT

Nonea pulla is an edible plant used in Turkish cuisine, where its leaves and roots are incorporated into traditional dishes. This study investigated the phytochemical composition, antioxidant activity, and molecular docking properties of *N. pulla* methanol extract. The extract exhibited significant antioxidant potential with an IC_{50} value of 5.87 ± 0.45 mg/mL and contained various biologically active compounds, such as flavonoids, phenols, and tannins. The total phenolic content was found to be 28.61 ± 0.27 mg GAE/g extract DW, which is relatively low compared to some other species, while the flavonoid content is higher. Antioxidant activity was assessed using the DPPH test, and the methanol extract demonstrated strong free radical scavenging activity. Additionally, the fatty acid profile of *N. pulla* was analyzed, revealing high concentrations of myristic, palmitic, and linoleic acids, compounds that play significant roles in reducing oxidative stress. Molecular docking studies suggested that fatty acids, such as linoleic and arachidic acids, had moderate binding affinities and could interact with antioxidants and metabolic pathways. Furthermore, in silico analyses for endocrine disruption potential indicated that these compounds did not directly bind to key nuclear receptors but showed significant interaction possibilities with receptors related to lipid metabolism and redox regulation. Reactome pathway analysis highlighted the vital role of superoxide dismutase 1 (SOD1) in antioxidant mechanisms, and both fatty acids showed potential modulation effects on SOD1 activity. Overall, these results provide preliminary evidence regarding the bioactive potential of *N. pulla* and its constituent compounds, particularly in relation to antioxidant mechanisms, oxidative stress modulation, and metabolic pathway interactions.

1 | Introduction

An antioxidant is defined as a compound, either organic or inorganic, that inhibits, stops, or controls the oxidation of a substrate; particularly in food science, it refers to substances present at low concentrations relative to an oxidizable substrate that delay or prevent oxidation. The inhibition of oxidation contributes to extending the shelf life of food by delaying the deterioration of food components [1].

Public health and societal well-being are significantly influenced by food safety, and cases of illness and death due to the consumption of contaminated foods are reported every year [2]; in this context, food packaging emerges as a key strategy for

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preserving quality [3], while conventional packaging generally provides only passive protection against moisture, oxygen, and contaminants [4]. In contrast, active packaging systems with antioxidant properties can extend shelf life by preventing oxidation and inhibiting microorganisms [5, 6]; for this purpose, natural polyphenols, natural extracts, and polymeric components are incorporated into packaging films [3, 7].

Additionally, natural antioxidants derived from plants have gained increasing attention as important food additives and nutraceutical supplements, with a significant rise in related scientific publications in recent years [8]. The primary purpose of antioxidants in foods is to delay oxidation by inhibiting the formation of free radicals or interrupting chain reactions [8–10]. Plants produce natural antioxidant compounds to protect against exogenous and endogenous oxidative stress; compounds, such as flavonoids, phenolic acids, carotenoids, and vitamins, are effective due to their abilities to scavenge free radicals, chelate metal ions, and act as reducing agents [11–13].

Nonea pulla is a plant species belonging to the Boraginaceae, found in Europe, the Far East of Russia, and Northwestern India. This plant typically grows in dry grasslands, hill slopes, and steppes, preferring sunny and dry areas. *N. pulla* can grow between 20 and 50 cm in height, with a straight, often branched stem. Its leaves are gray-green, covered with short, hair-like structures, and have a spiny texture. The lower leaves are 5–15 cm long and 1–2 cm wide, while the upper leaves encircle the stem. The flowers are five-parted and two-layered, and vary in color from purple to black, measuring between 10 and 14 mm in length. The plant is a hemicryptophyte, with its vegetation period lasting from April–May to August. Initially described by Carl von Linné in 1759 as *Lycopsis pulla*, it was later renamed *N. pulla* by Augustin Pyrame de Candolle in 1805. Ecologically, this plant prefers sunny environments and is commonly found in Central and Eastern Europe, as well as the western parts of Asia [14].

Nonea species are represented by approximately 35 taxa worldwide, with 23 species occurring in Türkiye. These species are commonly known as "Sormuk otu," and in Türkiye, the aerial parts are traditionally prepared as a porridge and consumed internally for the relief of stomach pain [15]. *Nonea persica* has long been utilized in Iran, where its leaves are consumed as a vegetable or incorporated into soups [16]. *Nonea melanocarpa* is also used as a vegetable and is mostly consumed after cooking [17]. Within the scope of this study, *N. pulla* is reported to have a notable place in Turkish culinary culture, as both its leaves and roots are used in the preparation of various traditional dishes and stews [18].

