World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

IN VITRO ANTIOXIDANT PROFILING AND IN SILICO KEAP1 DOCKING OF AN ELDERBERRY (*SAMBUCUS NIGRA*)-BASED NUTRACEUTICAL FORMULATION

Rajendran Aanaimuthu [1], Sudesh Raj [1,*], Ravetha Rajendran [1], Tejas Mutharikal [2], Muzhumathi Susithra [1], Sneha Srinivasan [1] and Reethi Suresh [1]

[1] Department of Clinical Research, Life Care Phyto Labs, Iyyappanthangal, Chennai, Tamil Nadu, India.
[2] Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Porur, Chennai, Tamil Nadu, India.
* Corresponding Author

ORCID Details
Sudesh Raj: https://orcid.org/0009-0000-0087-2202
Rajendran Aanaimuthu: https://orcid.org/0009-0001-3514-7200
Ravetha Rajendran: https://orcid.org/0009-0008-4483-9146
Tejas Mutharikal: https://orcid.org/0009-0003-5020-3295
Muzhumathi Susithra K: https://orcid.org/0009-0005-2799-8864
Sneha Srinivasan: https://orcid.org/0009-0005-5636-0093
Reethi Suresh: https://orcid.org/0009-0009-9189-6538

World Journal of Advanced Research and Reviews, 2026, 32(02), 106–115

Publication history: Received on 15 September 2026; revised on 26 October 2026; accepted on 28 October 2026

Article DOI: https://doi.org/10.30574/wjarr.2026.32.2.2708

Copyright © 2026 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution License 4.0

ABSTRACT

Multi-ingredient dietary supplements combining standardized botanicals, vitamins, and phytochemicals are frequently characterized using a single chemical assay, which offers limited insight into the relationship between composition, antioxidant capacity, and potential molecular mechanisms. This study characterized an elderberry (*Sambucus nigra*)-based nutraceutical formulation containing fruit extract standardized to 25% anthocyanins, together with cholecalciferol (vitamin D3), menaquinone-7 (vitamin K2), black garlic extract standardized to 1% S-allylcysteine, and *trans*-resveratrol (95%). Radical-scavenging activity was assessed with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay using ascorbic acid as the reference, and total phenolic content was determined by the Folin–Ciocalteu method. Cholecalciferol, cyanidin-3-glucoside, menaquinone-7, *trans*-resveratrol, and S-allylcysteine were docked against the Kelch domain of Kelch-like ECH-associated protein 1 (Keap1) by whole-domain docking in AutoDock Vina. The formulation showed concentration-dependent radical scavenging, increasing from 16 ± 2.8% at 250 µg/mL to 81 ± 4.7% at 1250 µg/mL, with an interpolated half-maximal inhibitory concentration of approximately 777.8 µg/mL (ascorbic acid, 6.43 µg/mL). Total phenolic content was 109.15 ± 3.24 mg gallic acid equivalents/g. Predicted binding affinities were −9.7, −8.8, −8.6, −8.3, and −4.8 kcal/mol for cholecalciferol, cyanidin-3-glucoside, menaquinone-7, *trans*-resveratrol, and S-allylcysteine, respectively. These complementary chemical and computational findings support characterization of the product as a multi-component, antioxidant-oriented formulation. However, they do not establish

activation of nuclear factor erythroid 2–related factor 2 or clinical efficacy, and cellular, pharmacokinetic, stability, and clinical studies are required to determine biological relevance.

Keywords: Elderberry; Nutraceutical formulation; Antioxidant activity; Total phenolic content; Keap1–Nrf2; Molecular docking

1 INTRODUCTION

Oxidative stress occurs when the generation of reactive oxygen species (ROS) and reactive nitrogen species exceeds the capacity of endogenous and exogenous antioxidant systems (1). It is a broad biochemical condition rather than a single disease mechanism. Its biological consequences depend on the type, concentration, and site of reactive species generation, the duration of exposure, and the capacity of cellular antioxidant and repair systems to mount adaptive responses (2,3). Accordingly, assessment of a dietary supplement intended for antioxidant support should ideally include more than one type of evidence.

Despite the increasing use of multi-ingredient dietary supplements containing botanicals, vitamins, and bioactive phytochemicals, their antioxidant potential is often evaluated using a single chemical assay, providing limited insight into the relationship between formulation composition, chemical antioxidant capacity, and potential molecular mechanisms (4,5). This is particularly relevant for formulations containing chemically diverse constituents, for which differences in polarity, structural characteristics, and redox properties may influence their behavior in conventional antioxidant assays (6,7). Therefore, the present study aimed to provide an integrated characterization of an immune-support and antioxidant formulation containing vitamin D3, vitamin K2 (menaquinone-7), elderberry extract, black garlic, and *trans*-resveratrol by combining compositional characterization with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity, total phenolic content (TPC), and molecular docking analysis. DPPH was employed to evaluate the chemical radical-scavenging capacity of the formulation, whereas TPC was used to estimate its overall phenolic content (8,9). Molecular docking was subsequently applied to investigate the predicted interactions of selected formulation constituents with Kelch-like ECH-associated protein 1 (Keap1), thereby providing a hypothesis-generating assessment of their potential relevance to the Keap1–nuclear factor erythroid 2–related factor 2 (Nrf2) antioxidant response pathway (10,11). The study further sought to contextualise the experimental findings with published evidence concerning the antioxidant constituents of elderberry, black garlic, and *trans*-resveratrol and their reported interactions with oxidative-stress-related pathways. Importantly, the computational analysis was not intended to establish direct Nrf2 activation or biological efficacy; rather, docking was used to compare the relative predicted binding characteristics of selected constituents under a consistent computational protocol (10,11). Similarly, DPPH radical-scavenging activity and TPC were interpreted as chemical and compositional indicators rather than direct surrogates of *in vivo* antioxidant activity (6,8,9). The central hypothesis was that integrating complementary chemical and *in silico* evidence would provide a more comprehensive characterization of the antioxidant-oriented potential of the multi-ingredient formulation than any single analytical approach, while maintaining appropriate distinction between chemical antioxidant capacity, predicted molecular interaction, biological activity, and clinical efficacy.

