Journal of Agriculture and Food Research 18 (2024) 101495

Contents lists available at ScienceDirect

Journal of Agriculture and Food Research

journal homepage: www.sciencedirect.com/journal/journal-of-agriculture-and-food-research

Exploring the potential molecular targets of hydroxymethylbutyrate and glucosamine fortified whey protein drink to modulate sarcopenia and Alzheimer's disease by *in silico* and *in vitro* studies

Fahrul Nurkolis [a,*], Vincent Lau [b], Trina Ekawati Tallei [c], Nurpudji Astuti Taslim [d], Son Radu [e], Apollinaire Tsopmo [f], Bonglee Kim [g], Rony Abdi Syahputra [h], Raymond Rubianto Tjandrawinata [i], Rini Arianti [j,k,1], Endre Kristóf [j,1]

[a] Department of Biological Sciences, Faculty of Sciences and Technology, State Islamic University of Sunan Kalijaga (UIN Sunan Kalijaga), Yogyakarta, 55281, Indonesia
[b] Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia
[c] Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado, 95115, Indonesia
[d] Division of Clinical Nutrition, Department of Nutrition, Faculty of Medicine, Hasanuddin University, Makassar, 90245, Indonesia
[e] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, UPM Serdang, 43400, Malaysia
[f] Food Science and Nutrition Program, Department of Chemistry, Carleton University, 1125 Colonel by Drive, Ottawa, ON, K1S 5B6, Canada
[g] Department of Pathology, College of Korean Medicine, Kyung Hee University, Kyungheedae-Ro 26, Dong-Daemun-Gu, Seoul, 05254, South Korea
[h] Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155, Indonesia
[i] Center for Pharmaceutical and Nutraceutical Research and Policy, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Jakarta, 12930, Indonesia
[j] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, H-4032, Debrecen, Hungary
[k] Universitas Muhammadiyah Bangka Belitung, Pangkalpinang, 33134, Indonesia

ARTICLE INFO

Keywords:
Hydroxymethylbutyrate
Glucosamine
Sarcopenia
Alzheimer's disease
Anti-aging
Milk

ABSTRACT

Fortified foods have garnered significant attention as potential therapeutic strategies with less adverse effects and ready accessibility. However, precise formulation is required to optimize their beneficial effects. Our study aimed to unravel the mechanisms of a functional protein beverage, hydroxymethylbutyrate and glucosamine fortified whey protein drink (HG-WPD), as a possible intervention combatting sarcopenia and Alzheimer's disease (AD) by network pharmacology, molecular docking, and experimental assays. This study investigates the molecular pathways through which HG-WPD acts, by combining *in silico* and *in vitro* analyses. Our *in silico* study predicted the important target proteins, including acetylcholinesterase (AChE), matrix metalloproteinase 9 (MMP9), and angiotensin-converting enzyme (ACE), linked to both diseases. The molecular docking analysis showed that hydroxymethylbutyrate and glucosamine exhibited a notable binding affinity to these proteins, therefore suggesting their possible use as multi-target medicinal agents. *In vitro* studies on C2C12 myotubes showed that HG-WPD reduced dexamethasone-induced cell death highlighting its potential anti-sarcopenic actions. Furthermore, confirming their possible function in reducing cognitive impairment in AD, the antioxidant tests showed great activity of both substances. The results imply that HG-WPD is potentially effective for future therapeutic approaches as a functional food product with dual benefits in combatting sarcopenia and AD.

1. Introduction

Sarcopenia, a disease characterized by the decrease of muscle strength and mass, triggers significant challenges due to its association with adverse health outcomes, such as falls, functional decline, frailty, and mortality [1–3]. Moreover, sarcopenia has been linked to poor postoperative prognosis, longer hospitalization, and increased healthcare expenses [4]. It is also associated with a higher risk of depression

* Corresponding author.
E-mail addresses: fahrul.nurkolis.mail@gmail.com (F. Nurkolis), vilauv92@gmail.com (V. Lau), trina_tallei@unsrat.ac.id (T.E. Tallei), pudji_taslim@yahoo.com (N.A. Taslim), sonradu@gmail.com (S. Radu), apollinaire_tsopmo@carleton.ca (A. Tsopmo), bongleekim@khu.ac.kr (B. Kim), rony@usu.ac.id (R.A. Syahputra), raytjan@yahoo.com (R.R. Tjandrawinata), ariantirini@med.unideb.hu (R. Arianti), kristof.endre@med.unideb.hu (E. Kristóf).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.jafr.2024.101495
Received 29 September 2024; Received in revised form 1 November 2024; Accepted 2 November 2024
Available online 6 November 2024

which can negatively impact physical functions, leading to further adverse health consequences [1,5]. Several studies have highlighted the urgency of identifying treatment for sarcopenia as a significant public health concern [6]. Various studies emphasize the importance of early diagnosis to facilitate effective prevention and treatment strategies [7]. The increasing prevalence of sarcopenia, especially among older populations, has emerged as a major health issue [8]. Sarcopenia is prevalent not only among individuals with age-related diseases but it is also linked to poor health outcomes in a broader spectrum [9].

Managing sarcopenia faces significant challenges due to the lack of approved pharmacological treatments. Current interventions primarily focus on physical therapy for muscle strengthening, gait training, and ensuring adequate protein intake [10]. However, there is growing interest in exploring alternative strategies, such as whole-body vibration training, blood flow restriction, and electrical stimulation, as potential therapies for sarcopenia [11]. It is crucial to establish a multipronged approach to treat sarcopenia, which includes nutritional support, exercise, and potential pharmacological interventions [12].

Sarcopenia, the progressive loss of muscle mass and strength, is increasingly recognized as a potential risk factor for Alzheimer's disease (AD), as it may exacerbate cognitive decline by contributing to frailty and reduced physical activity. AD is the most prevalent form of dementia, accounting for approximately 60–80 % of dementia cases [13–15]. The global incidence of AD is increasing, resulting in a significant public health challenge [16]. Age is the primary risk factor for AD, with other factors, such as geographical location, income, gender, and environmental influences also playing a role [17]. Studies have indicated a strong association between AD and biological aging, with leukocyte telomere length suggested as a critical biomarker for assessing AD risk [18]. Furthermore, epidemiological evidence has suggested that non-steroidal anti-inflammatory drugs may reduce the risk of developing AD [19]. AD has also been linked to other health conditions, such as type 2 diabetes, with shared molecular mechanisms including amyloid-beta peptide (Aβ) accumulation and tau protein hyperphosphorylation [20–22].

AD is a progressive neurodegenerative condition with limited definitive cure. Existing therapeutic approaches primarily focus on managing symptoms and attempting to inhibit disease progression [23]. A key aspect of AD management involves limiting the decline in acetylcholine levels and neurotransmitter destabilization, which are linked to cognitive and memory impairments. Cholinesterase inhibitors are commonly used to elevate endogenous acetylcholine levels in the brain, offering relief for cognitive and behavioral symptoms in mild to moderate AD cases [24]. The inhibition of the acetylcholinesterase (AChE) enzyme to enhance acetylcholine levels in the synaptic clefts is a well-established therapeutic strategy for AD [25] via modulating cholinergic neurotransmission [26]. Additionally, disease-modifying therapies (DMTs) are gaining attention as they aim to slow the progression of dementia symptoms in patients with AD [27]. Several therapeutic strategies including the potential use of low-dose ionizing radiation [23], phytochemical compounds, nanoparticles, and phytochemical delivery systems [28] have been explored to establish more effective treatments. The pharmaceutical benefit of plant extracts and their bioactive compounds, such as alpha-tocopherol, selegiline, and ginkgo biloba, has also been investigated for their therapeutic potential [29].

Hydroxymethylbutyrate (HMB) and glucosamine have gained attention for their potential benefits in various health conditions. HMB, a metabolite of the amino acid leucine, has been reported to positively affect muscle strength and size, particularly in older individuals [30]. A study reported that HMB supplementation, in combination with elastic-band resistance exercise or vibration treatment, could effectively manage conditions like sarcopenia in older populations [31]. In addition, HMB supplementation, when combined with physical exercise, was found to delay frailty and reduce cognitive impairment in older adults [32]. Glucosamine, an amino sugar and naturally found in cartilage, was associated with improved joint health and reduced inflammation in osteoarthritis. Studies have shown that glucosamine supplementation can maintain joint structure, prevent cartilage degradation, and enhance the chondrogenic potential of stem cells [33]. Furthermore, the combination of glucosamine and nutrients, such as milk-fat globule membrane, was found to improve joint function and physical performance [34]. Both HMB and glucosamine play crucial roles in maintaining or improving muscle and joint health. While HMB has more impact on muscle strength and size, glucosamine is primarily associated with joint integrity and cartilage maintenance. These supplements, when used in conjunction with appropriate exercise intervention and nutrition, can contribute to overall well-being, especially in aging populations or individuals with specific health concerns related to muscle or joint functions.

Innovative food products fortified with HMB and glucosamine may provide therapeutic benefits to support the health of muscles and joints. Whey Protein Drink (WPD; HiLo Platinum™), a ready-to-drink milk product fortified with both HMB and glucosamine (HG-WPD), is currently available in the market. We intended to uncover the potential effect of HMB and glucosamine in commercial WPD products (HiLo Platinum™) by investigating the molecular signaling pathways involved in combating sarcopenia and AD. This was achieved using *in silico* network pharmacology, protein-protein interaction studies, molecular docking, and *in vitro* assays. This study aims to fill the current gap of knowledge and support new functional food innovations that can fight against sarcopenia and AD.

2. Materials and Methods

2.1. Sample preparation

The nutritional content information of the commercially available HG-WPD (Table 1) was obtained from the official website of the company (https://www.hilo.co.id/products/hilo-gold/hilo-platinum-hmb; accessed August 4th, 2024).

2.2. Analyses of bioactive compounds activities, toxicity, and drug likeness

The PubChem compound identifier (CID) was obtained for all the substances of our interest for further *in silico* analysis [35]. Possible bioactivities of these substances as anti-sarcopenia or AD were investigated by using WAS2DRUG PASS prediction tool (www.pharmaexpert.ru). The target proteins or ligands of the subjected substances could be predicted by structure activity relationship (SAR) analysis [36]. Probability of activity (Pa) value above 0.5 was considered to have high potential as anti-sarcopenia or anti-AD [37]. Toxicity analysis was performed by using Protox (https://tox.charite.de/protox3/index.php?site=compound_input). Drug likeness analysis was conducted by subjecting the SMILES notation of each compound of interest, which was obtained from Pubchem, into ADMETLab 2.0 database (www.admetmesh.scbdd.com). Lipinski's Rule of Five (Ro5) guide was employed to evaluate the drug similarity properties of each ligand [38–40].