The main objective of this study was to comprehensively investigate the phytochemical composition, antioxidant capacity, fatty acid profile, and molecular interactions of compounds present in the methanol extract of *N. pulla*, for which no prior scientific study has been reported in the literature. Although similar phytochemical and antioxidant studies have been conducted on other *Nonea* species (e.g., *N. micrantha* and *N. vesicaria*), a comprehensive analysis specific to *N. pulla* is presented here for the first time. Therefore, the novelty of this study arises not only from the investigation of an unstudied species, but also from the integration of conventional phytochemical and antioxidant analyses with molecular docking studies targeting the superoxide dismutase 1 (SOD1) enzyme and Reactome pathway analysis. Furthermore, the potential effects of these fatty acids on biological pathways were evaluated using the Reactome pathway database. This integrated approach provides the first set of biological and chemical data for *N. pulla* and offers a scientific basis for future studies on its bioactive properties and antioxidant-related biological processes.

2 | Materials and Methods

2.1 | Plant Material and Extraction

N. pulla specimens were collected from Kastamonu in May 2023. The species was identified by Dr. Alper Durmaz, and its identification was confirmed using the Flora of Türkiye [19] and POWO [20] databases. Freshly harvested *N. pulla* aboveground parts were cleaned, dried at 40°C for 2 days, and ground into a fine powder. About 100 g of the dried sample was combined with 1 L of methanol and kept in the dark for 72 h. After filtration, the solvents were evaporated under reduced pressure at 40°C and stored at 4°C for later use.

2.2 | Total Phenolic Content

The Folin–Ciocalteu reagent method was used. Plant extracts were diluted to a concentration of 1 mg/mL. About 0.5 mL was taken from these solutions and mixed with 2.5 mL of Folin–Ciocalteu reagent and 2 mL of 7.5% $NaHCO_3$. The Folin–Ciocalteu reagent had been previously diluted 10-fold with water. The mixture was incubated at 45°C for 15 min. After incubation, the absorbance of the reaction mixture was measured at 765 nm using a UV spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). A calibration curve was prepared. Each experiment was performed in triplicate. The results were expressed as gallic acid equivalents (mg GAE extract DW) [21].

2.3 | Total Flavonoid Content

The total flavonoid content was measured using a spectrophotometric method. The procedure is as follows: Extracts from each plant material (1 mL containing 0.1 mg/mL) were diluted with water (4 mL) in a 10-mL volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 mL) was added to each flask. At the 5th minute, 10% $AlCl_3$ solution (0.3 mL) was added. At the 6th minute, 1.0 M NaOH (2 mL) was added. Then, water (2.4 mL) was added to the reaction flask and mixed well. The absorbance of the reaction mixture was measured at 510 nm using a UV spectrophotometer (Epoch; Bio-Tek, Winooski, VT, USA). A calibration curve was prepared. Each experiment was performed in triplicate. The results were expressed as quercetin equivalents (mg QE extract DW) [22].

2.4 | Total Flavanol Content

The total flavanol content was determined using a spectrophotometric method. The total flavonoid content was measured using the aluminum chloride method. In this method, 1 mL of the extract was mixed with $AlCl_3$ and sodium acetate. The mixture was incubated in the dark for 30 min. Absorbance was measured at 415 nm. Each experiment was performed in triplicate. Quercetin was used as the standard. The results were expressed as a quercetin equivalent (mg QE extract DW) [23].

2.5 | Total Tannin Content

The total tannin content was determined using a spectrophotometric method. Samples were mixed with the Folin–Ciocalteu reagent and Na_2CO_3. The mixture was incubated in the dark. Absorbance was measured at 760 nm. Each experiment was performed in triplicate. The total tannin content was expressed as gallic acid equivalents (mg GAE extract DW) [24].

2.6 | Total Proanthocyanidin Content

The total proanthocyanidin content was determined using a spectrophotometric method. A 0.25 mL aliquot of the extract was added to 3 mL of a 95% n-butanol/HCl (95:5 v/v) solution. Then, 0.1 mL of an $NH_4Fe\ (SO4)_2{}^{\bullet}12\ H_2O$ solution was added. The tubes were incubated at 95°C for 40 min. A red color developed, and absorbance was measured at 550 nm. Measurements were performed in triplicate. Assessed using the butanol acid assay, the content was expressed as catechin equivalent (CAE) in milligrams per gram of dried extract (mg CAE/g extract) [25].

2.7 | 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The DPPH test technique was used. Plant extract was prepared at different concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL). A DPPH methanol solution was prepared at 80 μg/mL. About 2 mL of the DPPH solution was added to each extract solution, and the mixtures were vortexed. The reaction mixtures were incubated at 27°C for 30 min. At the end of the incubation period, the absorbance of the reaction mixtures was measured at 517 nm using UV spectrophotometry (Epoch; Bio-Tek, Winooski, VT, USA). Butylated hydroxytoluene (BHT) was used as the standard control. BHT stock solution was prepared at different concentrations (500, 250, 125, 62.5, 32.25, and 16.125 μg/mL). All measurements were performed in triplicate. A calibration curve was prepared [26]. The antioxidant activity of the plant extract was evaluated using the following formula:

$$\text{DPPH scavenging activity}(\%\text{inhibition}) = \left[\frac{\left(A_\text{control} - A_\text{sample}\right)}{A_\text{control}}\right] \times 100. \quad (1)$$