2 MATERIALS AND METHODS

2.1 Formulation Composition and Standardization

The formulation comprised five key ingredients: elderberry, black garlic, vitamin D3, resveratrol, and vitamin K2, each characterized by specific marker compounds or defined chemical forms used for standardization. Elderberry (*Sambucus nigra*) is characterized by a high abundance of cyanidin-derived anthocyanins, particularly cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside, which are widely used as characteristic constituents for the chemical characterization and quality assessment of elderberry preparations. The concentrations of these anthocyanins can vary substantially according to cultivar, growing conditions, harvest year, and stage of fruit ripening (12–14). Anthocyanins constitute the major phenolic constituents of elderberry, with cyanidin-based glycosides, particularly cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside, predominating in the fruit (15,16). Black garlic is produced by controlled thermal aging of raw garlic (*Allium sativum*) under elevated temperature and humidity, during which the sulfur-containing profile of garlic undergoes substantial transformation, resulting in increased levels of relatively stable compounds such as S-allyl-L-cysteine (SAC), which has been proposed as an important marker for the quality and standardization of black garlic preparations (17,18). Vitamin D3 is present as cholecalciferol and can be quantified using chromatographic techniques such as high-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Following absorption, cholecalciferol undergoes 25-hydroxylation primarily in the liver to form 25-hydroxyvitamin D3 [25(OH)D3], followed by 1α-hydroxylation predominantly in the kidney to produce the biologically active metabolite 1α,25-dihydroxyvitamin D3 [1,25$(OH)_2$D3] (19,20). Vitamin K2 comprises several menaquinone homologues, including MK-4 and MK-7, which differ in their side-chain structure, pharmacokinetic properties, tissue distribution, and biological responses; therefore, the specific menaquinone form should be defined when characterizing

or standardizing vitamin K2-containing formulations (20). Based on the pre-launch formulation specification, the serving size was two capsules, providing 125 µg (5,000 IU) of vitamin D3 as cholecalciferol, 100 µg of vitamin K2 as menaquinone-7 (MK-7), 500 mg of *S. nigra* fruit extract standardized to 25% anthocyanins, 150 mg of aged *A. sativum* bulb extract standardized to 1% S-allylcysteine, and 25 mg of *Polygonum cuspidatum* root extract standardized to 95% *trans*-resveratrol.

The declared standardization corresponds to approximately 125 mg total anthocyanins in the elderberry component, 1.5 mg S-allylcysteine in the black garlic component, and 23.75 mg *trans*-resveratrol in the resveratrol component, assuming the labelled standardization values represent the relevant marker content. These calculated marker amounts are label-based estimates and should be confirmed by validated chromatographic methods before being presented as analytically measured concentrations.

2.2 DPPH Radical-Scavenging Assay

The DPPH radical-scavenging assay is widely used to evaluate the capacity of antioxidant constituents to reduce the stable DPPH radical. In elderberry, DPPH radical-scavenging activity has been reported to vary with fruit developmental and ripening stage, alongside changes in phenolic and anthocyanin composition. Similarly, controlled aging of black garlic has been associated with increases in total polyphenol content and DPPH radical-scavenging activity, with antioxidant activity reaching higher levels during the aging process (8,14,21).

Five grams of the formulation was extracted in 50 mL of 80% (v/v) methanol with sonication for 10 min at room temperature, followed by centrifugation at 3000 rpm for 30 min. The supernatant was collected and used for the assay. DPPH solution (0.1 mM in methanol) was prepared fresh and protected from light. Sample concentrations were 250, 500, 750, 1000, and 1250 µg/mL. Ascorbic acid was used as the reference antioxidant at 2, 4, 6, 8, and 10 µg/mL.

In brief, 100 µL of sample or standard was mixed with 900 µL of DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm against a methanol blank. A sample blank (extract without DPPH) was used to correct for intrinsic color. The percentage of radical scavenging was calculated using Equation 1:

$$\text{Scavenging (\%)} = \left(1 - \frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{control}}}\right) \times 100 \qquad (1)$$

where A_{sample} is the absorbance of the sample with DPPH, $A_{\text{sample blank}}$ is the absorbance of the sample without DPPH, and A_{control} is the absorbance of the DPPH control (methanol + DPPH).

