S. Dc International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 18, Issue 3, 2026

Original Article

NETWORK TOXICOLOGY–EXPLORATION OF SWEETENER EXPOSURE AND DEPRESSION WITH *IN VITRO* EXPERIMENTAL VALIDATION

SUJATHA DODOALA[1*], LATHA PUJARI[1], MADHAVI DUVURU[2], SAI SUNEEL ADEM[3], SWETHA PETLU[1], MOHITHA PILLA[1], CHANDNA SHRINIVAASINY THATHAPPAGARI[1]

[1*]Institute of Pharmaceutical Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh-517502, India. [2]Department of Home Science, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh-517502, India. [3]School of Engineering and Technology, Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh-517502, India

[*]Corresponding author: Sujatha Dodoala; [*]Email: drsujathasai@spmvv.ac.in

Received: 10 Dec 2025, Revised and Accepted: 19 Feb 2026

ABSTRACT

Objective: Sweeteners, a category of food additives are increasingly being incorporated into foods, yet their potential neurobiological safety issues remain underexplored. This study investigated the possible link between the european food safety authority (EFSA)-approved sweeteners and depression by integrating network toxicology, molecular docking, and experimental validation, with a particular focus on steviol glycoside.

Methods: Sweeteners collected from EFSA were initially screened using SwissADME and ADMETlab 3.0 for toxicity profiles. The potential targets, protein–protein interactions (PPI), molecular pathways, and binding affinities of the screened compounds were further analyzed using varioussuitable *in silico* tools (including SwissTargetPrediction, PharmMapper, STRING, shinyGO, cytoscape, cytoHubba, molecular docking) and *in vitro* studies.

Results: A total of 31 sweeteners were retrieved from the EFSA database and evaluated for their ADMET characteristics. Among them, 14 compounds were predicted to exhibit toxicological properties. Integration of target prediction data for these compounds with depression-related genes obtained from DisGeNET, GeneCards, Swiss Target Prediction and PharmMapper identified 225 overlapping targets, with network analysis highlighting AKT1, SRC, TP53, ALB, and ESR1 as major hub genes. Functional enrichment analysis using ShinyGO revealed significant associations with neuroactive ligand–receptor interactions, oxidative stress pathways, and mood-related signaling processes. Molecular docking analysis of the top five sweeteners indicated that steviol glycoside displayed the highest affinity and selectivity toward ESR1. Supporting these, differential scanning calorimetry (DSC) and Fourier transform infrared (FT-IR) spectroscopy confirmed steviol-induced conformational alterations in bovine serum albumin. Additionally, biochemical assays demonstrated increased protein carbonylation ($p<0.05$), elevated amadori product formation, reduced thiol content, and dose-dependent modulation of monoamine oxidase enzyme.

Conclusion: These findings suggest that certain EFSA-approved sweeteners, especially steviol glycoside, may influence depression-related pathways through modulation of key molecular targets. It also induced oxidative stress and altered activity of monoamine oxidases. These results underscore the need for further mechanistic and *in vivo* studies to evaluate potential neurobiological risks associated with increased sweetener exposure.

Keywords: Depression, EFSA, Food additives, Network pharmacology, Sweetener, Toxicology

DOI: https://dx.doi.org/10.22159/ijap.2026v18i3.57747 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Food additives are substances incorporated into foods or beverages for various purposes, including enhancing safety, freshness, flavor, texture, or appearance, as defined by the european food safety authority (EFSA). Artificial sweeteners are essential additives in contemporary diets, valued for providing sweetness with reduced caloric intake and managing diseases like diabetes and obesity [1, 2]. Globally, sweeteners have become central to the food and beverage sector, with an expanding market in regions like North America, Europe, China, and India. Projections estimate the artificial sweetener sector alone will surpass $12 billion by 2033, while the natural sweeteners market is also rising, propelled by consumer demand for clean-label products [3].