2.3. Virtual screening of protein targets

The predicted targets of glucosamine and HMB were analyzed by inputting the SMILES notations of each compound into SuperPred target analysis tool (www.prediction.charite.de). Cut-off score for this analysis was 80 %, which reflects the precision of the model (Table S1) [41,42]. The predicted targets which are linked to sarcopenia and AD were analyzed by using Open Targets database (www.opentargets.org). Proteins, which were predicted as targets of glucosamine and HMB and were found to be linked to sarcopenia and AD, were selected for pathway enrichment and network pharmacology analysis by using Kyoto

Table 1
Nutritional fact of HG-WPD (HiLo Platinum™).

Nutritional Content	Amount per Serving
Total Energy	150 kcal
Total Fat	4 g
Trans Fat	0 g
Cholesterol	10 mg
Omega 3 (α-linolenic acid)	259 mg
Omega 6 (linoleic acid)	120 mg
Saturated Fat	1 g
Protein	12 g
Total Carbohydrate	22 g
Dietary Fiber	1 g
Sugar (Sucrose)	0 g
Lactose	2 g
Salt (Sodium)	140 mg
Vitamin A	800 IU
Vitamin C	36 mg
Vitamin B1	1.40 mg
Vitamin B2	0.64 mg
Vitamin B3	15 mg
Vitamin B5	2 mg
Vitamin B6	1.30 mg
Vitamin B7	12 μg
Vitamin B12	6 μg
Folic Acid	160 μg
Vitamin D	600 IU
Vitamin E	6 mg
Potassium	95 mg
Calcium	660 mg
Iron	4 mg
Phosphorus	133 mg
Zinc	3.9 mg
Magnesium	61 mg
Iodine	30 μg
Selenium	60 μg
Chromium	6 μg
Glucosamine	250 mg
HMB	500 mg

Encyclopedia of Genes and Genomes (KEGG) [43] and STRING database (www.string-db.org) [44], respectively.

2.4. Molecular docking simulation

The predicted receptors with the highest centrality which have been recognized to have role in the pathophysiology of AD were selected for further analysis. The binding affinity value of the selected proteins and glucosamine or HMB was evaluated by molecular docking simulation by CB-Dock2 that probes interaction between proteins and ligands based on the cavity-detection-guided blind docking. This approach combines homologous template fitting, cavity identification, and docking [45]. Compounds that have been established as binding ligands of selected protein targets were used as references. The binding affinity was indicated as Vina score where the higher negative score reflects the stronger binding affinity. Next, the predicted structures of the selected proteins or their ligands were analyzed by using RSCB protein data bank (www.rscb.org).

2.5. Antioxidant activity measurement by DPPH and ABTS assays

The antioxidant activity of HG-WPD was evaluated by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging activity assays [43,44]. HG-WPD samples were added to 3 mL of DPPH reagent at final concentrations of 1, 10, 20, 30, and 40 mg/mL. The DPPH-sample solution was incubated at 22 °C for 30 min. The absorbance was measured with a nano-spectrophotometer (SPECTROstarNano BMG LABTECH) at 517 nm. For ABTS test, a working solution was generated by adding $K_2S_2O_8$ and ABTS at a 1:1 ratio. Next, the HG-WPD was mixed with working solution at final concentrations of 1, 10, 20, 30, and 40 mg/mL. The solution was incubated in dark at 22 °C for 14 h. The absorbance of the solution was measured at a wavelength of 734 nm using a nano-spectrophotometer (SPECTROstarNano BMG LABTECH). Trolox was used as control for both DPPH and ABTS tests. The percentage of inhibition of DPPH and ABTS was quantified with this formula:

$$\text{Inhibition Activity } (\%) = \frac{A0 - A1}{A0} \times 100\%$$

where A_0 refers to the blank while A_1 to the standard or sample abdorbance values.

To ensure the reliability of the DPPH and ABTS assay results, each sample was tested in triplicates.

2.6. In vitro anti-sarcopenia activity assesment

C2C12 mouse skeletal myoblasts (American Type Culture Collection, Manassas, VA, USA) were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, and 200 mM L-glutamine (Life Technologies Italy, Milan, Italy). Cells were maintained at 37 °C in incubator at 5 % CO_2 and grown until they reached 80 % confluence. Next, cells were differentiated by using differentiation medium (DM), which contained 2 % horse serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 200 mM L-glutamine. The DM was refreshed every two days.

The viability of the myotubes was assessed by evaluating the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as an indicator of mitochondrial functionality [46]. The cells were seeded into a 96-well cell culture plate (3×10^{3} cells/well) and grown to 80 % confluence. After 7 days of differentiation, the cells were treated with 1 μM dexamethasone (DEX), either alone or in combination with HG-WPD at concentrations of 30 μM and 150 μM, for 48 h. After washing, 1 mg/mL of MTT was added to each well and incubated for 30 min at 37 °C. After the formazan crystals formed, 150 μL of dimethyl sulfoxide was added to each well to dissolve the crystals. Absorbance was measured at 550 nm. The experiments were performed in quadruplicate and repeated in three independent experimental sets.

2.7. Anti-Alzheimer's activity by in vitro inhibition of acetylcholinesterase (AChE) enzyme

The activity of AChE was assessed using a modified version of the spectrophotometric method developed by Ellman *et al.* and Rajan *et al.* [47,45]. A mixture containing 500 μL of 3 mM 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB) solution (in 0.1 M potassium phosphate buffer, pH 8), 100 μL of 15 mM acetylthiocholine iodide (AChI) (in water), 275 μL of 0.1 M potassium phosphate buffer (pH 8), 100 μL of HG-WPD solution (in water) at various concentrations (1, 10, 20, 30, and 40 mg/mL, respectively), was used a blank (control for non-enzymatic hydrolysis of acetylcholine) [48]. In the reaction cuvettes, 25 μL of buffer was replaced with an AChE solution at the activity of 0.16 U/mL. Thiocholine, generated from the hydrolysis of acetylcholine, reacted with DTNB during which a yellow compound was generated. The absorbance was recorded every minute as the reaction was observed at 405 nm for 5 min. After calculating the reaction rate, enzyme activity was expressed as a percentage of the rate relative to the control test containing buffer solution instead of HG-WPD as the inhibitor. The experiment was carried out in triplicate.

2.8. Statistical analysis and data management

For *in vitro* antioxidant assays and in the case of *in vitro* inhibition of AChE, two-way ANOVA (CI 95 %, 0.05) statistical test was carried out using GrapPad Prism Premium Licensed version 10.3.0 (GraphPad Software, Inc; CA, USA). For *in vitro* study, values are expressed as the

mean ± S.E.M.

3. Results

3.1. Nutritional contents/facts information

The nutritional information obtained from the official website is presented in Table 2. The chemical characteristics of each compound, including the substance/compound name, molecular formula, molecular weight (g/mol), and PubChem CID, were obtained and documented.

3.2. Prediction of HG-WPD bioactive compound activities, toxicity analysis, and drug likeness

First, we performed a comprehensive analysis of HG-WPD bioactive compounds targeting processes, genes, and proteins linked to sarcopenia and AD. The Pa value assessment, toxicity computational analysis, drug-likeness evaluation, and network pharmacology were performed to identify the relevant pathways, which were further examined in the molecular docking stage (Table 3). Our evaluation revealed that two compounds, which are C15 and C16, showed a promising potential. Specifically, C15 demonstrated potential for the treatment of acute neurologic disorders. Additionally, C16 exhibited potential as an acetylcholine neuromuscular blocking agent, neurotransmitter antagonist, and myosin ATPase inhibitor. The term LD_{50} refers to the quantification of the amount of substances or compounds that, under control conditions, will be a lethal dose to 50 % of a large number of test animals or *in vitro* of a particular object. The value is expressed in milligrams of the substance being tested per kilogram of animal body weight (mg/kg). Next, we determined the predicted LD_{50} values to classify these molecules according to the oral toxicity GHS Classification Scheme [49]. We found that both C15 and C16 had toxicity class 4 and 5, respectively, indicating their safety for oral consumption. Furthermore, both compounds met Lipinski's Rule of Five, indicating good drug-likeness, as described in Table 3. These findings validate the therapeutic potential of the two HG-WPD molecules for the potential treatment reference of sarcopenia and AD.

We employed Swiss Target Prediction to determine the detailed chemical characteristics of the compounds, along with a radar chart that visually displays the multidimensional properties of the molecules (Fig. 1). The axes of the radar chart typically represent different properties, such as lipophilicity (LIPO), size, polarity (POLAR), solubility (INSOLU), flexibility (FLEX), and saturation (INSATU). Additionally, information regarding the chemical structure, water solubility, and pharmacokinetics of the compounds were also shown in Fig. 1.

Table 2
Chemical characteristics from observed compounds of HG-WPD.

No	Substance/ Compounds in	Molecular Formula	Molecular Weight (g/mol)	PubChem CID
C1	Omega 3 (α-linolenic acid)	$C_{18}H_{30}O_2$	278.4	5280934
C2	Omega 6 (linoleic acid)	$C_{18}H_{32}O_2$	280.4	5280450
C3	Vitamin A	$C_{20}H_{30}O$	286.5	445354
C4	Vitamin C	$C_6H_8O_6$	176.12	54670067
C5	Vitamin B1	$C_{12}H_{17}C_lN_4OS$	300.81	6042
C6	Vitamin B2	$C_{17}H_{20}N_4O_6$	376.4	493570
C7	Vitamin B3	$C_6H_5NO_2$	123.11	928
C8	Vitamin B5	$C_9H_{17}NO_5$	219.23	6613
C9	Vitamin B6	$C_8H_{11}NO_3$	169.18	1054
C10	Vitamin B7	$C_{10}H_{16}N2O_3S$	244.31	171548
C11	Vitamin B12	$C_{63}H_{88}CoN_{14}O_{14}P$	1355.4	5311498
C12	Folic Acid	$C_{19}H_{19}N_7O_6$	441.4	135398658
C13	Vitamin D	$C_{27}H_{44}O$	384.6	5280795
C14	Vitamin E	$C_{29}H_{50}O_2$	430.7	14985
C15	Glucosamine	$C_6H_{13}NO_5$	179.17	439213
C16	HMB	$C_5H_{10}O_3$	118.13	69362

3.3. Protein target identification and network pharmacology analysis

Protein target identification and analysis were conducted to identify the primary proteins involved in the pathogenesis of both sarcopenia and AD. This study included an analysis of proteins and genes identified from disease-related targets and compounds found in HG-WPD (HMB and glucosamine). The results are depicted in the quadruple Venn diagram (Fig. 2A), which shows that two genes/proteins, angiotensin-converting enzyme (ACE) and matrix metalloproteinase 9 (MMP9), were common between HG-WPD, sarcopenia, and AD. Protein-protein interactions provided supporting evidence, as shown in Fig. 2B. Further investigation of the target proteins from HG-WPD identified several potential targets within the GO-Biological Process (Fig. 2C), GO-Molecular Function (Fig. 2D), and KEGG analysis (Fig. 2E). Our results showed that HMB and glucosamine affected genes which are involved in various biological functions, mainly related to skeletal muscle development, such as skeletal muscle atrophy, skeletal muscle adaptation, and myotube development (Fig. 2C). Those affected genes are also involved in various molecular functions including cholinesterase activity, signaling receptor binding, and proteoglycan binding (Fig. 2D). Furthermore, KEGG pathway enrichment analysis revealed that glucosamine and HMB affected the genes which are involved in numerous important pathways, such as longevity regulating pathway, endocrine resistance, and growth hormone synthesis, secretion, and action (Fig. 2E).