2.8 | Extraction and GC-MS Analysis of Plant Fatty Acids

Oil extraction from the plant's aerial parts was performed using the Soxhlet method. The analysis was conducted using the SHIMADZU GCMS-QP2010 system. Compound identification was performed by comparing spectral data with the NIST database. GC-MS analysis was performed using a SHIMADZU GCMS-QP2010 Mass Spectrometer equipped with an Rxi-5MS column (30m × 0.25 mm x 0.25 μm). The oven temperature began at 70°C for 10 min, then increased gradually at 3°C/min to 150°C, maintained for 5 min, followed by a further increase at 10°C/min to 250°C, where it remained for 5 min. The solvent delay varied from 0 to 2 min, and the total GC/MS runtime was 56.67 min. The crude oil was converted to FAMEs and analyzed using a Thermo Scientific Trace 1310 GC-MS system. Fatty acids were quantified as relative percentages and identified by comparing retention times with a standard FAME mixture.

2.9 | Molecular Docking Studies

In this study, the human-derived SOD1 enzyme was selected as the target protein to evaluate the potential antioxidant effects of the tested compounds (PDB ID: 1CB4). SOD1 plays a central role in cellular defense against oxidative stress by catalyzing the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen. As one of the primary endogenous antioxidant enzymes, SOD1 is critical for preventing biomolecular damage induced by reactive oxygen species (ROS). The molecular docking studies were designed to investigate the binding affinity of selected natural compounds to the active site of SOD1, thereby assessing their potential contribution to radical scavenging activity. In this context, the computational analyses conducted using the 1CB4 structure provide a theoretical framework for understanding the antioxidant capabilities of the investigated compounds.

To assess the potential interactions between plant-derived metabolites and antioxidant-related targets, molecular docking simulations were conducted. The three-dimensional (3D) structures of the selected compounds were retrieved from the PubChem database. Before docking, energy minimization was performed to ensure that the ligands possessed stable and optimized conformations.

The crystal structure of the selected antioxidant enzyme, human copper–zinc superoxide dismutase (PDB ID: 1CB4), was obtained from the RCSB Protein Data Bank. In preparation for docking, all crystallographic water molecules and nonstandard residues were removed. Polar hydrogen atoms were added, and Gasteiger partial charges were assigned using AutoDock Tools (Version 1.5.7) to prepare the protein structure.

Docking studies were executed using AutoDock Vina, a widely used open-source software tool for molecular docking. A grid box was strategically positioned to encompass the active site of the enzyme, ensuring sufficient space for ligand binding. Multiple docking conformations were generated for each metabolite, and the pose with the most favorable (i.e., lowest) binding energy value (in kcal/mol) was chosen for further evaluation.

For advanced visualization and interpretation of the docking results, Discovery Studio Visualizer (Version 21.1.0) was utilized. This program provided 3D interaction maps and helped identify key amino acid residues involved in ligand recognition, thereby contributing to the understanding of potential antioxidant mechanisms [27].

2.10 | Reactome-Based Pathway Analysis

To evaluate the potential effects of molecular interactions on biological pathways, an *in silico* pathway mapping analysis was performed using the Reactome Pathway Browser (https://reactome.org/). Following molecular docking studies, Cu,Zn--SOD1 was identified as the primary target protein, and its

UniProt ID (P00441) was used for querying within the Reactome database.

The biological processes and metabolic pathways involving SOD1 were explored in the Reactome database, with particular focus on pathways associated with ROS detoxification (Detoxification of ROS), cellular responses to stress, and neurodegenerative processes, such as amyotrophic lateral sclerosis (ALS).

The pathway diagrams were interactively examined, and the graphical outputs were enhanced by incorporating the binding potential of linoleic acid and arachidic acid. These compounds were integrated as external molecular modulators within the relevant pathways, and their possible interactions with SOD1 were visually represented. The resulting pathway schematics were interpreted to assess the potential systemic biological consequences of these molecule–protein interactions.

2.11 | Statistical Analysis

To determine the relationship between two variables, correlation coefficients (R) were calculated using the CORREL statistical function in MS Excel software. Data are expressed as mean ± SD obtained from three separate observations.

3 | Results

The methanol extract of *N. pulla* demonstrated free radical scavenging capacity (IC_{50}: 5.87 ± 0.45 mg/mL) and exhibited moderate antioxidant activity when compared to standard antioxidants (e.g., BHT: 0.23 mg/mL). The analysis of the phytochemical profile revealed that the extract contains total flavonol compounds (28.47 ± 2.08 mg QE/g extract DW), total flavonoid compounds (79.01 ± 2.99 mg QE/g extract DW), total phenolic compounds (28.61 ± 0.27 mg GAE/g extract DW), total proanthocyanidin content (126.05 ± 18.57 mg CAE/g extract DW), and total tannin content (42.88 ± 4.01 mg GAE/g extract DW) (Table 1).