2.3 Total Phenolic Content Determination

TPC was determined to provide an estimate of the phenolic constituents present in the formulation. The Folin–Ciocalteu colorimetric method was selected because it is a widely used and standardized approach for estimating total phenolic content in botanical materials and dietary supplement matrices. In this assay, phenolic and other reducing constituents react with the Folin–Ciocalteu reagent under alkaline conditions to produce a blue-colored complex, the intensity of which is measured spectrophotometrically at approximately 765 nm. Results are commonly quantified against a gallic acid calibration curve and expressed as gallic acid equivalents (GAE). Because the Folin–Ciocalteu reagent is not completely specific to phenolic compounds and can also respond to other reducing substances, the resulting value was interpreted as an estimate of total reducing phenolic constituents rather than an absolute measurement of individual phenolic compounds (9,22).

Briefly, 100 µL of appropriately diluted sample extract was mixed with 500 µL of Folin–Ciocalteu reagent (1:10 in distilled water) and incubated for 5 min at room temperature. Then, 400 µL of 7.5% sodium carbonate solution was added, and the mixture was incubated in the dark for 2 h. Absorbance was measured at 765 nm against a reagent blank. A gallic acid calibration curve (10–100 µg/mL) was prepared, and TPC was calculated and expressed as mg GAE/g sample.

2.4 Molecular Docking Analysis

The Keap1–Nrf2 signaling axis was selected as the molecular target because Keap1 acts as a principal negative regulator of Nrf2 by promoting its ubiquitination and proteasomal degradation under basal conditions. The Kelch domain of Keap1 is responsible for recognizing the ETGE and DLG motifs of Nrf2 and therefore represents a functionally relevant region for investigating compounds that may interfere with Keap1–Nrf2 interactions. Natural products and phytochemicals have previously been investigated computationally as potential modulators of the Keap1–Nrf2 interface, supporting the use of molecular docking as an initial hypothesis-generating approach for evaluating ligand–

Keap1 interactions (10,11). However, docking scores represent predicted binding affinities and should not be interpreted as direct evidence of target engagement, Nrf2 activation, or biological efficacy.

Molecular docking was performed against the Kelch domain of Keap1 using AutoDock Vina. The crystal structure of the human Keap1 Kelch domain (PDB ID: 1U6D; 1.85 Å resolution) was obtained from the Protein Data Bank. The protein structure was prepared by removing water molecules, adding polar hydrogens, and assigning Gasteiger charges using AutoDock Tools (version 1.5.7). The ligand structures (vitamin D3, cyanidin-3-glucoside, menaquinone-7, *trans*-resveratrol, and S-allylcysteine) were obtained from PubChem, energy-minimized using the MMFF94 force field, and saved in PDBQT format.

Because 1U6D is an apo structure without a co-crystallized ligand, a whole-domain (blind) docking approach was used. The grid box (center coordinates: 28.423, 8.392, 23.263 Å; dimensions: 40 × 40 × 40 Å) was set large enough to encompass both the Nrf2-binding pocket on the top face of the Kelch β-propeller and its central channel, so that ligands could sample either site without bias. Nine poses were generated per ligand, and the pose with the lowest (most negative) predicted binding energy was taken as the docking score. Interaction maps were visualized using Discovery Studio Visualizer, and three-dimensional poses were visualized using PyMOL (version 3.1.8).

2.5 Statistical Analysis

Experimental results are expressed as mean ± standard deviation (SD) of three independent experiments (n = 3). Half-maximal inhibitory concentration (IC_{50}) values for DPPH radical scavenging were estimated by linear interpolation between the two tested concentrations bracketing 50% scavenging. The repeatability of TPC measurements was expressed as the coefficient of variation (CV).

3 RESULTS AND DISCUSSION

3.1 Formulation Composition Overview

The formulation comprises five declared active components with distinct chemical and functional characteristics. The elderberry component is present at the highest mass per serving and is standardized to anthocyanins, whereas the black garlic and resveratrol components are standardized to SAC and *trans*-resveratrol, respectively. Vitamin D3 and vitamin K2 are provided as defined chemical forms, namely cholecalciferol and menaquinone-7, rather than as non-standardized botanical materials. This chemical diversity is relevant to the interpretation of antioxidant potential because antioxidant effects may arise through direct radical-scavenging reactions, metal-ion interactions, modulation of endogenous antioxidant defenses, or regulation of redox-sensitive cellular signaling pathways (23). Conventional chemical antioxidant assays primarily capture specific chemical reactivity under defined experimental conditions and therefore cannot distinguish among these underlying biological mechanisms (6). Accordingly, the compositional characteristics of the formulation provide a rationale for integrating DPPH radical-scavenging activity and TPC with molecular docking as complementary approaches, while recognizing that confirmation of pathway-level effects would require subsequent cell-based or other biological assays (10).

3.2 Radical-Scavenging Activity

The radical-scavenging activity of the formulation was evaluated at increasing concentrations using the DPPH assay, with ascorbic acid included as the reference antioxidant (Figure 1). The sample showed a concentration-dependent increase in DPPH radical scavenging. Scavenging was 16 ± 2.8% at 250 µg/mL, 22 ± 3.2% at 500 µg/mL, 49 ± 3.7% at 750 µg/mL, 58 ± 4.1% at 1000 µg/mL, and 81 ± 4.7% at 1250 µg/mL (Figure 1B). The ascorbic acid reference showed 18%, 35%, 47%, 61%, and 91% scavenging at 2, 4, 6, 8, and 10 µg/mL, respectively (Figure 1A). Based on linear interpolation between the concentrations bracketing 50% scavenging, the IC_{50} values were approximately 777.8 µg/mL for the formulation and 6.43 µg/mL for ascorbic acid. Under the tested conditions, the formulation exhibited a concentration-dependent increase in DPPH radical-scavenging activity, reaching 81% at the highest tested concentration.