3.4. Molecular docking simulation

In drug development and discovery, molecular docking is a widely-used computational method to predict the interactions between a small molecule (ligand) and a target macromolecule (receptor). This method allows for the evaluation of the binding activity of substances identified in HG-WPD, particularly glucosamine and HMB, through their interactions with various proteins, including ACE, MMP9, and AChE. Next, we performed molecular docking simulation targeting specific drug receptors including ACE, MMP9, and AChE (Table 4). Established targeted agents—captopril for ACE, marimastat for MMP9, and rivastigmine for AChE—were used as controls, with their affinity values also displayed in Table 4. The findings indicated that C15 exhibited binding affinity values comparable to those of captopril and marimastat for ACE and MMP9, respectively. On the other hand, Compound C16 demonstrated weaker affinity for both ACE and MMP9 compared to captopril and marimastat. Additionally, the binding affinities of both C15 and C16 to AChE were relatively weaker than that of the control rivastigmine/physostigmine. These results suggest that C15 has a stronger overall binding affinity to the target receptors compared to C16, with its affinity for MMP9 being particularly comparable to that of marimastat. However, both HG-WPD compounds exhibit potential as multi-target ligands for these enzymes. Hence, the results suggest that C15 has the potential to be a viable alternative, with a binding profile that is not significantly weaker than the established controls. The compound clearly shows negative ΔG or Vina score values comparable to the control based on the molecular docking parameters, although with different degrees, which implies that the molecules in the HG-WPD might have synergistic qualities.

The evaluation is based on the ability of these compounds to inhibit signal binding to the specific receptors (Fig. 3). Table S2 provides a graphical representation of the predicted interactions between amino acids and the compounds derived from HG-WPD, excluding glucosamine and HMB, as well as the control, against specific receptor proteins. The effectiveness of these drugs is determined by the degree and strength of amino acid interactions that inhibit signal transmission to the receptors. The extent of amino acid binding may be linked to the adaptability of certain drugs. Analyzing the strength of these binding interactions also aids in understanding a compound's affinity, particularly through the formation of hydrogen bonds. Most drugs identified in HG-WPD form

Table 3
The evaluation of HG-WPD potential for anti-sarcopenia and anti-Alzheimer's based on structure–activity relationship (SAR) predictions via Pa score, toxicity prediction, and drug-likeness analysis.

No	Pa Level[a] Target with >0.4	Toxicity Model Computation Analysis[b]		Drug Likeness[b]		
		Predicted LD_{50} (mg/kg BW)	Toxicity Class	Lipinski	Pfizer	GSK
C15	Acute neurologic disorders treatment (0.539)	1.804	4	Accepted	Accepted	Accepted
C16	Acetylcholine neuromuscular blocking agent (0.517), Neurotransmitter antagonist (0.599), and Myosin ATPase inhibitor (0.718)	3.056	5	Accepted	Accepted	Accepted

[a] Way2Drug.
[b] Protox; ***ADMET.

hydrogen bonds with amino acids involved in disease pathways, indicating varying levels of docking activity, which can be correlated with their chemical structures and functional characteristics. Furthermore, molecular docking studies have shown that glucosamine exhibits a binding affinity comparable to that of certain controls against specific receptors, suggesting its potential bioactive properties.

3.5. Antioxidant activities of HG-WPD, HMB, and glucosamine assesed by DPPH and ABTS

Next, we measured the antioxidant activity of HG-WPD, glucosamine, and HMB to evaluate the nutritional effects of these compounds. The results are compared to the Trolox control as the standard antioxidant. Our data showed that based on ABTS test, HG-WPD, glucosamine, and HMB exhibited a comparable antioxidant activity as compared to Trolox at 1 mg/mL low concentration (Fig. 4A). At higher concentrations, only HG-WPD showed a comparable antioxidant activity to Trolox, whereas glucosamine and HMB alone showed a lower antioxidant activity (Fig. 4A). Similar results were also observed for the antioxidant activity using DPPH test (Fig. 4B). Our results suggested that HG-WPD possessed an antioxidant property that may enhance its nutritional values and therapeutical benefits.

3.6. HG-WPD, HMB, and glucosamine protect C2C12 myocytes from DEX-induced cell death

Next, we evaluated the effect of HG-WPD, HMB, and glucosamine on the viability of C2C12 myotubes (Table 5). DEX treatment decreased their viability to 90.50 ± 1.5 %. However, when DEX and HG-WPD at 10 mg/mL and 40 mg/mL were co-administered, the cell viability was increased to 105.30 ± 0.6 % and 110.55 ± 0.5 %, respectively. Similarly, the combination of DEX with 10 mg/mL HMB raised cell viability to 106.50 ± 0.4 %, while the addition of 10 mg/mL glucosamine led to a slight increase to 96.90 ± 0.5 %. These findings suggest that HG-WPD, especially at higher concentrations, prevents the reduction in cell viability induced by DEX, with effects comparable to those observed with HMB alone, suggesting their potential beneficial effects against sarcopenia.

3.7. HG-WPD, HMB, and glucosamine potently inhibit AChE activity In vitro

The results shown in Fig. 5 presents the potential anti-Alzheimer's activities of HG-WPD, HMB, and glucosamine, evaluated *in vitro* by measuring their inhibitory effects on AChE at increasing concentrations (1, 10, 20, 30, and 40 mg/mL). Physostigmine was used as a positive control. At all tested concentrations, HG-WPD, HMB, and glucosamine demonstrated AChE inhibition activity comparable to the control. No significant differences in inhibition were observed between the compounds and the control, as indicated by p-values greater than 0.05 across all concentrations. This suggests that HG-WPD, HMB, and glucosamine exhibit potential anti-Alzheimer's activity similar to that of the standard treatment, physostigmine, via inhibiting AChE.

4. Discussion

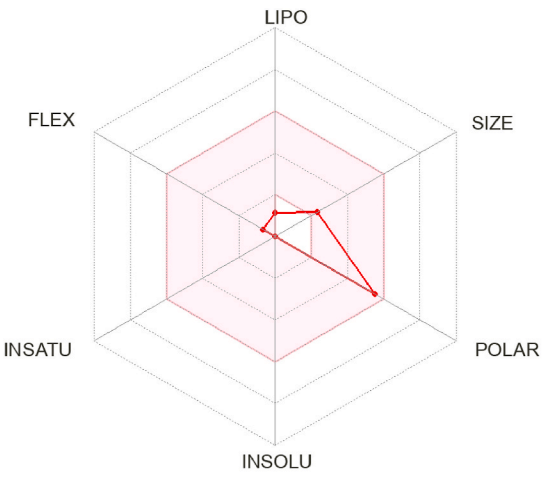
Sarcopenia, a condition characterized by the loss of muscle mass and function, is commonly associated with aging. Various studies have investigated potential therapeutic interventions for sarcopenia, ranging from natural compounds to pharmacological agents. Melatonin, a molecule derived from tryptophan, has garnered attention for its potential in treating sarcopenia due to its chronobiotic, antioxidant, and anti-inflammatory properties [50]. Moreover, interventions targeting NAD^+ metabolism and downregulating specific pathways, such as SIRT1, IL-6, TNF, and AKT1, have shown promise in mitigating the effects of sarcopenia [51,46]. Compounds like tomatidine, curcumin, and other plant bioactives also demonstrated anti-sarcopenic activity through mechanisms including antioxidant and anti-inflammatory effects [52–54].

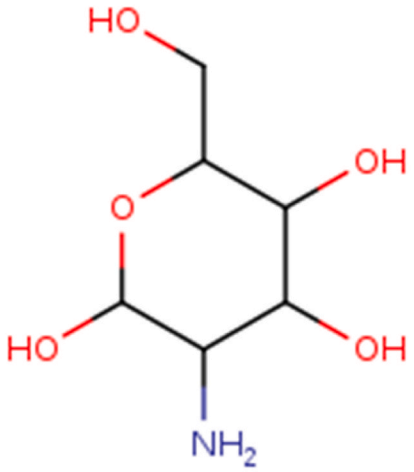
Recent studies have shed light on the potential role of the ACE in sarcopenia and AD. The *ACE* gene has been identified as a risk locus for AD, and ACE inhibitors have been suggested as a potential therapy for sarcopenia in older adults [55,56]. Additionally, the classical renin-angiotensin system (RAS) pathway has been implicated in muscle wasting, while the non-classical RAS pathway, involving angiotensin 1–7 and the Mas receptor, may have protective effects against sarcopenia [57]. The LACE study investigated the effects of the ACE inhibitor Perindopril on physical performance and muscle mass in older individuals with sarcopenia, showing promising results [58]. However, a separate study found that the *ACE* I/D genotype was associated with strength in men with sarcopenia but did not correlate with the response to ACE inhibitor therapy in older adults with sarcopenia [59]. This highlights the complexity of the relationship between ACE and sarcopenia. Moreover, genetic evidence suggests a protective effect of cerebral ACE against AD, while the therapeutic use of ACE inhibitors has been proposed to prevent cognitive impairment and neurodegeneration in animal models of AD [56,60]. Additionally, carriers of heterozygous loss-of-function *ACE* mutations may be at risk for AD due to the involvement of ACE in the cleavage of amyloid Aβ42, a key component in the pathogenesis of AD [61].

MMP9 has been identified as a significant factor in various neurological disorders, including AD. Elevated levels of MMP9 was found in the brain and cerebrovasculature of individuals with AD [62]. In the context of sarcopenia, research has explored the association between primary sarcopenia and serum MMP9 levels [63]. Additionally, MMP9 has been implicated in the development of sensory circuits during early postnatal life and is linked to neurodegenerative disorders, such as traumatic brain injury, multiple sclerosis, and AD [64]. Studies have also highlighted the involvement of MMP9 in inflammatory processes in cerebrovascular diseases, further emphasizing its role in neurological conditions [65].