3.1 | Fatty Acid Composition of *N. pulla*

The fatty acid composition of *N. pulla* is presented in Table 2. The analysis reveals that myristic acid constitutes the highest percentage of the total area, accounting for 29.88%. It is followed by palmitic acid (25.19%) and linoleic acid (9.11%). Butyric acid makes up 8.46% of the total area, while alpha-linolenic acid and cis-8,11,14-eicosatrienoic acid methyl ester are present at 6.11% and 4.74%, respectively. Stearic acid and arachidic acid are found in lower amounts, representing 3.93% and 1.36%, respectively. Lauric acid is the least abundant fatty acid, with a percentage of 1.33%. These findings provide a comprehensive profile of the fatty acid composition of *N. pulla*, offering valuable data for further studies on its nutritional value and potential health benefits.

3.2 | Result of Molecular Docking

Molecular docking analysis of the selected natural compounds revealed that binding energies ranged from −4.0 to −5.7 kcal/mol. The highest binding affinity was observed for arachidic acid (−5.7 kcal/mol), followed by linoleic acid (−5.2 kcal/mol) and alpha-linolenic acid (−5.0 kcal/mol). 8,11,14-Eicosatrienoic acid methyl ester also exhibited a comparable binding energy (−5.0 kcal/mol). In contrast, stearic acid (−4.8 kcal/mol), palmitic acid (−4.7 kcal/mol), myristic acid (−4.3 kcal/mol), and butyric acid (−4.0 kcal/mol) demonstrated comparatively lower binding affinities (Table 3).

Table 4 presents the noncovalent interactions observed between linoleic acid and arachidic acid and the target protein. Linoleic acid did not form any hydrogen bonds, van der Waals, or π–π stacking interactions. However, it engaged in multiple alkyl interactions with several hydrophobic residues, including A: VAL7, B: CYS55 (twice), B: CYS144 (twice), and B: VAL146 (twice), as well as through its own carbon atoms ([001:C17] repeated twice). These interactions suggest that the binding of linoleic acid is predominantly governed by hydrophobic contacts (Figure 1A).

In contrast, arachidic acid formed a hydrogen bond between its H30 atom and the carboxylate oxygen (OD2) of ASP11. Similar to linoleic acid, it did not display van der Waals or π–π stacking interactions but showed extensive alkyl interactions with hydrophobic residues, such as A:VAL146 (twice), B:VAL7, B:LYS9, B:CYS55, and B:CYS144, along with its own carbon atoms ([011:C17] repeated twice). These findings indicate that both fatty acids primarily interact with the protein through hydrophobic forces, with arachidic acid showing a slightly stronger interaction profile due to its additional hydrogen bond (Figure 1B).

In this study, the potential endocrine-disrupting properties of linoleic acid and arachidic acid were evaluated based on their predicted binding affinities to various nuclear receptors and transcriptional regulators using ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and Endocrine Disruptome analyses. Both compounds showed no direct binding (0) to key endocrine-related targets, such as the androgen receptor (AR), estrogen receptor (ER), androgen receptor ligand-binding domain (AR-LBD), and estrogen receptor ligand-binding domain (ER-LBD) (Table 5). However, they exhibited low probabilities of interaction, with binding likelihoods for linoleic acid calculated as 9.30%, 6.20%, 4.70%, and 7.20%, respectively, and for arachidic acid as 8.80%, 5.20%, 2.80%, and 6.50%.

The predicted interaction with aromatase, a key enzyme in steroidogenesis, was slightly higher for linoleic acid (9.60%) than for arachidic acid (7.80%). Both compounds demonstrated similar probabilities of interaction with the aryl hydrocarbon receptor (AhR)—7.30% for linoleic acid and 7.50% for arachidic acid. Notably, interaction with the antioxidant response element (ARE) was relatively high for both compounds, at 22.60% and 20.50%, respectively, indicating possible involvement in redox-regulated signaling pathways.

For ATPase family AAA domain–containing protein 5 (ATAD5), the binding probabilities were low (2.50% for linoleic acid and 4.00% for arachidic acid). Interaction with the heat shock element (HSE), a stress response pathway, was moderately high, particularly for arachidic acid (18.70%) compared to linoleic acid (11.40%). Interaction probabilities with p53, a tumor suppressor protein, were minimal (3.10% and 3.90%, respectively).

A particularly noteworthy result was observed for peroxisome proliferator–activated receptor gamma (PPARγ), with linoleic acid showing a high binding probability of 39.30% and arachidic acid 26.70%. This suggests that linoleic acid may have a stronger modulatory role in lipid metabolism and energy homeostasis.

TABLE 1 | Antioxidant activity and phenolic content of *N. pulla*.

Plant name	DPPH (IC_{50} mg/mL)	Total flavonol compound (mg QE/g extract)	Total flavonoid compound (mg QE/g extract)	Total phenolic compound (mg GAE/g extract)	Total proanthocyanidin content (mg CAE/g extract)	Total tannin content (mg GAE/g extract)
Nonea pulla	5.87 ± 0.45	28.47 ± 2.08	79.01 ± 2.99	28.61 ± 0.27	126.05 ± 18.57	42.88 ± 4.01
BHT	0.23 ± 0.01	—	—	—	—	—

Predicted binding to matrix metalloproteinases (MMPs) was comparable for both compounds (15.10% and 15.50%, respectively).