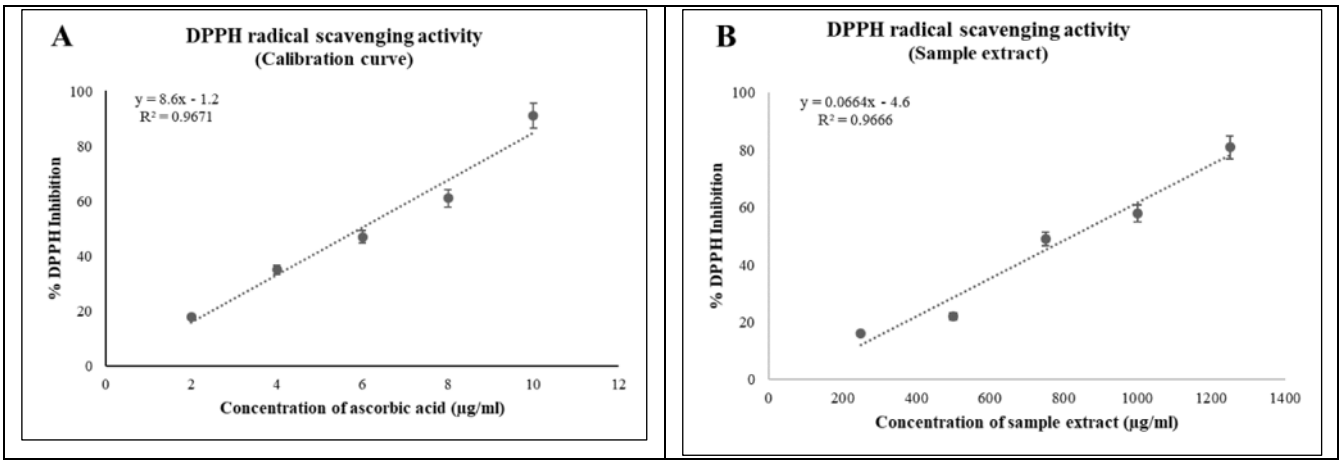

Figure 1: DPPH radical-scavenging activity. (A) Concentration-dependent DPPH scavenging by the ascorbic acid reference standard (2–10 µg/mL). (B) DPPH scavenging activity of the formulation extract (250–1250 µg/mL). Values are expressed as mean ± SD (n = 3).

3.3 Phenolic Content Analysis

TPC was determined to estimate the overall reducing phenolic constituents extracted from the formulation under the selected extraction conditions. The Folin–Ciocalteu assay is widely used for this purpose and expresses the response relative to a gallic acid calibration standard (Figure 2); however, the reagent is not completely specific to phenolic compounds and may also react with other reducing substances present in complex matrices (9,24). Therefore, the measured TPC value was interpreted as an estimate of Folin–Ciocalteu-reactive constituents rather than as a direct measure of the concentration of individual phenolic compounds.

The TPC was 109.15 ± 3.24 mg GAE/g sample. The replicate values were 111.99, 109.85, and 105.61 mg GAE/g, corresponding to a CV of approximately 3.0%, indicating relatively good repeatability of the triplicate measurements. The result may reflect phenolic compounds from elderberry and *trans*-resveratrol, as well as other reducing constituents extracted under the selected methanol conditions.

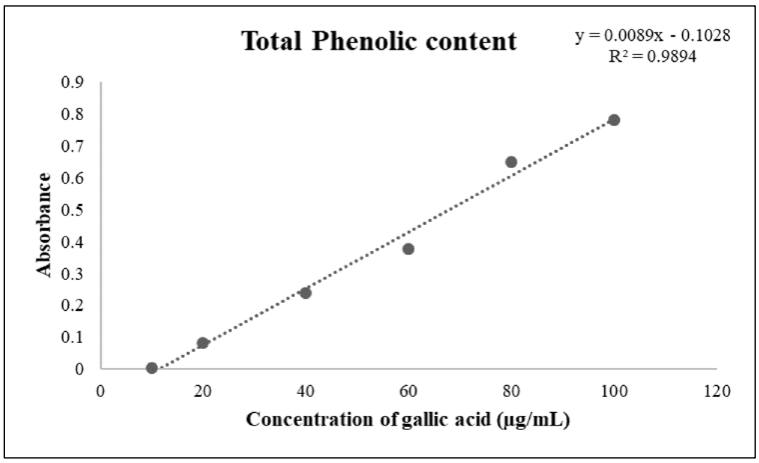

Figure 2: Gallic acid calibration curve used for the determination of total phenolic content. Absorbance was measured at 765 nm.