AChE is a crucial enzyme involved in various neurological disorders, including AD, and sarcopenia. In AD, AChE hyper-activation leads to a decrease in acetylcholine levels, contributing to memory loss and

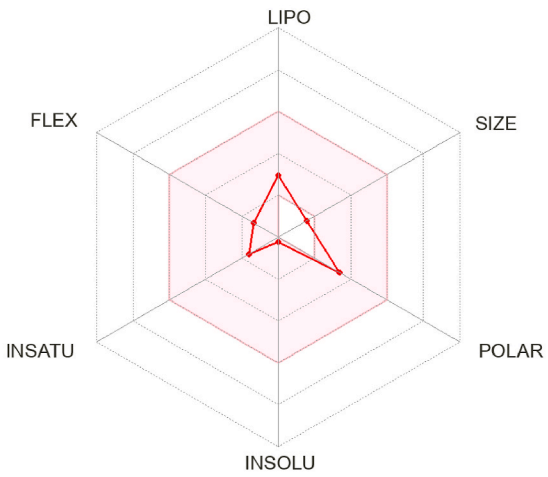
Glucosamine

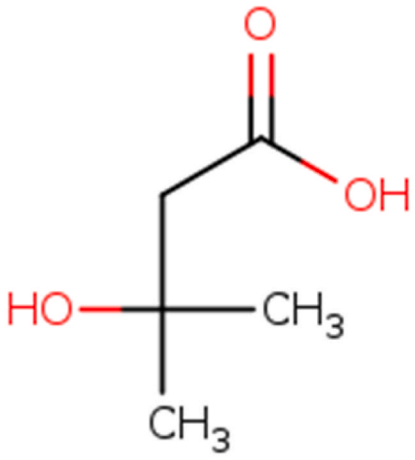




Water Solubility	
Log *S* (ESOL)	1.32
Solubility	3.74e+03 mg/ml ; 2.09e+01 mol/l
Class	Highly soluble
Log *S* (Ali)	1.64
Solubility	7.84e+03 mg/ml ; 4.38e+01 mol/l
Class	Highly soluble
Log *S* (SILICOS-IT)	2.40
Solubility	4.49e+04 mg/ml ; 2.51e+02 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	Low
BBB permeant	No
P-gp substrate	Yes
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-9.88 cm/s

HMB





Water Solubility	
Log *S* (ESOL)	-0.23
Solubility	7.02e+01 mg/ml ; 5.94e-01 mol/l
Class	Very soluble
Log *S* (Ali)	-0.41
Solubility	4.63e+01 mg/ml ; 3.92e-01 mol/l
Class	Very soluble
Log *S* (SILICOS-IT)	0.14
Solubility	1.65e+02 mg/ml ; 1.40e+00 mol/l
Class	Soluble
Pharmacokinetics	
GI absorption	High
BBB permeant	No
P-gp substrate	No
CYP1A2 inhibitor	No
CYP2C19 inhibitor	No
CYP2C9 inhibitor	No
CYP2D6 inhibitor	No
CYP3A4 inhibitor	No
Log K_p (skin permeation)	-7.26 cm/s

Fig. 1. Radar chart, chemical structure, water solubility, and pharmacokinetics of glucosamine (left panel) and HMB (right panel).

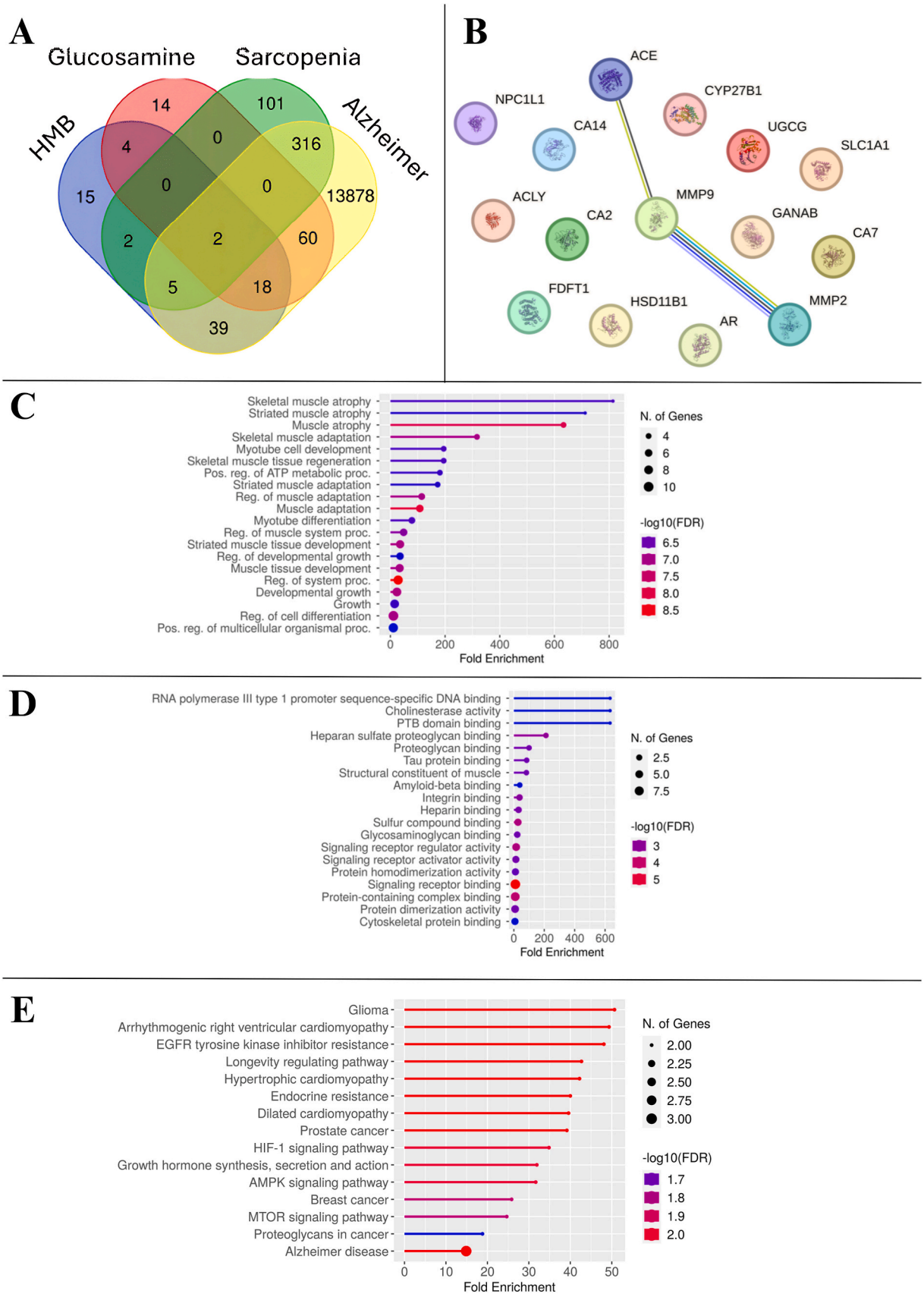


Fig. 2. *In Silico* Protein-protein or Network Pharmacology Analysis. (A) The quadruple Venn diagram shows shared HG-WPD targets and genes associated with Sarcopenia and Alzheimer's; (B) Predicted protein-protein interactions (PPIs) of HG-WPD targets in Sarcopenia and Alzheimer's; (C) GO-Biological Process; (D) GO-Molecular Function; (E) KEGG Analysis.

Table 4
The Vina score of the molecular docking parameter for the HG-WPD compounds of identification.

Drug/Nutritional Content	*Sarcopenia- and Alzheimer's-Related Proteins*		
	ACE	MMP9	AChE
Captopril	*−5.9*	-	-
Marimastat	-	*−6.1*	-
Rivastigmine	-	-	*−7.2*
C15 (Glucosamine)	*−5.5*	*−6.5*	*−5.9*
C16 (HMB)	*−4.5*	*−5.3*	*−4.6*

neurobehavioral abnormalities [66]. Additionally, AChE inhibition plays a significant role in increasing cholinergic transmission, reducing Aβ aggregation, and preventing the generation of neurotoxic fibrils in AD [67]. The selective vulnerability of basal forebrain cholinergic neurons, regulated by AChE, is a key factor in AD pathophysiology [68]. In the context of AD, AChE is a primary target for therapeutic intervention, with drugs such as donepezil acting as AChE inhibitors to enhance cholinergic activity and reduce Aβ aggregation [69]. Furthermore, the inhibition of AChE is a strategy in the treatment of AD and similar disorders due to its role in acetylcholine hydrolysis [70]. Studies have also shown that elevated AChE activity may contribute to the development of AD [71]. Regarding sarcopenia, the degeneration of acetylcholine receptors (AChRs) at the neuromuscular junction is a significant factor in aging-associated sarcopenia [72]. Targeting AChRs-related genes involved in acetylcholine signaling may offer a potential treatment approach for sarcopenia. Moreover, in the aging process, imbalances in acetylcholine levels are linked to neurodegenerative diseases, such as AD and Parkinson's Disease [73].

Interventions like exercise, nutritional supplements, and the use of natural, such as bee, products have also been suggested for their potential in preventing and treating sarcopenia [74]. Specifically, resistance training combined with protein supplementation, amino acids, especially leucine, vitamin D, and omega-3 fatty acids hold a promise in attenuating sarcopenia. This finding is supported by our results, since HMB, a metabolite of leucine, was studied for its positive effects on muscle strength and size, particularly in older individuals. Protein intake, in particular, plays a significant role in counteracting sarcopenia, with deficiencies in protein or vitamin D being major contributors to the condition [75,76]. Recently, branched-chain amino acid (BCAA) supplementation was proposed as a potential treatment for improving sarcopenia in elderly individuals [77].

AD is a complex neurodegenerative disorder in which developing effective treatments is challenging to date. Several studies have identified novel compounds with potential anti-Alzheimer's properties. In a previous study, thiazole-pyridiniums were synthesized as potential anti-Alzheimer's agents, which show AChE inhibitory activities and inhibition of Aβ self-aggregation [78]. Additionally, compounds from the methanolic extract of *Lawsonia inermis* seeds exhibited anti-Alzheimer's properties by modulating acetylcholine content and AChE activity [79]. Furthermore, compound 80l, a cinnamic acid derivative, demonstrated strong neuroprotective activity against Aβ 1–42 toxicity, making it a promising novel compound for the treatment of AD [80]. Additionally, the use of transthyretin stabilization as a strategy for AD treatment has been proposed, emphasizing the importance of targeting specific proteins in neurodegenerative diseases [81]. The development of novel compounds from both synthetic and natural sources holds promise for