The evolution of food sweeteners reflects a history of technological innovation and regulatory challenges. The first artificial sweetener, saccharin, was synthesized in 1879, followed by others like cyclamate, aspartame, and sucralose. Safety concerns led to cycles of approval, ban, and re-evaluation. For example, saccharin was temporarily banned due to toxicity reports but was later reintroduced. These early discoveries laid the foundation for today's widespread use and monitoring of both natural and synthetic sweeteners in food processing [4, 5].

Natural and synthetic sweeteners are employed for distinct purposes, from calorie reduction and metabolic control to product formulation and shelf-life extension. Natural sweeteners, such as stevia, honey, and maple syrup, are often favored for perceived health benefits and antioxidant content, though they still showing memory, cognitive impairment and neurological impacts. Synthetic sweeteners, including aspartame and sucralose, are preferred for their high sweetness potency and utility in weight management and specialized diets, but can sometimes cause side effects like headaches or digestive concerns in sensitive individuals [4, 6].

The joint food and agriculture organization of the united nations (FAO)/WHO expert committee on food additives (JECFA) sets acceptable daily intake (ADI) values based on extensive toxicological reviews, including studies on toxicity, carcinogenicity, metabolism, primarily on systemic toxicity and overt adverse outcomes. However, ADI assessments are not designed to capture subtle, long-term neurobiological effects or redox-sensitive molecular alterations that may arise under habitual, lifelong exposure conditions. Given that mood regulation involves finely tuned signaling pathways highly sensitive to oxidative and thiol-mediated modifications, depression-related mechanisms represent a biologically plausible endpoint for exploratory evaluation. Accordingly, the present study adopts a hypothesis-generating approach to examine whether

exposure levels within regulatory safety margins may intersect with molecular pathways relevant to mood regulation, without implying exceedance of ADI or direct neurotoxicity.

Although commonly used sweeteners comply with JECFA-defined ADI thresholds, growing scientific evidence indicates the possibility of toxicities, that are not fully addressed in existing regulatory frameworks, underscoring the need for mechanistic and systems-level investigations. However, some products have already been reported to be consumed beyond the ADI limits. Recent studies also suggested a relationship between excessive sweetener usage and harmful neurological, metabolic, and cardiovascular effects [7-9]. But studies are lacking to explain the mechanisms of toxicities, targets and biological pathways involved in such toxicities. Among the spectrum of reported toxicities, the current investigation prioritized neurological effects, especially depression, given the consistently reported rise in its incidence [10].

In this context, the present research aimed to explore the association between the use of sweeteners and their potential effects on the depression. Network toxicology assessment was included to understand probable pathways responsible for toxicity. Based on the results of network analysis, further validation was done using *in vitro* models where, bovine serum albumin was used as test system.

MATERIALS AND METHODS

Chemicals and reagents

Bovine serum albumin (BSA),2,4-dinitrophenylhydrazine (DNPH), guanidine hydrochloride (GdnHCl), ascorbic acid, nitro blue tetrazolium (NBT), 5,5'-dithiobis2-nitrobenzoic acid (DTNB), Reduced glutathione (GSH) was purchased from Sigma Aldrich Pvt Ltd, India. Total protein estimation kit was obtained from Erba diagnostics private limited, India. All other chemicals and reagents used in the study were of analytical grade and purchased from sisco research laboratories (SRL)/Hi Media/Merck Pvt Ltd, India. The steviol glycoside (SG) used in the study was of food grade and obtained from commercial suppliers.

EFSA approved sweeteners

The sweeteners authorized by the EFSA (https://www.efsa.europa.eu/en) with regulatory approval and supporting scientific literature were collected. PubChem ID (PCID) and recognized simplified molecular input line entry system (SMILES) sequence of each sweetener was systematically retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) to enable computational analysis [11, 12].