Lastly, binding probabilities to the thyroid receptor (TR) and glucocorticoid receptor (GR) were moderate, with linoleic acid showing values of 15.90% and 15.60%, and arachidic acid showing 12.70% and 13.70%, respectively.

In summary, although neither linoleic acid nor arachidic acid showed direct binding to the evaluated nuclear receptors, they demonstrated moderate to high interaction probabilities with several key targets, particularly PPARγ, ARE, MMP, and GR. These results suggest that both fatty acids may exert indirect biological effects through modulation of metabolic, inflammatory, and antioxidant pathways.

3.3 | Reactome-Based Pathway Interpretation

The pathway analysis conducted via the Reactome Pathway Browser revealed that SOD1 plays a central role in several redox-related biological processes. Notably, SOD1 was found to be actively involved in key pathways, such as detoxification of ROS, cellular responses to stress, and ALS. In line with molecular docking results, linoleic acid and arachidic acid exhibited notable binding potentials to the active site of SOD1, suggesting their possible influence on redox homeostasis. The graphical representations of these pathways were enhanced by integrating both compounds as external molecular modulators targeting SOD1 (Figure 2). These interactions were evaluated in the context of antioxidant defense mechanisms, particularly the enzymatic neutralization of ROS. Given the binding affinities (ΔG: -5.2 kcal/mol for linoleic acid and -5.7 kcal/mol for arachidic acid), both molecules appear to contribute to redox regulation by potentially modulating SOD1 activity within these biological pathways.

4 | Discussion

4.1 | Antioxidant Activity and Phytochemical Content

The total polyphenol content of the methanol extract obtained from the aerial parts of *N. pulla* was determined using the Folin–Ciocalteu reagent. The total phenolic content of the methanol extract was found to be 28.61 ± 0.27 mg GAE/g extract DW. This value is lower than the values reported by Mohammed and Abdullah [28] for *Nonea pulmonarioides* (113.33 ± 1.06 μg GAE/mg). The ethyl acetate and chloroform fractions from *Nonea micrantha* (total phenolic content 280.00 ± 3.78 and 270.33 ± 2.60 mg GAE/g) were also higher than the values obtained from *N. pulla* [29]. The total flavonoid content of the aerial parts of *N. pulla* was determined to be 79.01 ± 2.99 mg QE/g extract DW, which is higher than the value reported by Mohammed and Abdullah [28], 19.80 mg QE/g DW. Compared to the methanol extract of *N. pulla*, *N. melanocarpa* was found to have a lower phenolic content (22.2 mg GAE/g) [30]. According to the literature, these variations in total polyphenols, flavonoids, and tannins vary depending on the species, plant tissues evaluated, season, cultivation conditions, sample preparation, and methods of analysis used [31].

TABLE 2 | Results of fatty acid composition in *N. pulla*.

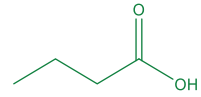
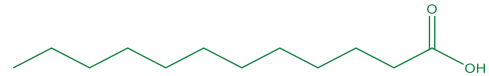
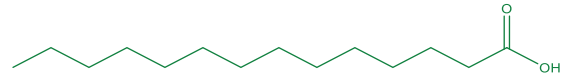
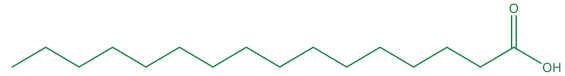
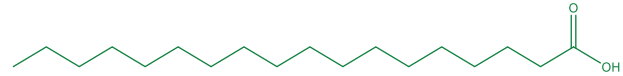
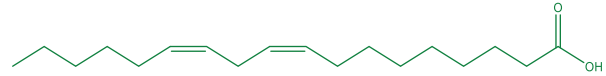
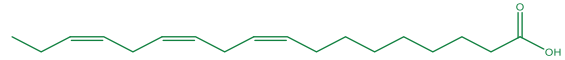
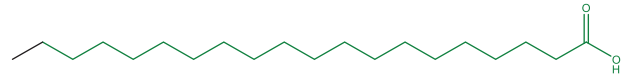
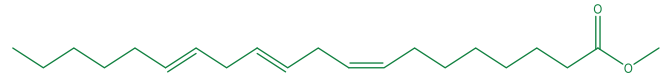
No	Retention time (min)	Name of the fatty acid	Area %	Chemical structure
1	4.88	Butyric acid	8.46	
2	16.24	Lauric acid	1.33	
3	20.48	Myristic acid	29.88	
4	24.39	Palmitic acid	25.19	
5	27.94	Stearic acid	3.93	
6	29.65	Linoleic acid	9.11	
7	30.93	Alpha-linolenic acid	6.11	
8	31.19	Arachidic acid	1.36	
9	33.50	*cis*-8,11,14-Eicosatrienoic acid methyl ester	4.74	

TABLE 3 | Docking-based binding and efficiency metrics of selected natural compounds.