3.4 Keap1 Binding Interactions

The Kelch domain of Keap1 contains the binding site for the ETGE and DLG motifs of Nrf2, with residues including Arg415 and Arg483 contributing to recognition of the ETGE motif (25). Compounds that occupy this Nrf2-binding pocket could potentially interfere with the Keap1–Nrf2 interaction, whereas other Nrf2 activators can act through modification of regulatory Keap1 cysteine residues or through upstream signaling mechanisms (10).

In the present formulation, the selected ingredients may contribute to antioxidant-related responses through complementary mechanisms. *trans*-Resveratrol and cyanidin-3-glucoside have been associated with modulation of Nrf2-related antioxidant responses (26), while vitamin D3 has been reported to increase Nrf2 expression in neuronal cells and to dock favorably to Keap1 *in silico* (27). S-Allylcysteine from aged garlic has been reported to modulate Nrf2

signalling in cultured cells (28), whereas elderberry anthocyanins may contribute to chemical radical-scavenging activity (13,14).

Molecular docking predicted favorable interactions with Keap1, with vitamin D3 showing the most favorable docking score (−9.7 kcal/mol), followed by cyanidin-3-glucoside (−8.8 kcal/mol), menaquinone-7 (−8.6 kcal/mol), *trans*-resveratrol (−8.3 kcal/mol), and S-allylcysteine (−4.8 kcal/mol) (Table 1). The predicted binding pose of vitamin D3, the highest-scoring ligand, is shown in Figure 3. The top-ranked vitamin D3 pose was located in the central channel of the Kelch β-propeller, where it formed alkyl-type contacts with Ala366 and Val606 and van der Waals contacts with residues including Gly417, Leu557, and Gly605, rather than at the Arg415/Arg483-containing Nrf2-binding site. Because this site does not overlap the Nrf2-recognition residues, the pose does not by itself indicate competitive inhibition of the Keap1–Nrf2 interaction. These docking results provide computational evidence for the predicted interaction of the selected constituents with the Keap1 Kelch domain; however, docking scores alone do not establish target engagement, inhibition of the Keap1–Nrf2 interaction, Nrf2 activation, or the precise biological mechanism. Experimental validation is therefore required.

Table 1: Predicted binding affinities of selected formulation constituents with the Keap1 Kelch domain (whole-domain docking, PDB 1U6D)

Compound	Docking score (kcal/mol)
Vitamin D3 (cholecalciferol)	−9.7
Cyanidin-3-glucoside	−8.8
Menaquinone-7	−8.6
trans-Resveratrol	−8.3
S-Allylcysteine	−4.8

Note: Scores are the lowest predicted binding energies obtained with AutoDock Vina; more negative values indicate more favourable predicted binding.

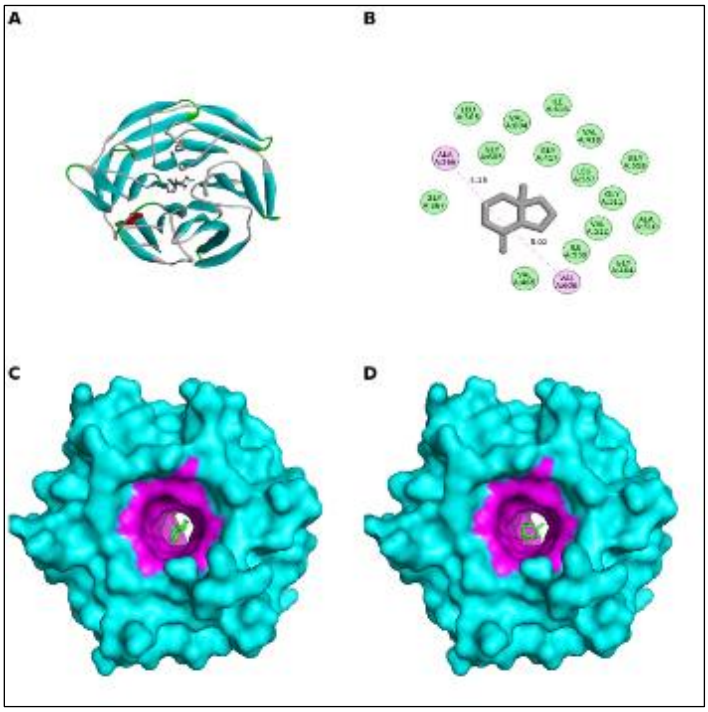

Figure 3: Top-ranked predicted binding pose of vitamin D3 from whole-domain docking against the Keap1 Kelch domain (PDB 1U6D). (A) Ribbon representation of the Kelch domain with the docked ligand (Discovery Studio). (B) Two-dimensional interaction map showing amino acid residues within the predicted binding site; dashed lines indicate predicted ligand–residue interactions with distances in Å. (C, D) Three-dimensional surface representation of the docked ligand within the central channel of the β-propeller in two orientations (PyMOL); the ligand is shown as sticks, the protein surface in cyan, and the channel in magenta.