Prediction of pharmacokinetic and toxicity parameters

Pharmacokinetic and toxicological characteristics of the sweeteners were analyzed using their canonical SMILES in ADMETlab3.0 (https://admetlab3.scbdd.com/) to predict absorption, distribution, metabolism, and excretion (ADME) parameters including Lipinski's rule, log P, Caco-2 permeability, blood brain barrier (BBB) penetration, and CYP enzyme interactions (CYP2C9 substrate, CYP2C19, CYP3A4 inhibitors). Toxicity predictions included assessments of hERG blockade, carcinogenic potential, AMES mutagenicity, hepatotoxicity, and the risks of drug-induced nephrotoxicity and neurotoxicity [13].

Target fishing and mapping

Sweeteners-related targets library was performed using swiss target prediction (version2019; http://www.swisstargetprediction.ch) and from PharmMapper (version 2017; https://www.lilab-ecust.cn) with "Z-score" of 1.0 or 1.5 to accept a target prediction and to obtain target names, UniProt IDs, chembl identifiers, and gene types [14-16].

Genes associated with depression were collected from GeneCards (version 5.26; https://www.genecards.org/) and DisGeNET (version 25.4; https://disgenet.com/). From GeneCards the targets were collected considering only protein-coding genes with functional annotations, while collection from DisGeNET includes gene name, full name, score gda, uniprot, gene type, eigda, and N chemicals. After merging and refined by removing duplicates, isoforms, and uncharacterized entries from the datasets the list of targets were finalised. The intersecting genes between predicted targets of sweeteners and genes linked with depression were identified by an online bioinformatic venn diagram tool (https://bioinformatics.psb.ugent.be/webtools/Venn/). These overlapped gene clusters were employed as the basis for subsequent network research.

Network construction and analysis

The network was constructed for sweeteners-PCID vs PCID-target genes vs disease–target genes. The resulting networks were visualized and analysed using cytoscape (version 3.10.3) (https://cytoscape.org/) for all the overlapping genes, but network was constructed for the top 30 gene targets. Later on top 5 hub genes together with the top five sweeteners were identified based on network connectivity through the degree technique via the cytohubba plug-in. The degree method (degree centrality) was applied to evaluate the topological importance of nodes within the interaction network. Degree centrality represents the number of direct connections (edges) associated with a node, reflecting its level of interaction with other nodes in the network. Protein protein interaction (PPI) networks of gene clusters were generated using the STRING (version 12.0) (https://string-db.org/) restricting the species to "*Homo sapiens* "and applying a confidence score threshold of ≤0.7 [17].

Functional GO and KEGG pathway enrichment analysis

Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses were performed using ShinyGO version 0.82 (https://bioinformatics.sdstate.edu/go/) to determine the biological relevance of the overlapping genes. The analysis was carried out using *homo sapiens* as the reference species with an FDR cut off of 0.05. Pathways containing 2–5000 genes were filtered based on fold enrichment, and the top ten enriched pathways were highlighted. GO analysis categorized target genes into biological processes, cellular components, and molecular functions, while KEGG analysis identified the associated signalling pathways. Bubble plots generated from both enrichment analyses were exported for further interpretation.

Molecular docking studies

Molecular docking was performed to validate the network toxicology predictions and to characterize the interactions between selected sweeteners and key depression-related proteins. Based on the degree centrality analysis, five sweeteners thaumatin (CAS: 53850-34-3), isomalt (CAS: 64519-82-0), steviol glycosides (CAS: 57817-89-7), lactitol (CAS: 585-86-4), and steviol (CAS: 471-80-7) were identified as the top candidates for further *in silico* evaluation. Similarly, the top five hub targets identified from the PPI network were selected for docking: AKT1 (PDB ID: 8UW7), SRC (PDB ID: 1Y57), TP53 (PDB ID: 8SWJ), ALB (PDB ID: 9IK6), and ESR1 (PDB ID: 6VPF). Protein structures in their highest-resolution forms were retrieved from the research collaboratory for structural bioinformatics (RCSB) protein data bank. These ligand–protein pairs were then used to perform molecular docking to assess binding affinity and interaction profiles.