	Binding energy (kcal/mol)
Myristic acid	−4.3
Palmitic acid	−4.7
Butyric acid	−4.0
Stearic acid	−4.8
Linoleic acid	−5.2
Alpha-linolenic acid	−5.0
Arachidic acid	−5.7
8,11,14-Eicosatrienoic acid, methyl ester	−5.0

TABLE 4 | Summary of noncovalent interactions between ligands and protein.

Compounds	H-bond	Van der Waals	Pi–Pi stacking	Alkyl interactions
Linoleic acid	—	—	—	A: VAL7, B: CYS55 (× 2), B: CYS144 (× 2), B: VAL146 (× 2), [001:C17] (× 2)
Arachidic acid	[011:H30] – ASP11: OD2	—	—	A: VAL146 (× 2), B: VAL7, B: LYS9, B: CYS55, B: CYS144, [011:C17] (× 2)

It is known that the antioxidant capacity of plant extracts is influenced by various factors, so it is important to use different antioxidant tests [31]. In this study, *N. pulla* extracts were examined using DPPH radical scavenging tests. The DPPH test measures the ability of antioxidants to donate an electron or hydrogen to reduce the stable DPPH free radical.

According to Table 1, the smaller the IC_{50} value, the stronger the antiradical effect of the plant. The methanol extract of *N. pulla* ($IC_{50} = 5.87 \pm 0.45$ mg/mL) exhibited moderate antioxidant activity. Studies have reported similar results for the acetone extract of *N. pulmonarioides* [28]. The antioxidant activity results of *Nonea vesicaria* showed a significant effect, and these values are consistent with our findings [32]. The IC_{50} values of *Nonea micrantha* for the aqueous, chloroform, hexane fractions, and methanolic extract were determined to be 3, 5, 93, and 120 μg/mL, respectively [33], and these values are significantly higher than the results we obtained.

In the DPPH radical scavenging activity, the *Nonea lutea* extract ($IC_{50} = 99.14 \pm 0.3$ μg/mL) [34] exhibited significantly higher activity compared to the *N. pulla* extracts. In another study by Gharib et al. [35], the DPPH radical scavenging activity was observed in the ethanol extract of *Nonea caspica* ($IC_{50} = 23.13 \pm 1.90$ μg/mL), which exhibited higher activity compared to our study.

Free radicals are generated within the body and can also be present in fried and highly spiced foods, leading to the oxidation of cellular lipids and damage to DNA [36]. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), BHT, tertiary butylhydroquinone, and gallic acid esters, have raised concerns due to their potential adverse health effects, resulting in restrictions on their use and an increasing shift toward safer and more effective natural antioxidants. In addition, these synthetic compounds are reported to exhibit limited solubility and moderate antioxidant capacity [29, 37]. Accordingly, there has been a growing interest in the antioxidant potential of medicinal plants for mitigating free radical–induced damage. In this context, natural antioxidants derived from tea, wine, fruits, vegetables, and spices, as well as rosemary and sage, are widely utilized as food additives and dietary supplements [38, 39].

The methanol extract of *N. pulla* evaluated in this study exhibited notable antioxidant activity with an IC_{50} value of 5.87 ± 0.45 mg/mL in the DPPH radical scavenging assay. Although this value is lower than that of the standard antioxidant BHT (0.23 ± 0.01 mg/mL), the findings indicate that *N. pulla* possesses potential as a natural antioxidant source. In this context, the extract may be considered a natural preservative in food systems due to its ability to delay oxidative deterioration. For example, the antioxidant effects of clove in meat products have been reported by Abd El-Alim et al. [40]. Similarly, the findings of the present study indicate that *N. pulla* extract also exhibits antioxidant activity. In this context, the extract may be considered to exert a protective effect against oxidative deterioration in meat and other food systems.

4.2 | Molecular Docking and Reactome-Based Pathway Interpretation

The disruption of the balance between ROS levels and the antioxidant defense system leads to oxidative stress. Oxidative stress causes changes in macromolecules, such as proteins, lipids, and DNA, and is associated with various chronic diseases, such as aging, diabetes, arthritis, cancer, and cardiovascular diseases [41]. To maintain the balance between oxidants and antioxidants, cells possess enzymatic systems, such as SOD [42]. This enzyme belongs to an important class of antioxidant enzymes. SOD is one of the key cellular defense systems against intracellular superoxide radicals. It contains redox metals at its catalytic center and functions by converting superoxide into hydrogen peroxide, thereby enhancing transcriptional activity. Three different isoforms of SOD have been reported: cytosolic copper/zinc-containing SOD (SOD-1), mitochondrial manganese-containing SOD (SOD-2), and extracellular SOD (SOD-3). SOD expression