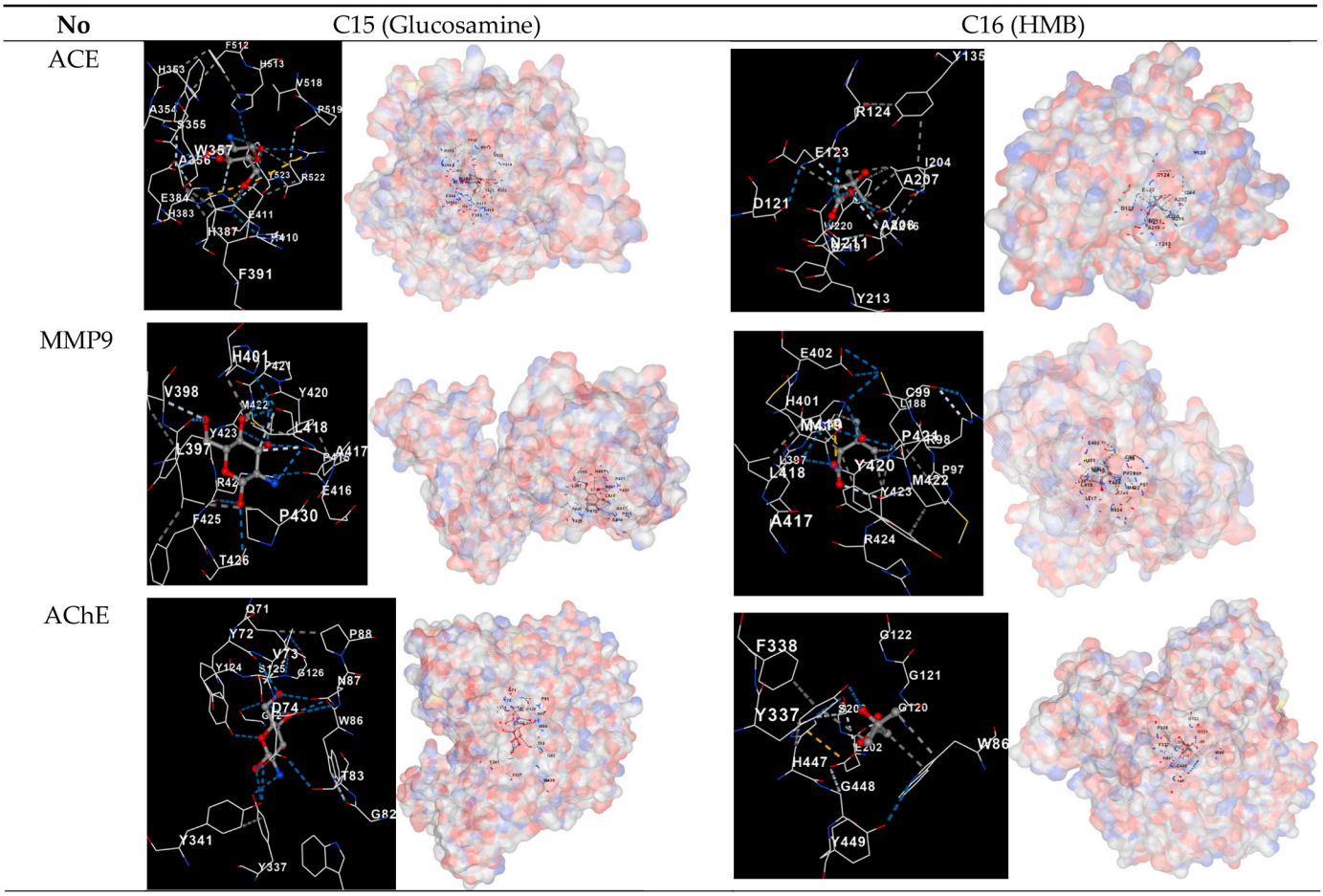


Fig. 3. Illustration of the predicted interactions between amino acid residues and glucosamine (left panel) or HMB (right panel) with ACE, MMP9, and AChE.

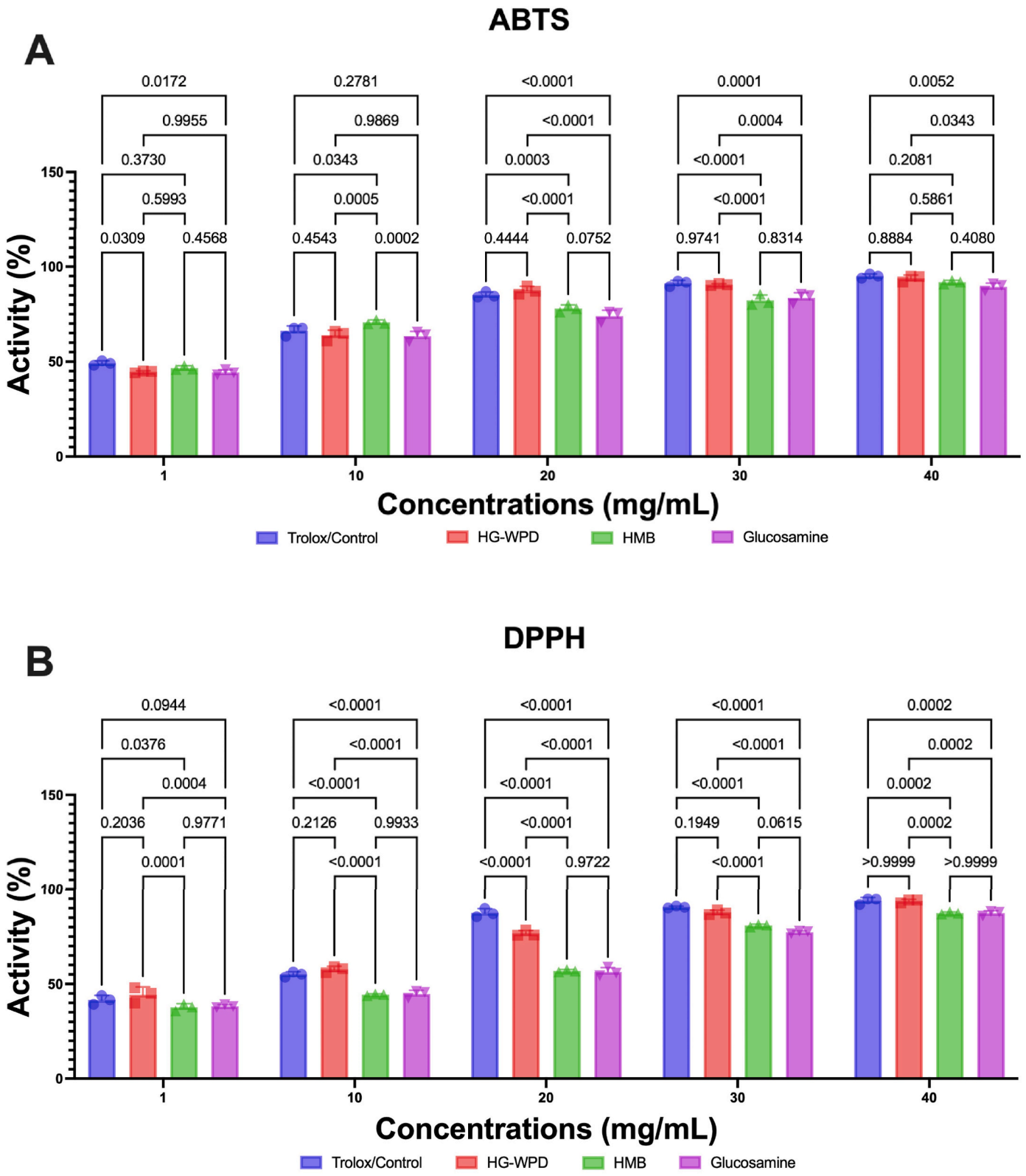


Fig. 4. Antioxidant activities of HG-WPD, C15 (glucosamine), and C16 (HMB) measured by ABTS (**A**) or DPPH (**B**) assays. n = 3, statistical analysis was performed by two-way ANOVA. HG-WPD: HMB and glucosamine fortified Whey Protein Drink.

the treatment of AD. These compounds target various pathways involved in the disease, including AChE inhibition, Aβ aggregation, and neuroprotection.

Various studies have explored the potential benefits of different types of supplementations in preventing or delaying the onset of AD. Phytochemicals from plant-based herbal treatments have been investigated for their interactions with critical proteins involved in amyloidogenesis, a key process in AD pathogenesis [82]. Probiotic and vitamin co-supplementation has also gained attention as a complementary strategy for AD prevention [83]. Additionally, omega-3 polyunsaturated

Table 5
The effect of HG-WPD, HMB, and glucosamine on the viability of C2C12 myocytes. Cell death was induced by 1 μM DEX.

Treatment	Cell Viability
Control/Without Treatment	100 ± 5.0
dexamethasone 1 μM	90.50 ± 1.5
dexamethasone 1 μM + 10 mg/mL HG-WPD	105.30 ± 0.6
dexamethasone 1 μM + 40 mg/mL HG-WPD	110.55 ± 0.5
dexamethasone 1 μM + 10 mg/mL HMB	106.50 ± 0.4
dexamethasone 1 μM + 30 mg/mL glucosamine	96.90 ± 0.5

Cell viability was evaluated by MTT assay. Values are expressed as the mean ± S.E.M of 3/triplicates experiments. We arbitrarily set the control condition as 100 %.

fatty acids, such as those found in fish oil, have shown promise in slowing cognitive decline in older adults at risk for AD [84]. Interestingly, antioxidants, such as those found in novel antioxidative supplements, have been proposed as a means to prevent cognitive decline in AD by targeting oxidative stress and the Aβ cascade [85]. Our results indicate that HG-WPD, HMB, and glucosamine possess potent antioxidant properties, particularly at higher concentrations. These findings support the potential of these compounds to prevent cognitive decline in AD. The targeted proteins included ACE, MMP9, and AChE. Based on these results, we propose a model that illustrates the potential pathogenesis and interconnection between these targeted proteins, demonstrating how the compounds in HG-WPD may act as potential treatments for both sarcopenia and AD (Fig. 6).

5. Conclusions

The results of this study highlight the strong potential of a hydroxymethylbutyrate and glucosamine-strengthened whey protein drink (HG-WPD) as a multivarious nutrient mix for fighting against both sarcopenia and Alzheimer's disease (AD). By combining *in silico* studies consisting of network pharmacology and molecular docking, and *in vitro* assays, the present work emphasizes the capacity of HG-WPD to target important proteins involved in sarcopenia and AD, including ACE, MMP9, and AChE. Significant binding affinities of the HG-WPD compounds to these target proteins were predicted by the molecular docking simulations which imply their possible use as multi-target therapeutic agents. Indicative of their anti-sarcopenic properties, *in vitro* experiment showed that compounds enriched in the drink preserved muscle cell viability under a stress condition. Furthermore, the strong antioxidant activity demonstrated in this study points to a preventive function of the functional food in reducing oxidative stress which is a recognized factor causing AD-related cognitive deterioration. These results, taken together, point to HG-WPD as a creative and useful dietary intervention for the management and prevention of AD and sarcopenia. Nevertheless, further clinical research is required to confirm these results and investigate the therapeutic possibilities of this fortified whey protein drink in human populations.

Supplementary Materials: Table S1: The SMILES notation for each compound of HG-WPD obtained from PubChem; Table S2: The visual representation of the interaction between amino acids and the compounds identified from HG-WPD against specific receptor proteins.