Docking was performed using the SwissDock (2024; https://www.swissdock.ch/) integrated with the AutoDock vina algorithm by uploading the ligand and receptor files and setting the binding site grid parameters to efficiently predict the interactions. SwissDock generated multiple binding conformations and calculated affinities.

The results of docking were viewed and analyzed with PyMOL (version 3.1.4.1) (https://www.pymol.org/) to identify ligand binding to the protein active site [18, 19]. Further interaction profiling was carried out using BIOVIA discovery studio 2025 (https://www.3ds.com/products/biovia/discovery-studio), focusing on stabilization interactions such as hydrogen bonds, hydrophobic interactions, and van der waals forces [20].

***In vitro* studies using BSA as model protein**

Differential scanning calorimetry (DSC)

DSC experiments were carried out for BSA and physical mixture of BSA with equimolar ratio of steviol glycoside. The study was conducted using a Mettler-Toledo calorimeter (DSC 1/500), with 4-4.5 mg of each sample sealed in aluminium pans and heated between 20-100 °C to measure their melting and thermal properties [21].

Fourier transform infrared (FT-IR) analysis

BSA and physical mixture of BSA with equimolar ratio of steviol glycoside (10 µM each) were precisely blended with potassium bromide (KBr) in a 100:1 ratio, thereafter subjected to vacuum pressing to produce translucent pellets. Subsequently, these samples were transmitted to FT-IR (Bruker, Alpha-T model) over a wavenumber range of 400-4000 $cm^{-1}$to assess their changes in secondary structure [22].

Effect of steviol glycoside on protein carbonyl content

Protein carbonylation was quantified using a standard 2,4-dinitrophenylhydrazine (DNPH) spectrophotometric assay with minor modifications from Colombo *et al.* (2016) and Yan *et al.* (2009). Briefly, BSA (100 µM), either alone or in combination with steviol glycoside (10-160 µg/ml), was incubated with 10 mmol DNPH prepared in 2 N HCl to obtain derivatized protein carbonyl groups [23, 24]. For the positive control, BSA was treated with 1 ml of 10% H_2O_2 along with 10 mmol DNPH in 2 N HCl, a condition known to reliably induce protein carbonylation. Following incubation, proteins were precipitated by adding 20% (w/v) trichloroacetic acid (TCA) and centrifuged to obtain the pellet. The pellet was then washed three times with a chilled ethanol: ethyl acetate (1:1, v/v) mixture to remove excess DNPH and other interfering compounds. The final pellet was then dissolved in 6 M guanidine hydrochloride (prepared in 20 mmol phosphate buffer, pH 8.0), vortexed, and incubated at 37 °C until completely solubilised

Absorbance of the DNPH-derivatized samples was measured at 360 nm using guanidine hydrochloride as the reference blank. Protein carbonyl content was calculated using the DNPH molar extinction coefficient, ε = 22,000 M^{-1} cm^{-1} and expressed as nM carbonyls/mg protein.

Advanced glycation end-product assay

The glycation assay was performed following previously reported methods with minor modifications. BSA (10 mg/ml) was prepared in phosphate buffer solution (PBS 50 mmol, pH 7.4). Three reaction mixtures were set up, which included BSA, BSA incubated with glucose (100 mg/ml), and BSA incubated with both glucose (100 mg/ml) and steviol glycoside (10 mg/ml). All mixtures contained sodium azide (0.02%) to prevent microbial growth. The samples were incubated at 37 °C for 3 w in a biochemical oxygen demand (BOD) incubator (Remi CI-10 PLUS) [25].

Spectrophotometric analysis of absorbance changes

Following the incubation period, the UV–visible absorption spectra of BSA, BSA incubated with glucose, and BSA exposed to both glucose and steviol glycoside were recorded using a UV–Vis spectrophotometer (Shimadzu UV-1900i). Spectra were collected in the wavelength range of 200–400 nm to assess glycation-induced structural alterations.