4 DISCUSSION

Compared with studies evaluating isolated elderberry infusions or single antioxidant endpoints, the present work provides a broader characterization of a defined multi-ingredient dietary supplement. The formulation exhibited concentration-dependent DPPH radical scavenging and a TPC of 109.15 ± 3.24 mg GAE/g sample. The observed TPC was numerically higher than values reported for several elderberry preparations; however, direct comparison is limited by differences in extraction solvent, sample normalization, assay conditions, and formulation matrix (13,24). Similarly, the maximum DPPH inhibition was comparable in magnitude to values reported for some elderberry preparations, although the comparison should be interpreted cautiously because antioxidant activity depends on sample concentration, extraction conditions, reaction kinetics, and assay methodology (6,8). Molecular docking identified favorable predicted Keap1 interactions for vitamin D3, cyanidin-3-glucoside, menaquinone-7, and *trans*-resveratrol. The favorable score for vitamin D3 is consistent with a previous *in silico* study in which cholecalciferol docked favorably to Keap1 (27), although in the present whole-domain docking its top-ranked pose occupied the central channel rather than the Nrf2-binding site. *trans*-Resveratrol is further supported by published literature describing its association with Keap1–Nrf2-related antioxidant responses (11,26). Collectively, these findings position the formulation as a candidate with complementary chemical and computational antioxidant-related evidence. They do not establish universal superiority over other supplements, and direct head-to-head studies, chromatographic standardization, cellular validation, and ultimately clinical evaluation are required.

4.1 Limitations and Future Perspectives

The principal limitation is the absence of a direct comparator formulation. Demonstrating comparative performance would require testing the product alongside at least two commercially available antioxidant supplements or matched ingredient mixtures using identical extraction, DPPH, TPC, and reporting procedures, with anonymized comparators and serving-size normalization. Comparative evaluation of commercial antioxidant supplements using multiple complementary antioxidant assays and chemical profiling has been shown to provide more informative characterization than reliance on a single assay (24,29).

The second limitation is the lack of complete chromatographic confirmation. HPLC with diode-array detection (HPLC-DAD) or LC–MS/MS should be used to quantify cyanidin-3-glucoside or another validated anthocyanin marker, *trans*-resveratrol, and S-allylcysteine. Vitamin D3 and MK-7 may require suitable chromatographic methods because of their distinct chemical properties and relatively low concentrations. Such analyses would establish whether the labelled standardizations are met in the tested batch.

The third limitation is the absence of a complete DPPH concentration–response model. Although the data support concentration dependence, the IC_{50} was estimated by linear interpolation; future work should report replicate-level data, regression-based concentration–response modelling, and an IC_{50} with a confidence interval where the response curve permits. Complementary assays such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), or a cell-based intracellular ROS assay could provide orthogonal evidence because antioxidant assays are based on different chemical reaction mechanisms and may not provide interchangeable estimates of antioxidant capacity (30,31).

The fourth limitation concerns interpretation of TPC. The Folin–Ciocalteu reaction is not specific to phenolic compounds and primarily reflects reducing capacity under the assay conditions. Therefore, TPC should be complemented by HPLC-based phenolic profiling and, where relevant, total anthocyanin determination. The relationship between TPC and DPPH activity should be statistically examined rather than assumed to be causal (32,33).

The fifth limitation concerns docking validation. Because a whole-domain search on an apo structure was used, site-directed docking restricted to the Nrf2-binding pocket, preferably using a ligand-bound Keap1 structure (for example, PDB 4IQK), is needed to test whether the constituents could compete directly with Nrf2. Future work should also include a co-crystallized ligand or a validated Keap1 inhibitor as a positive control, redocking analysis with root-mean-square deviation (RMSD) where an experimentally determined ligand pose is available, and assessment of docking reproducibility. These procedures can help determine whether the selected docking protocol is capable of reproducing a known binding mode rather than relying solely on predicted affinity scores. Docking affinity scores should also not be treated as directly comparable across different scoring functions. Molecular dynamics simulations could provide additional information regarding the stability of the predicted ligand–Keap1 complexes, although they would still not substitute for experimental target-engagement studies; previous Keap1 computational studies have used RMSD and related trajectory analyses to examine complex stability (34). Cellular experiments should subsequently assess Nrf2 nuclear translocation, heme oxygenase-1 (HO-1) and NAD(P)H quinone dehydrogenase 1 (NQO1) expression, antioxidant response element (ARE) reporter activity, and intracellular ROS to determine whether predicted Keap1 interactions translate into pathway-level biological responses. Such endpoints have been used experimentally to demonstrate Nrf2 activation and downstream antioxidant-response signaling (35,36).

Abbreviations

ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); ARE: antioxidant response element; CV: coefficient of variation; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power; GAE: gallic acid equivalents; HO-1: heme oxygenase-1; HPLC: high-performance liquid chromatography; HPLC-DAD: high-performance liquid chromatography with diode-array detection; IC_{50}: half-maximal inhibitory concentration; IU: international units; Keap1: Kelch-like ECH-associated protein 1; LC–MS/MS: liquid chromatography–tandem mass spectrometry; MK-4: menaquinone-4; MK-7: menaquinone-7; NQO1: NAD(P)H quinone dehydrogenase 1; Nrf2: nuclear factor erythroid 2–related factor 2; ORAC: oxygen radical absorbance capacity; RMSD: root-mean-square deviation; ROS: reactive oxygen species; SAC: S-allylcysteine; SD: standard deviation; TPC: total phenolic content.