CRediT authorship contribution statement

Fahrul Nurkolis: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vincent Lau:** Writing – original draft, Visualization, Software, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Trina Ekawati Tallei:** Writing – review & editing, Validation, Supervision, Methodology. **Nurpudji Astuti Taslim:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Data curation, Conceptualization. **Son Radu:** Writing – review & editing, Supervision. **Apollinaire Tsopmo:** Writing – review & editing. **Bonglee Kim:** Writing – review & editing, Validation, Supervision, Methodology, Funding acquisition. **Rony Abdi Syahputra:** Writing – original draft, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data

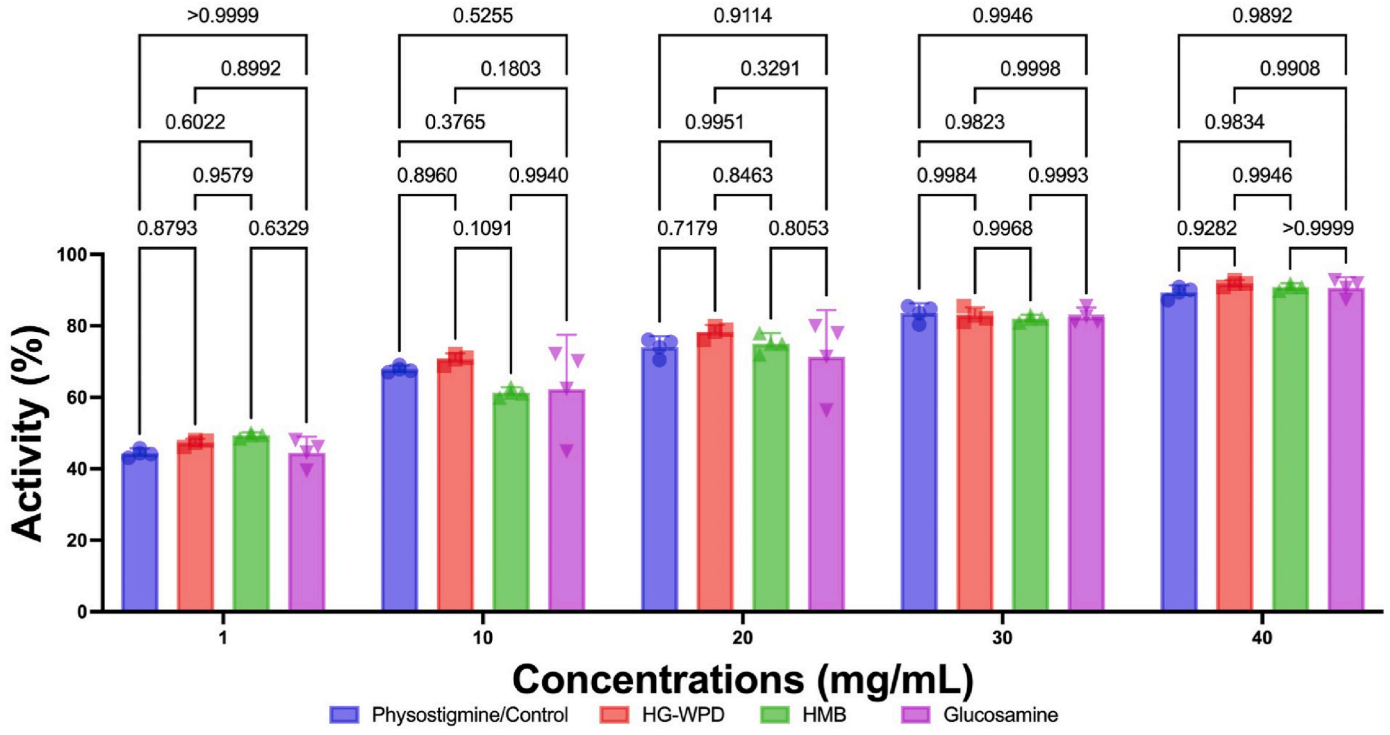


Fig. 5. *In vitro* inhibition of acetylcholinesterase enzyme (AChE) activity by HG-WPD, C15 (glucosamine), and C16 (HMB). n = 3, statistical analysis by two-way ANOVA. HG-WPD: HMB and glucosamine fortified Whey Protein Drink.

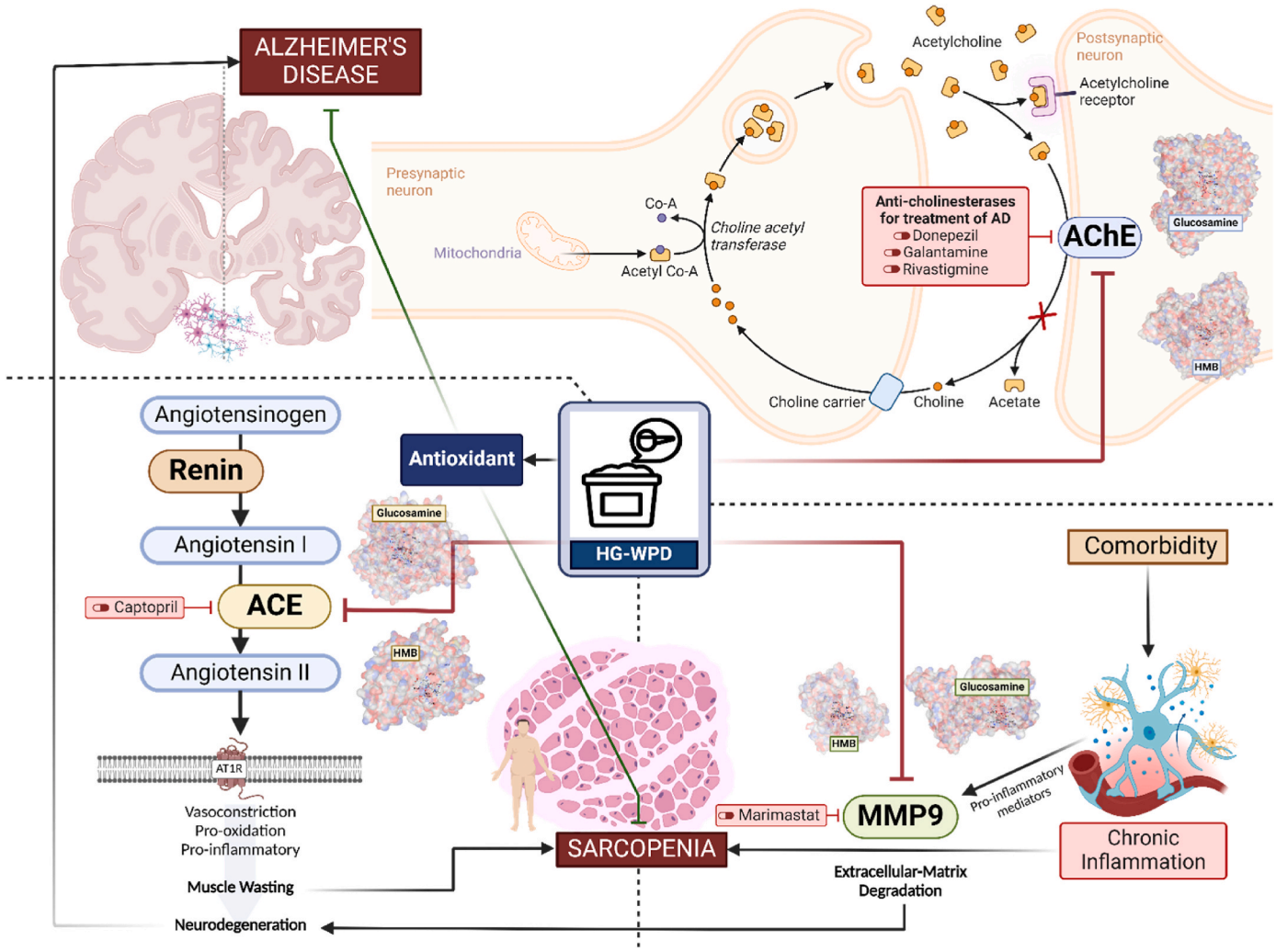


Fig. 6. Potential molecular mechanism of action of hydroxymethylbutyrate and glucosamine fortified whey protein drink in modulating sarcopenia and Alzheimer's disease from *in silico* and *in vitro* insights. Created with BioRender.com Premium License by Fahrul Nurkolis (https://app.biorender.com, accessed on August 12, 2024).

curation, Conceptualization. **Raymond Rubianto Tjandrawinata:** Writing – review & editing, Writing – original draft, Validation, Supervision. **Rini Arianti:** Writing – review & editing, Writing – original draft, Validation, Supervision, Funding acquisition. **Endre Kristóf:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

Informed consent statement

Not applicable.

Institutional review board statement

Not applicable.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Funding

This research was supported by a grant from Kyung Hee University in 2023 (KHU-20230914), Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2020R1I1A2066868), the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2020R1A5A2019413), a grant of the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), and the innovation network support Program through the INNOPOLIS funded by Ministry of Science and ICT (2022-IT-RD-0205-01-101) by B.K. R.A. and E.K. were supported by the National Research, Development and Innovation Office (NKFIH-PD146202 and FK145866) of Hungary and the University of Debrecen Program for Scientific Publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors and contributors also declare that this is not an endorsement of the product that is the subject of this study HG-WPD (HiLo™); all claims in this article are in accordance with the study/research conducted. The researcher did not receive any support from the manufacturer of HG-WPD (HiLo™) or Nutrifood Indonesia Company.

Acknowledgments

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jafr.2024.101495.