Determination of amadori products

At the end of the incubation period, amadori product formation was quantified based on the reaction between nitroblue tetrazolium (NBT) and ketoamines. Briefly, 100 µl** of each sample was mixed with 100 µl** of NBT solution (250 µM prepared in 0.1 M carbonate buffer, pH 10.8) and incubated at 37 °C for 45 min. The absorbance of the resulting chromophore was measured at 525 nm. The procedure followed the method described by Emami *et al.* (2024) [25]. The results were given as % fructosamine content compared to control.

Total thiol content assay

In this assay, 100 µl** of each test sample was reacted with 200 µl** of freshly prepared DTNB solution in PBS (pH 8.5). Reduced glutathione was used to generate the standard calibration curve. Absorbance was recorded at 420 nm, following the protocol reported by Emami *et al.* (2024) [25]. The results were given as total thiol content in nM/mg protein.

***In vitro* MAO inhibition assay**

Monoamine oxidase (MAO) was isolated from rat liver tissues obtained from a committee for control and supervision of experiments on animals (CCSEA) approved breeder, and all experimental procedures were conducted with prior approval from the institutional animal ethics committee (IAEC) of Sri Padmavathi Mahila Visvavidyalayam (Approval No. CCSEA/1677/SPMVV/IAEC/VI-15). MAO activity was evaluated using rat liver homogenate as the enzyme source, following the protocol described by Guili Huang *et al.* (2016) [26]. The modulatory effect of steviol glycoside at different concentrations (10 to 160µg/ml) on MAO enzyme activity was subsequently assessed, with selegiline (10 to 160 µg/ml) serving as the reference standard. Protein concentrations in the homogenates were quantified using the erba total protein estimation kit and the results were given as % inhibition of enzyme activity compared to control.

Statistical analysis

Statistical analysis was carried out using GraphPad prism 10.6.1 and the results were expressed as mean±standard deviation (SD). All statistical analyses were carried out using either one-way or two-way ANOVA followed by post-hoc tukey's multiple comparison test with a significance level set at $p<0.05$. As the *in vitro* experiments were conducted in triplicate, given the low sample size, normality testing was not informative; thus, one-way or two-way ANOVA was applied based on the need, due to its robustness to minor deviations from normality.

RESULTS

Screening of approved sweeteners

Initially, 31 sweeteners approved by the EFSA were collected and screened for the essential physicochemical properties and were compiled (supplementary table1).

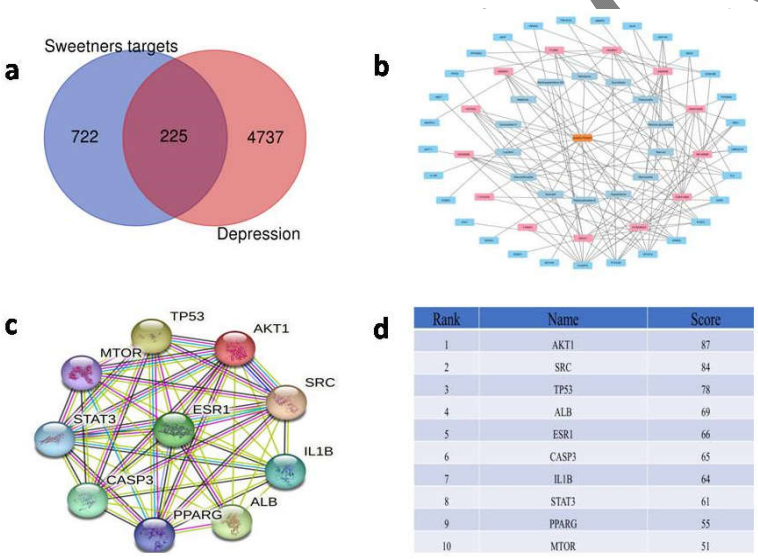
Computational assessment of ADMET properties

Using ADMETlab 3.0, the toxicity profiles of the shortlisted sweeteners were predicted using a green–yellow–red risk scale. Compounds exhibiting moderate to high toxicity were retained, while sweeteners identified as salts and showing no toxicity were excluded from further analysis. This refinement resulted in a final panel of 14 sweeteners. Detailed profiling data for these selected sweeteners were provided in supplementary tables 2 and 3.