5 CONCLUSION

The present study provided an integrated preliminary characterization of a defined multi-ingredient dietary supplement containing elderberry, black garlic, vitamin D3, *trans*-resveratrol, and vitamin K2. The formulation demonstrated concentration-dependent DPPH radical-scavenging activity, with an estimated IC_{50} of approximately 777.8 µg/mL, while TPC indicated the presence of appreciable Folin–Ciocalteu-reactive constituents. Molecular docking further identified favorable predicted interactions between selected formulation constituents and the Keap1 Kelch domain, with vitamin D3 showing the most favorable predicted docking score among the compounds evaluated. These findings provide complementary chemical and computational evidence supporting the antioxidant-related potential of the formulation.

However, the results should not be interpreted as evidence of direct Nrf2 activation, superior clinical antioxidant efficacy, or therapeutic effectiveness. DPPH and TPC provide chemical and compositional information under defined assay conditions, whereas molecular docking provides predicted ligand–target interactions rather than experimentally confirmed biological activity. The findings therefore support further investigation rather than definitive efficacy claims. Future studies should incorporate chromatographic verification of the declared marker compounds, comparison with appropriate commercial or formulation controls, additional antioxidant assays, and cell-based evaluation of Nrf2 pathway activation, including Nrf2 nuclear translocation and downstream markers such as HO-1 and NQO1. Collectively, such approaches would establish whether the chemical and computational findings observed in the present study translate into reproducible biological antioxidant responses and, ultimately, clinically relevant effects.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Acknowledgments

During the preparation of this work, the authors used QuillBot and Grammarly for language refinement and grammar. After using these tools, the authors reviewed and edited all content as necessary and take full responsibility for the content of the published article.

Funding Source

This study was conducted as internal product testing by Life Care Phyto Labs, Chennai, India. No external grant or funding was received.

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this manuscript.

Statement of ethical approval

This study involved only *in vitro* chemical assays and *in silico* molecular docking; no human or animal subjects were involved, and ethical approval was therefore not required.

REFERENCES

[1] Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, et al. Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol. 2020;11:694. doi:10.3389/fphys.2020.00694

[2] Navarro-Yepes J, Burns M, Anandhan A, Khalimonchuk O, Del Razo LM, Quintanilla-Vega B, et al. Oxidative stress, redox signaling, and autophagy: cell death versus survival. Antioxid Redox Signal. 2014;21(1):66-85. doi:10.1089/ars.2014.5837

[3] Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763. doi:10.1155/2017/8416763

[4] Hermans N, Cos P, Maes L, De Bruyne T, Vanden Berghe D, Vlietinck AJ, et al. Challenges and pitfalls in antioxidant research. Curr Med Chem. 2007;14(4):417-30. doi:10.2174/092986707779941005

[5] Viktorova J, Stranska-Zachariasova M, Fenclova M, Vitek L, Hajslova J, Kren V, et al. Complex evaluation of antioxidant capacity of milk thistle dietary supplements. Antioxidants (Basel). 2019;8(8):317. doi:10.3390/antiox8080317

[6] Xie J, Schaich KM. Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. J Agric Food Chem. 2014;62(19):4251-60. doi:10.1021/jf500180u

[7] Dawidowicz AL, Olszowy M, Jóźwik-Dolęba M. Importance of solvent association in the estimation of antioxidant properties of phenolic compounds by DPPH method. J Food Sci Technol. 2015;52(7):4523-9. doi:10.1007/s13197-014-1451-2

[8] Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol. 1995;28(1):25-30. doi:10.1016/S0023-6438(95)80008-5

[9] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 1999;299:152-78. doi:10.1016/S0076-6879(99)99017-1

[10] Cuadrado A, Rojo AI, Wells G, Hayes JD, Cousin SP, Rumsey WL, et al. Therapeutic targeting of the NRF2 and KEAP1 partnership in chronic diseases. Nat Rev Drug Discov. 2019;18(4):295-317. doi:10.1038/s41573-018-0008-x

[11] Li M, Huang W, Jie F, Wang M, Zhong Y, Chen Q, et al. Discovery of Keap1-Nrf2 small-molecule inhibitors from phytochemicals based on molecular docking. Food Chem Toxicol. 2019;133:110758. doi:10.1016/j.fct.2019.110758

[12] Mikulic-Petkovsek M, Schmitzer V, Slatnar A, Todorovic B, Veberic R, Stampar F, et al. Investigation of anthocyanin profile of four elderberry species and interspecific hybrids. J Agric Food Chem. 2014;62(24):5573-80. doi:10.1021/jf5011947

[13] Sidor A, Gramza-Michałowska A. Advanced research on the antioxidant and health benefit of elderberry (Sambucus nigra) in food – a review. J Funct Foods. 2015;18:941-58. doi:10.1016/j.jff.2014.07.012

[14] Marţiş (Petruţ) GS, Mureşan V, Marc (Vlaic) RM, Mureşan CC, Pop CR, Buzgău G, et al. The physicochemical and antioxidant properties of Sambucus nigra L. and Sambucus nigra Haschberg during growth phases: from buds to ripening. Antioxidants (Basel). 2021;10(7):1093. doi:10.3390/antiox10071093

[15] Młynarczyk K, Walkowiak-Tomczak D, Staniek H, Kidoń M, Łysiak GP. The content of selected minerals, bioactive compounds, and the antioxidant properties of the flowers and fruit of selected cultivars and wildly growing plants of Sambucus nigra L. Molecules. 2020;25(4):876. doi:10.3390/molecules25040876