References

[1] L. Ye, Y. Wen, Y. Chen, J. Yao, X. Li, Y. Liu, J. Song, Z. Sun, Diagnostic reference values for sarcopenia in Tibetans in China, Sci. Rep. 10 (2020), https://doi.org/10.1038/s41598-020-60027-0.
[2] Y. Zhang, Y. Zhu, Sarcopenia and its influencing factors in patients with colorectal cancer, Gastroenterol. Nurs. 47 (2024) 138–147.
[3] X. Sun, J. Xu, X. Chen, W. Zhang, W. Chen, C. Zhu, J. Sun, X. Yang, X. Wang, Y. Hu, et al., Sarcopenia in patients with normal body mass index is an independent predictor for postoperative complication and long-term survival in gastric cancer, Clin. Transl. Sci. 14 (2021) 837–846.
[4] Z. Li, B. Liu, X. Tong, Y. Ma, T. Bao, C. Wu, J. Yue, The association between sarcopenia and incident of depressive symptoms: a prospective cohort study, Research Square (2023).
[5] J. He, J. Wang, B. Pan, H. Zhang, S. Shen, X. Zhao, Association between nocturnal sleep duration and midday napping and the incidence of sarcopenia in middle-aged and older adults: a 4-year longitudinal study, Environ. Health Prev. Med. 29 (2024) 29, 29.
[6] G. Bahat, D. Erbaş Sacar, SARC-F test in sarcopenia and frailty: a narrative review, Nutrition and Food Processing 4 (2021) 1–3.
[7] K. Yuenyongchaiwat, C. Akekawatchai, Prevalence and incidence of sarcopenia and low physical activity among community-dwelling older Thai people: a preliminary prospective cohort study 2-year follow-up, PeerJ 10 (2022) e13320.
[8] J. Pacifico, E.M. Reijnierse, W.K. Lim, A.B. Maier, The association between sarcopenia as a comorbid disease and incidence of institutionalisation and mortality in geriatric rehabilitation inpatients: REStORing health of acutely unwell AdulTs (RESORT), Gerontology 68 (2022) 498–508.
[9] S. Liu, J. Zhao, Y. Jiang, R. Deng, Q. Mu, Predictive models of sarcopenia in patients with chronic liver disease: a cross-sectional study, Research Square (2023).
[10] J.Y. Jang, D. Kim, N.D. Kim, Pathogenesis, intervention, and current status of drug development for sarcopenia: a review, Biomedicines 11 (2023) 1635.
[11] J. Hu, Y. Wang, X. Ji, Y. Zhang, K. Li, F. Huang, Non-pharmacological strategies for managing sarcopenia in chronic diseases, Clin. Interv. Aging 19 (2024) 827–841.
[12] E.J. Carey, J.C. Lai, C. Sonnenday, E.B. Tapper, P. Tandon, A. Duarte-Rojo, M. A. Dunn, C. Tsien, E.R. Kallwitz, V. Ng, et al., A north American expert opinion statement on sarcopenia in liver transplantation, Hepatology 70 (2019) 1816–1829.
[13] A.A.A.P. Laksmidewi, C.P. Vania Rau, Late onset alzheimer dementia in patients with genotype E3/E4: a case report, Open Access Maced. J. Med. Sci. 9 (2021) 5–9.
[14] H.W. Kim, J. Hong, J.C. Jeon, Cerebral small vessel disease and Alzheimer's disease: a review, Front. Neurol. 11 (2020), https://doi.org/10.3389/fneur.2020.00927.
[15] N. Mu, H. Wu, P. Ma, D. Rao, J. Yu, Q. Huang, M. Tang, R. Zhang, J. Pan, Gender difference in behavioral and psychological symptoms of Alzheimer's disease, Alzheimers Dis. Dement. 3 (2019), https://doi.org/10.36959/734/375.
[16] K. Sak, High intake of sunflower seeds and low mortality from Alzheimer's disease and dementia: is there a correlation? Explor Foods Foodomics 2 (2024) 101–106.
[17] Y. Zhang, The risk factors and causes for Alzheimer's disease, in: Proceedings of the Advances in Social Science, Education and Humanities Research, Atlantis Press, Paris, France, 2022.
[18] B. Rodríguez-Fernández, G. Sánchez-Benavides, P. Genius, C. Minguillon, K. Fauria, I.D. Vivo, A. Navarro, J.L. Molinuevo, J.D. Gispert, A. Sala-Vila, et al., Association between telomere length and cognitive function among cognitively unimpaired individuals at risk of Alzheimer's disease, bioRxiv (2023).
[19] J. Rivers-Auty, A.E. Mather, R. Peters, C.B. Lawrence, D. Brough, Anti-inflammatories in Alzheimer's disease—potential therapy or spurious correlate? Brain Commun 2 (2020) https://doi.org/10.1093/braincomms/fcaa109.
[20] M. Madmoli, M. Madmoli, M. Khodadadi, Y. Madmoli, The communication between diabetes and Alzheimer's disease: a study on diabetic patients referring to health centers in three cities of khuzestan province, Int Res in Med and Heal Sci (2019), https://doi.org/10.36437/irmhs.2019.2.2.l.
[21] R. Hamzé, E. Delangre, S. Tolu, M. Moreau, N. Janel, D. Bailbé, J. Movassat, Type 2 diabetes mellitus and Alzheimer's disease: shared molecular mechanisms and potential common therapeutic targets, Int. J. Mol. Sci. 23 (2022) 15287.
[22] G.D. Stanciu, V. Bild, D.C. Ababei, R.N. Rusu, A. Cobzaru, L. Paduraru, D. Bulea, Link between diabetes and Alzheimer's disease due to the shared amyloid aggregation and deposition involving both neurodegenerative changes and neurovascular damages, J. Clin. Med. 9 (2020) 1713.
[23] J. Jebelli, M.C. Hamper, D. Van Quelef, D. Caraballo, J. Hartmann, J. Kumi-Diaka, The potential therapeutic effects of low-dose ionizing radiation in Alzheimer's disease, Cureus (2022), https://doi.org/10.7759/cureus.23461.
[24] Suneesha, T.V. Kumar, Evaluation of anti-alzheimer activity of ethanolic and methanolic extracts of polygonum glabrum against aluminum chloride-induced Alzheimer's in experimental rats, Asian J. Pharm. Clin. Res. (2021) 118–125.
[25] G.D. Stanciu, A. Luca, R.N. Rusu, V. Bild, S.I. Beschea Chiriac, C. Solcan, W. Bild, D. C. Ababei, Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement, Biomolecules 10 (2019) 40.
[26] Y. Liu, G. Wu, X. Shu, X. Wang, Targeting the transnitrosylation cascade provides a novel therapeutic strategy for Alzheimer's disease, Neurosci. Bull. 37 (2021) 1373–1376.
[27] T.A. Hassan, Breakthrough in Alzheimer's disease, J Islamabad Med Dental Coll 11 (2022) 135–137.
[28] S. Bakrim, S. Aboulaghras, N. El Menyiy, N. El Omari, H. Assaggaf, L.-H. Lee, D. Montesano, M. Gallo, G. Zengin, Y. AlDhaheri, et al., Phytochemical compounds and nanoparticles as phytochemical delivery systems for Alzheimer's disease management, Molecules 27 (2022) 9043.
[29] S. Goorah, G.D. Dorkhy, Non-pharmacological management of Alzheimer's disease a qualitative study, in Mauritius, bioRxiv (2020).
[30] J.A.E. Pohlenz-Saw, J.L. Merriweather, L. Wandrag, Mal)Nutrition in critical illness and beyond: a narrative review, Anaesthesia 78 (2023) 770–778.
[31] S.K.-H. Chow, Y.-N. Chim, K.Y.-K. Cheng, C.-Y. Ho, W.-T. Ho, K.C.-C. Cheng, R.M.-Y. Wong, W.-H. Cheung, Elastic-band resistance exercise or vibration treatment in combination with hydroxymethylbutyrate (HMB) supplement for management of sarcopenia in older people: a study protocol for a single-blinded randomised controlled trial in Hong Kong, BMJ Open 10 (2020) e034921.
[32] J. Courel-Ibáñez, T. Vetrovsky, K. Dadova, J.G. Pallarés, M. Steffl, Health benefits of β-Hydroxy-β-Methylbutyrate (HMB) supplementation in addition to physical exercise in older adults: a systematic review with meta-analysis, Nutrients 11 (2019) 2082.
[33] V.G. Goni, M. Mishra, S. Kadam, S.S. Gandhi, Effect of nucart vg (boswellia serrata in combination with veg glucosamine Sulphate) in Comparison with glucosamine Sulphate to improve quality of life of knee osteoarthritis patients: a randomized controlled trial, Int. J. Res. Orthop. 7 (2021) 976.
[34] C. Suzukamo, K. Ishimaru, R. Ochiai, N. Osaki, T. Kato, Milk-fat globule membrane plus glucosamine improves joint function and physical performance: a randomized, double-blind, placebo-controlled, parallel-group study, J. Nutr. Sci. Vitaminol. 65 (2019) 242–250.
[35] S. Kim, J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B.A. Shoemaker, P. A. Thiessen, B. Yu, et al., PubChem in 2021: new data content and improved web interfaces, Nucleic Acids Res. 49 (2021) D1388–D1395.
[36] D.S. Druzhilovskiy, A.V. Rudik, D.A. Filimonov, T.A. Gloriozova, A.A. Lagunin, A. V. Dmitriev, P.V. Pogodin, V.I. Dubovskaya, S.M. Ivanov, O.A. Tarasova, et al., Computational platform Way2Drug: from the prediction of biological activity to drug repurposing, Russ. Chem. Bull. 66 (2017) 1832–1841.
[37] V. Lau, F. Nurkolis, M.N. Park, D.S. Heriyanto, N.A. Taslim, T.E. Tallei, H. K. Permatasari, R.R. Tjandrawinata, S. Moon, B. Kim, Green seaweed caulerpa racemosa as a novel non-small cell lung cancer inhibitor in overcoming tyrosine kinase inhibitor resistance: an analysis employing network pharmacology, molecular docking, and in vitro research, Mar. Drugs 22 (2024) 272.
[38] P. Banerjee, A.O. Eckert, A.K. Schrey, R. Preissner, ProTox-II: a webserver for the prediction of toxicity of chemicals, Nucleic Acids Res. 46 (2018) W257–W263.
[39] U. Norinder, C.A.S. Bergström, Prediction of ADMET properties, ChemMedChem 1 (2006) 920–937.
[40] J. Dong, N.-N. Wang, Z.-J. Yao, L. Zhang, Y. Cheng, D. Ouyang, A.-P. Lu, D.-S. Cao, ADMETlab: a platform for systematic ADMET evaluation based on a comprehensively collected ADMET database, J. Cheminform. 10 (2018), https://doi.org/10.1186/s13321-018-0283-x.
[41] J. Nickel, B.-O. Gohlke, J. Erehman, P. Banerjee, W.W. Rong, A. Goede, M. Dunkel, R. Preissner, SuperPred: update on drug classification and target prediction, Nucleic Acids Res. 42 (2014) W26–W31.
[42] K. Gallo, A. Goede, R. Preissner, B.-O. Gohlke, SuperPred 3.0: drug classification and target prediction—a machine learning approach, Nucleic Acids Res. 50 (2022) W726–W731.
[43] F. Nurkolis, A.F. Purnomo, D. Alisaputra, W.B. Gunawan, F.R. Qhabibi, W. Park, M. Moon, N.A. Taslim, M.N. Park, B. Kim, In silico and in vitro studies reveal a synergistic potential source of novel anti-ageing from two Indonesian green algae, J. Funct.Foods 104 (2023) 105555.
[44] M. Kuswari, F. Nurkolis, N. Mayulu, F.M. Ibrahim, N.A. Taslim, D.S. Wewengkang, N. Sabrina, G.R. Arifin, K.E.K. Mantik, M.R. Bahar, et al., Sea grapes extract improves blood glucose, total cholesterol, and PGC-1α in rats fed on cholesterol- and fat-enriched diet, F1000Res 10 (2021) 718.
[45] J. Ferreira, S. Santos, H. Pereira, *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of *quercus suber* cork and corkback extracts, Evid. Based. Complement. Alternat. Med. (2020), https://doi.org/10.1155/2020/3825629. **2020**.
[46] J. Dai, H. Wang, L. Jiang, F. Zhang, J. Lin, L. Wang, J. Yang, X. Wang, Exploring the therapeutic mechanisms of sijunzi decoction in the treatment of sarcopenia: key targets and signaling pathways, Biomed. Chromatogr. 37 (2023), https://doi.org/10.1002/bmc.5722.
[47] G.L. Ellman, K.D. Courtney, V. Andres jr., R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (1961) 88–95.
[48] M. Mathew, S. Subramanian, In vitro screening for anti-cholinesterase and antioxidant activity of methanolic extracts of ayurvedic medicinal plants used for cognitive disorders, PLoS One 9 (2014) e86804.
[49] Committee on the Design and Evaluation of Safer Chemical Substitutions, A Framework to Inform Government and Industry Decision; Board on Chemical Sciences and Technology; Board on Environmental Studies and Toxicology; Division on Earth and Life Studies; National Research Council *Overview Of the GHS*