Network analysis

A total of 4,142 targets was predicted using PharmMapper, while 1,402 targets were obtained from SwissTargetPrediction. After merging the datasets and eliminating duplicate entries, 997 unique targets were identified. A total of 4,962 depression-associated targets were retrieved from DisGeNET (283 targets) and GeneCards (4835 targets), after eliminating the duplicates. A bioinformatic venn analysis revealed 225 overlapping genes between the sweetener-related targets and depression-related targets (fig. 1a).

A network comprising all common genes and the top 30 targets was constructed using cytoscape 3.10.3 and analyzed with the cytoHubba plug-in. To improve clarity, the network was refined to include only the top 30 targets and the 14 sweeteners (fig. 1b). This analysis also enabled the identification of the top five sweeteners based on the degree method, which were subsequently selected for further validation. The 196 intersecting genes were then evaluated using the STRING database to construct a protein–protein interaction (PPI) network, and the top 10 hub targets were identified and ranked (fig. 1c and fig. 1d).



Rank	Name	Score
1	AKT1	87
2	SRC	84
3	TP53	78
4	ALB	69
5	ESR1	66
6	CASP3	65
7	IL1B	64
8	STAT3	61
9	PPARG	55
10	MTOR	51

Fig. 1: Network analysis of overlapping targets between sweeteners and targets of depression. a. Indicates venn diagram for overlapping targets between sweeteners (blue) and depression (red), b. Represents an interaction network of sweeteners-top 30 targets related to depression. Orange represents food additive, blue shows sweetener list, pink presents PubChem ID, and associated targets in light blue, c. Depicts the PPI network of shared depression related targets, d. Summarizes the top 10 targets with their respective ranks and degree centrality scores

GO/KEGG pathway enrichment analysis

GO enrichment analysis of the 225 intersecting genes revealed that sweeteners may primarily influence pathways related to responses to oxygen-containing compounds (biological processes), intrinsic components of the plasma membrane (cellular components), and signalling receptor and molecular transducer activities (molecular functions) (fig. 2a–c). KEGG pathway analysis further indicated that these targets are predominantly associated with the neuroactive ligand–receptor interaction pathway, highlighting a potential connection to neurological and behavioural processes and may the major reason for causing depression (fig. 2d).