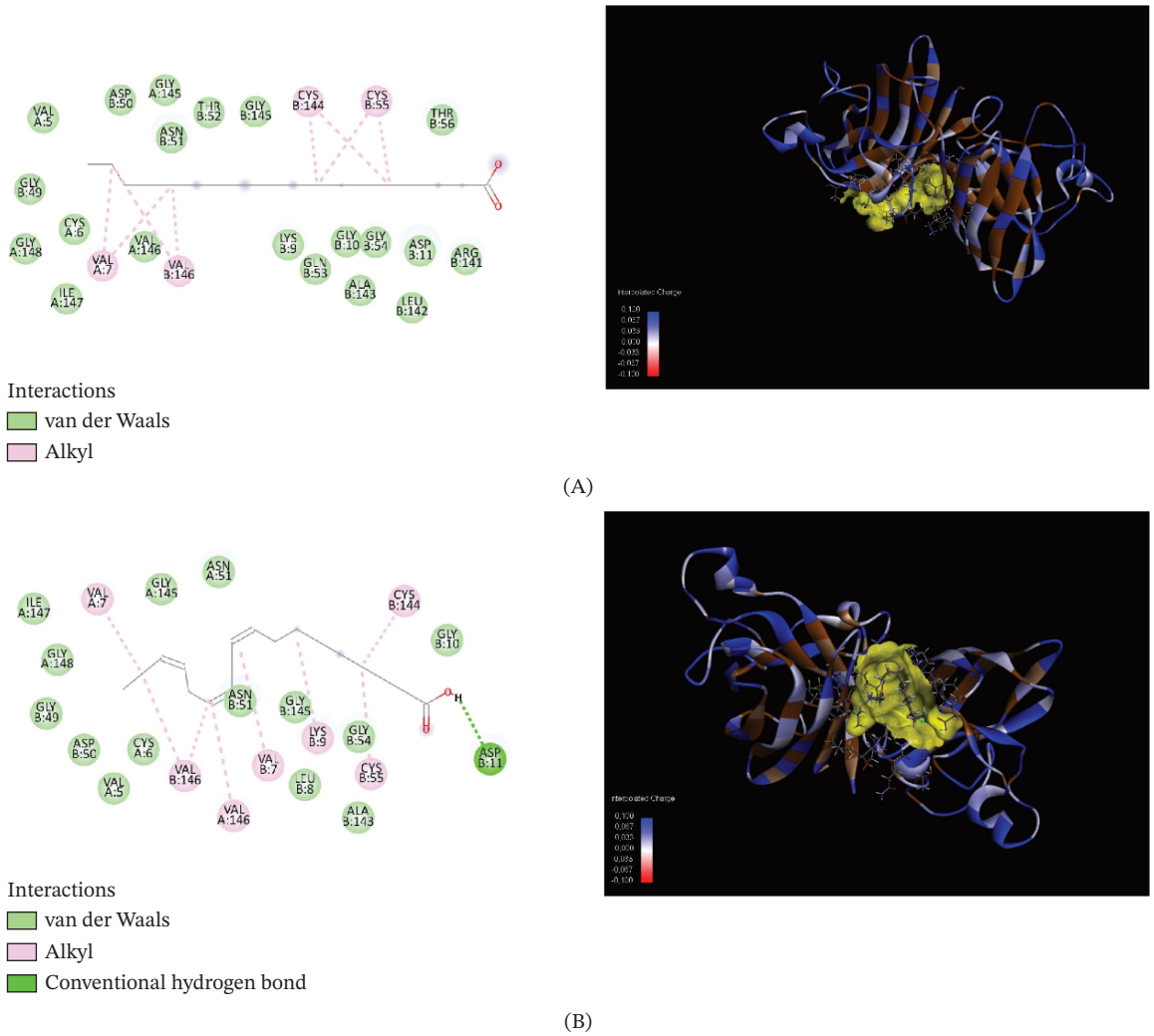


FIGURE 1 | 2D and 3D interactions of (A) linoleic acid and (B) arachidic acid with the superoxide dismutase enzyme (SOD1) (PDB ID: 1CB4).

TABLE 5 | Predicted endocrine disruption profiles of linoleic and arachidic acids.

ADMET	Endocrine disruption			
	Linoleic acid		Arachidic acid	
AR	0	%9.30	0	%8.80
ER	0	%6.20	0	%5.20
AR-LBD	0	%4.7	0	%2.80
ER-LBD	0	%7.20	0	%6.50
Aromatase	0	%9.60	0	%7.80
AhR	0	%7.30	0	%7.50
ARE	0	%22.60	0	%20.50
ATAD5	0	%2.50	0	%4.00
HSE	0	%11.40	0	%18.70
p53	0	%3.10	0	%3.90
PPARγ	0	%39.30	0	%26.70
MMP	0	%15.10	0	%15.50
TR	0	%15.90	0	%12.70
GR	0	%15.60	0	%13.70

levels are closely related to oxidative stress [43]. According to Le Chatelier's principle, when the initial equilibrium of a system is disturbed, the system responds to restore this equilibrium [44].