Classification Scheme In Hazard Classification, National Academies Press, Washington, D.C., DC, 2014.
[50] J. Fernández-Martínez, Y. Ramírez-Casas, Y. Yang, P. Aranda-Martínez, L. Martínez-Ruiz, G. Escames, D. Acuña-Castroviejo, From chronodisruption to sarcopenia: the therapeutic potential of melatonin, Biomolecules 13 (2023) 1779.
[51] D. Liu, Y.-B. Fan, X.-H. Tao, W.-L. Pan, Y.-X. Wu, X.-H. Wang, Y.-Q. He, W.-F. Xiao, Y.-S. Li, Mitochondrial quality control in sarcopenia: updated overview of mechanisms and interventions, Aging Dis 12 (2021) 2016.
[52] T.B. Waltz, E.M. Fivenson, M. Morevati, C. Li, K.G. Becker, V.A. Bohr, E.F. Fang, Sarcopenia, aging and prospective interventional strategies, Curr. Med. Chem. 25 (2019) 5588–5596.
[53] S.L. Saud Gany, K.-Y. Chin, J.K. Tan, A. Aminuddin, S. Makpol, Curcumin as a therapeutic agent for sarcopenia, Nutrients 15 (2023) 2526.
[54] N. Majeda Alfarafisa, K. Kitaguchi, T. Yabe, The aging of skeletal muscle and potential therapeutic effects of extracts from edible and inedible plants, Rev. Agric. Sci. 8 (2020) 70–88.
[55] M. Mile, L. Balogh, G. Papp, J.M. Pucsok, K. Szabó, L. Barna, Z. Csiki, I. Lekli, Effects of functional training on sarcopenia in elderly women in the presence or absence of ACE inhibitors, Int. J. Environ. Res. Public Health 18 (2021) 6594.
[56] D.K. Ryan, V. Karhunen, B. Su, M. Traylor, T.G. Richardson, S. Burgess, I. Tzoulaki, D. Gill, Genetic evidence for protective effects of angiotensin-converting enzyme against alzheimer disease but not other neurodegenerative diseases in European populations, Neurol. Genet. 8 (2022), https://doi.org/10.1212/nxg.0000000000200014.
[57] B. Afsar, R.E. Afsar, Y. Caliskan, K.L. Lentine, J.C. Edwards, Renin angiotensin system-induced muscle wasting: putative mechanisms and implications for clinicians, Mol. Cell. Biochem. (2024), https://doi.org/10.1007/s11010-024-05043-8.
[58] A. Shrestha, T. Bashir, M. Achison, S. Adamson, A. Akpan, T. Aspray, A. Avenell, M. M. Band, L.A. Burton, V. Cvoro, et al., Association of bradykinin receptor 2 (BDKRB2) variants with physical performance and muscle mass: findings from the LACE sarcopenia trial, PLoS One 19 (2024) e0307268.
[59] C. Rossios, T. Bashir, M. Achison, S. Adamson, A. Akpan, T. Aspray, A. Avenell, M. M. Band, L.A. Burton, V. Cvoro, et al., ACE I/D genotype associates with strength in sarcopenic men but not with response to ACE inhibitor therapy in older adults with sarcopenia: results from the LACE trial, PLoS One 18 (2023) e0292402.
[60] M.-H. Yang, T.-C. Ho, C.-C. Chang, Y.-S. Su, C.-H. Yuan, K.-P. Chuang, Y.-C. Tyan, Utilizing proteomic approaches to uncover the neuroprotective effects of ACE inhibitors: implications for Alzheimer's disease treatment, Molecules 28 (2023) 5938.
[61] S.M. Danilov, I.A. Adzhubei, A.J. Kozuch, P.A. Petukhov, I.A. Popova, A. Choudhury, D. Sengupta, S.M. Dudek, Carriers of heterozygous loss-of-function ACE mutations are at risk for Alzheimer's disease, Biomedicines 12 (2024) 162.
[62] C. Ringland, J.E. Schweig, M. Eisenbaum, D. Paris, G. Ait-Ghezala, M. Mullan, F. Crawford, L. Abdullah, C. Bachmeier, MMP9 modulation improves specific neurobehavioral deficits in a mouse model of Alzheimer's disease, BMC Neurosci. 22 (2021), https://doi.org/10.1186/s12868-021-00643-2.
[63] V. Suzan, H. Yavuzer, R. Bag Soytas, B. Bektan Kanat, P. Arman, T. Emiroglu Gedik, D. Unal, O. Atar, I.M. Bolayirli, A. Doventas, The relationship between primary sarcopenia and SARC-F, serum MMP9, TIMP1 levels, and MMP9/TIMP1 ratio in the geriatric patients, Eur. Geriatr. Med. 12 (2021) 1229–1235.
[64] A.R. Vieira, R. Scariot, J.T. Gerber, J. Arid, E.C. Küchler, A.M. Sebastiani, M. Palinkas, K.V. Díaz-Serrano, C.P. Torres, S.C.H. Regalo, et al., Bruxism throughout the lifespan and variants in MMP2, MMP9 and COMT, J. Pers. Med. 10 (2020) 44.
[65] X. Yang, Z. Wang, Identification of novel immune-related biomarker and therapeutic drugs in Parkinson disease via integrated bioinformatics analysis, Medicine (Baltim.) 102 (2023) e34456.
[66] L. Sadeghi, R. Yekta, G. Dehghan, The inhibitory effects of bile acids on catalytic and non-catalytic functions of acetylcholinesterase as a therapeutic target in Alzheimer's disease, Acta Neurobiol. Exp. 80 (2020) 108–116.
[67] M.G. Mahmoud, M.E.E. Awady, M.S. Selim, A.Y. Ibrahim, F.M. Ibrahim, S. S. Mohamed, Characterization of biologically active exopolysaccharide produced by streptomyces sp. NRCG4 and its anti-alzheimer efficacy: in-vitro targets, J. Genet. Eng. Biotechnol. 21 (2023) 76.
[68] M. Moreno-Rodríguez, J. Martínez-Gardeazabal, I.B. de Tena, A. Llorente-Ovejero, L. Lombardero, E.G. de San Román, L. Giménez-Llort, I. Manuel, R. Rodríguez-Puertas, Cortical lipids containing choline mediate cannabinoid-induced cognitive improvement, bioRxiv (2024).
[69] B.A. Akhoon, S. Choudhary, H. Tiwari, A. Kumar, M.R. Barik, L. Rathor, R. Pandey, A. Nargotra, Discovery of a new donepezil-like acetylcholinesterase inhibitor for targeting Alzheimer's disease: computational studies with biological validation, J. Chem. Inf. Model. 60 (2020) 4717–4729.
[70] M. Bektasevic, M. Jurin, M. Roje, O. Politeo, Phytochemical profile, antioxidant activity and cholinesterase inhibition potential of essential oil and extracts of teucrium montanum from Bosnia and Herzegovina, Separations 10 (2023) 421.
[71] Z.A.S. Hawash, E.M. Yassien, B.S. Alotaibi, A.M. El-Moslemany, M. Shukry, Assessment of anti-alzheimer pursuit of jambolan fruit extract and/or choline against AlCl3 toxicity in rats, Toxics 11 (2023) 509.
[72] Z. Bao, C. Cui, S.K.-H. Chow, L. Qin, R.M.Y. Wong, W.-H. Cheung, AChRs degeneration at NMJ in aging-associated sarcopenia–A systematic review, Front. Aging Neurosci. 12 (2020), https://doi.org/10.3389/fnagi.2020.597811.
[73] S. Pitiphattharabun, K. Auewattanapun, T.L. Htet, M.M. Thu, G. Panomsuwan, R. Techapiesancharoenkij, J. Ohta, O. Jongprateep, Reduced graphene oxide/zinc oxide composite as an electrochemical sensor for acetylcholine detection, Sci. Rep. 14 (2024), https://doi.org/10.1038/s41598-024-64238-7.
[74] A.M. Ali, H. Kunugi, Apitherapy for age-related skeletal muscle dysfunction (sarcopenia): a review on the effects of royal jelly, propolis, and bee pollen, Foods 9 (2020) 1362.
[75] Y.J. Jang, The effects of protein and supplements on sarcopenia in human clinical studies: how older adults should consume protein and supplements, J. Microbiol. Biotechnol. 33 (2023) 143–150.
[76] S. Jia, W. Zhao, F. Hu, Y. Zhao, M. Ge, X. Xia, J. Yue, B. Dong, Sex differences in the association of physical activity levels and vitamin D with obesity, sarcopenia, and sarcopenic obesity: a cross-sectional study, BMC Geriatr. 22 (2022), https://doi.org/10.1186/s12877-022-03577-4.
[77] K. Narita, E. Amiya, Is branched-chain amino acid nutritional supplementation beneficial or detrimental in heart failure? World J. Cardiol. 13 (2021) 163–169.
[78] T. Umar, R. Meena, P. Mustehasan Kumar, A.A. Khan, Recent updates in the development of small molecules as potential clinical candidates for Alzheimer's disease: a review, Chem. Biol. Drug Des. 100 (2022) 674–681.
[79] P. Jyothi, K. Yellamma, Anti-Alzheimer's activity of compounds from the methanolic extract of Lawsonia inermis seeds: in vivo and in silico molecular docking studies, Ind. J. Pharm. Educ. 55 (2021) 463–473.
[80] N. Ruwizhi, B.A. Aderibigbe, Cinnamic acid derivatives and their biological efficacy, Int. J. Mol. Sci. 21 (2020) 5712.
[81] F. Saponaro, J.H. Kim, G. Chiellini, Transthyretin stabilization: an emerging strategy for the treatment of Alzheimer's disease? Int. J. Mol. Sci. 21 (2020) 8672.
[82] D.H. O'Day, Phytochemical interactions with calmodulin and critical calmodulin binding proteins involved in amyloidogenesis in Alzheimer's disease, Biomolecules 13 (2023) 678.
[83] O.F. Sultana, R.A. Hia, P.H. Reddy, A combinational therapy for preventing and delaying the onset of Alzheimer's disease: a focus on probiotic and vitamin Co-supplementation, Antioxidants 13 (2024) 202.
[84] H.N. Yassine, C.E. Finch, APOE alleles and diet in brain aging and Alzheimer's disease, Front. Aging Neurosci. 12 (2020), https://doi.org/10.3389/fnagi.2020.00150.
[85] K. Tadokoro, Y. Ohta, H. Inufusa, A.F.N. Loon, K. Abe, Prevention of cognitive decline in Alzheimer's disease by novel antioxidative supplements, Int. J. Mol. Sci. 21 (2020) 1974.