[16] Domínguez R, Pateiro M, Munekata PES, Santos López EM, Rodríguez JA, Barros L, et al. Potential use of elderberry (Sambucus nigra L.) as natural colorant and antioxidant in the food industry. A review. Foods. 2021;10(11):2713. doi:10.3390/foods10112713

[17] Kimura S, Tung YC, Pan MH, Su NW, Lai YJ, Cheng KC. Black garlic: a critical review of its production, bioactivity, and application. J Food Drug Anal. 2017;25(1):62-70. doi:10.1016/j.jfda.2016.11.003

[18] Woo HJ, Cha GS, Kang MJ, Kyung KH. Assessment of standardization of domestic commercial black garlic extract for S-allyl-L-cysteine and S-1-propenyl-L-cysteine. Food Sci Biotechnol. 2022;31(2):253-60. doi:10.1007/s10068-021-01028-1

[19] Ramasamy I. Vitamin D metabolism and guidelines for vitamin D supplementation. Clin Biochem Rev. 2020;41(3):103-26. doi:10.33176/AACB-20-00006

[20] Yan Q, Zhang T, O'Connor C, Barlow JW, Walsh J, Scalabrino G, et al. The biological responses of vitamin K2: a comprehensive review. Food Sci Nutr. 2023;11(4):1634-56. doi:10.1002/fsn3.3213

[21] Choi I, Cha H, Lee Y. Physicochemical and antioxidant properties of black garlic. Molecules. 2014;19(10):16811-23. doi:10.3390/molecules191016811

[22] Kupina S, Fields C, Roman MC, Brunelle SL. Determination of total phenolic content using the Folin-C assay: single-laboratory validation, First Action 2017.13. J AOAC Int. 2018;101(5):1466-72. doi:10.5740/jaoacint.18-0031

[23] Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21(7):363-83. doi:10.1038/s41580-020-0230-3

[24] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-302. doi:10.1021/jf0502698

[25] Canning P, Sorrell FJ, Bullock AN. Structural basis of Keap1 interactions with Nrf2. Free Radic Biol Med. 2015;88:101-7. doi:10.1016/j.freeradbiomed.2015.05.034

[26] Di Liu, Fengguang Pan, Jiyun Liu, Ying Wang, Ting Zhang, Erlei Wang, Jingbo Liu; Individual and combined antioxidant effects of ginsenoside F2 and cyanidin-3-O-glucoside in human embryonic kidney 293 cells. RSC Adv. 2016; 6 (84): 81092–81100

[27] Uthaiah CA, Devaru NC, Shivakumar NH, Rajalakshmi R, Madhunapantula SV. Vitamin D mitigates hyperglycemia-induced cognition decline in Danio rerio (zebrafish) through the activation of antioxidant mechanisms. Antioxidants (Basel). 2022;11(11):2114. doi:10.3390/antiox11112114

[28] Lin W, Lai Y, Nagabhushanam K, Ho C, Pan M. S-Allylcysteine potently protects against PhIP-induced DNA damage via Nrf2/AhR signaling pathway modulation in normal human colonic mucosal epithelial cells. Mol Nutr Food Res. 2022;66(16):e2101141. doi:10.1002/mnfr.202101141

[29] Mirończuk-Chodakowska I, Kujawowicz K, Cyuńczyk M, Sejbuk M, Witkowska AM. Multi-method screening of commercial antioxidant supplements: antioxidant activity and selected antioxidant compound profiles. Foods. 2026;15(8):1303. doi:10.3390/foods15081303

[30] Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005;53(6):1841-56. doi:10.1021/jf030723c

[31] Bartosz G. Non-enzymatic antioxidant capacity assays: limitations of use in biomedicine. Free Radic Res. 2010;44(7):711-20. doi:10.3109/10715761003758114

[32] Platzer M, Kiese S, Herfellner T, Schweiggert-Weisz U, Eisner P. How does the phenol structure influence the results of the Folin-Ciocalteu assay? Antioxidants (Basel). 2021;10(5):811. doi:10.3390/antiox10050811

[33] Raposo F, Borja R, Gutiérrez-González JA. A comprehensive and critical review of the unstandardized Folin-Ciocalteu assay to determine the total content of polyphenols: the conundrum of the experimental factors and method validation. Talanta. 2024;272:125771. doi:10.1016/j.talanta.2024.125771

[34] Adelusi TI, Abdul-Hammed M, Idris MO, Oyedele QK, Adedotun IO. Molecular dynamics, quantum mechanics and docking studies of some Keap1 inhibitors – an insight into the atomistic mechanisms of their antioxidant potential. Heliyon. 2021;7(6):e07317. doi:10.1016/j.heliyon.2021.e07317

[35] Hsieh CY, Hsiao HY, Wu WY, Liu CA, Tsai YC, Chao YJ, et al. Regulation of shear-induced nuclear translocation of the Nrf2 transcription factor in endothelial cells. J Biomed Sci. 2009;16(1):12. doi:10.1186/1423-0127-16-12

[36] Liang L, Gao C, Luo M, Wang W, Zhao C, Zu Y, et al. Dihydroquercetin (DHQ) induced HO-1 and NQO1 expression against oxidative stress through the Nrf2-dependent antioxidant pathway. J Agric Food Chem. 2013;61(11):2755-61. doi:10.1021/jf304768p