Unsaturated fatty acids, particularly polyunsaturated fatty acids, such as linoleic acid (C18:2) and alpha-linolenic acid (C18:3), are known to play a significant role in combating oxidative stress [45]. These fatty acids can neutralize free radicals, reduce oxidative damage at the cellular level, and may therefore be beneficial in preventing inflammation, cardiovascular diseases, and certain types of cancer [46]. Linoleic acid, present in the composition of *N. pulla*, may support cardiovascular health by exhibiting anti-inflammatory properties through its conversion to arachidonic acid in the body [47, 48]. The compound arachidic acid, a 20-carbon alkane, has been found to have protective effects against oxidative damage and to reduce inflammation [49]. Arachidic acid, produced via the cyclooxygenase and lipoxygenase pathways, has been shown to be associated with oxidative stress, and their production increases under shear stress conditions [50].

In this context, molecular docking analyses revealed that linoleic acid and arachidic acid exhibited binding affinities of −5.2 kcal/mol and −5.7 kcal/mol, respectively, toward the active site of

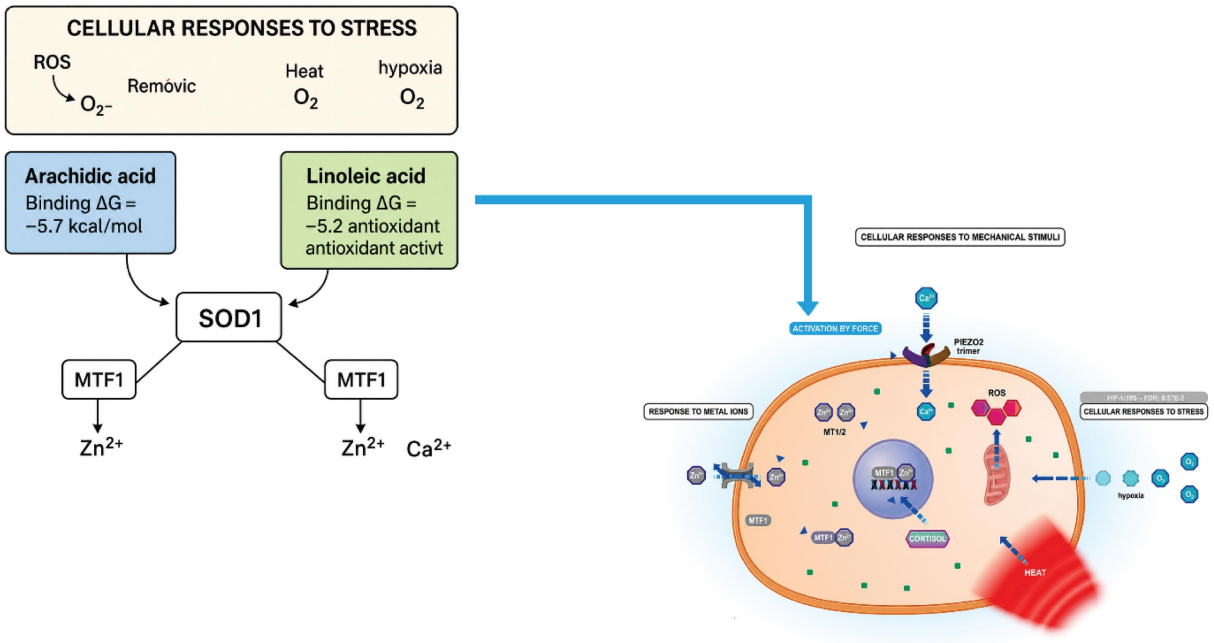


FIGURE 2 | Interaction of linoleic and arachidic acids with superoxide dismutase (SOD1) and their potential involvement in cellular stress responses (Figure adapted from Reactome).

SOD1. These values may suggest that the compounds could have a limited interaction potential with SOD1. This may indicate that the antioxidant effects of these fatty acids could be associated with mechanisms other than direct enzyme inhibition.

5 | Conclusion

The methanol extract of *N. pulla* exhibited antioxidant activity due to its rich phytochemical content, including flavonoids, phenolic compounds, and tannins. Fatty acid analysis revealed the presence of beneficial unsaturated fatty acids, such as linoleic acid and alpha-linolenic acid, which may contribute to its antioxidant and anti-inflammatory effects. Linoleic acid and arachidic acid identified in the *N. pulla* extract were predicted to bind to the active site of SOD1 with low binding affinities. These low binding affinities indicate a limited interaction potential and do not provide direct evidence for any inhibitory or regulatory effect on SOD1 activity. The findings are based solely on computational predictions and reflect possible interaction tendencies rather than confirmed biological effects. Therefore, no definitive conclusions can be drawn regarding the functional impact of these compounds on SOD1 activity. These interactions were interpreted within the context of antioxidant defense mechanisms associated with the enzymatic neutralization of ROS, and the results obtained from molecular docking and Reactome-based analyses were considered predictive and exploratory in nature. Accordingly, to establish the biological relevance of these findings, further advanced experimental studies are required, including enzyme activity assays (e.g., SOD1 activity), cell-based oxidative stress models, and protein expression analyses.

Author Contributions

Erdi Can Aytar: conceptualization, methodology, formal analysis, investigation, supervision, and writing–review and editing. Betül Aydın: methodology, formal analysis, investigation, data curation, and writing–review and editing. Esin Çolak: methodology, investigation, data curation, writing–original draft, and writing–review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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