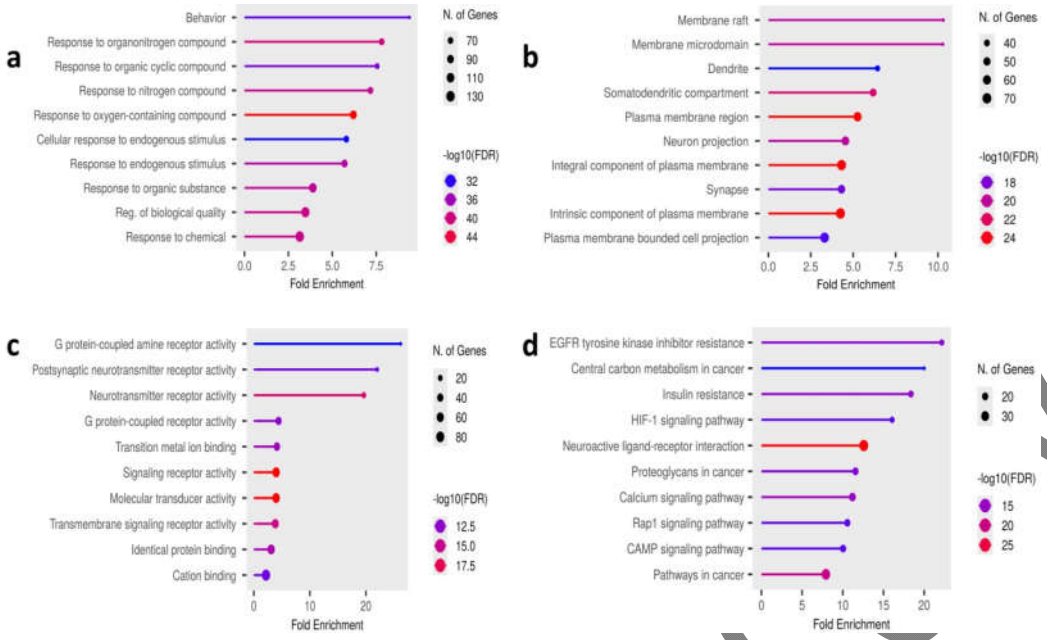


Fig. 2: GO/KEGG enrichment analysis of sweeteners-depression: shows the network plots of the top ten enriched pathways identified through ShinyGO. Panels (2a, 2b, 2c) represent the leading pathways in the biological process, cellular components, and molecular function categories respectively in GO analysis, while panel (2d) displays the KEGG pathway enrichment results

Molecular docking analysis

The network analysis was validated using molecular docking analysis, wherein the top 5 sweeteners with predicted toxicity and top 5 targets involved in the disease depression were selected. The docking results of top 5 targets with top 5 sweeteners with interaction score, interacting amino acids were mentioned in the supplementary data table 4. From the docking results it was clear that steviol glycoside recorded the best binding score of-8.159 towards oestrogen receptor ESR1 (PDB ID: 6VPF) among all the studied targets, by forming Conventional H bonds and Pi–Alkyl interactions. The docking poses were exhibited in detail in fig. 3a-e.

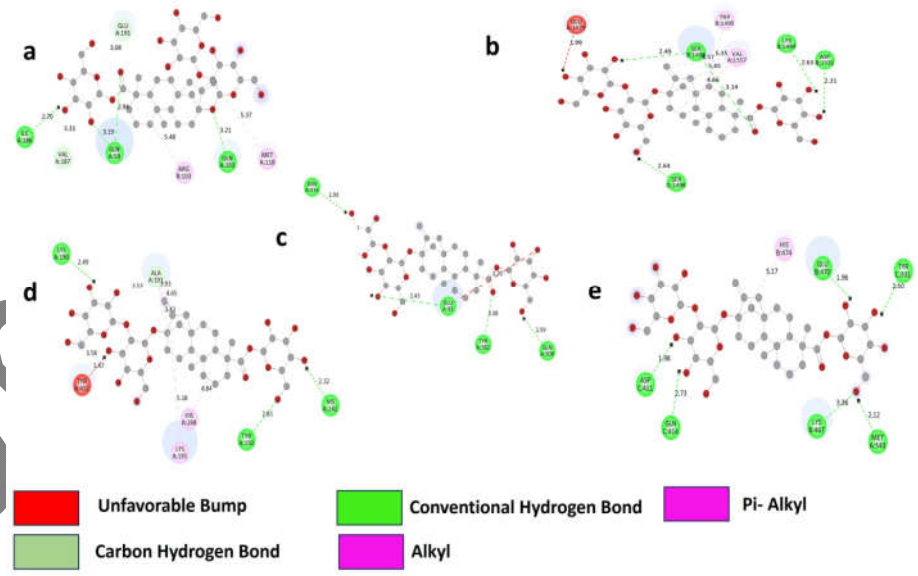


Fig. 3: Molecular docking outlines of steviol glycoside with various core proteins. Panel 3(a). Shows the 2D interaction profile for AKT1 (PDB: 8UW7/interaction score: -6.460 kcal/mol.), 3(b). Represents the 2D binding interactions with SRC (PDB: 1Y57/interaction score: -7.949 kcal/mol.), 3(c). Depicts the 2D interaction map within the TP53 active site (PDB: 8SWJ/interaction score:-6.294 kcal/mol.), 3(d). Displays the 2D interaction pattern for ALB (PDB: IK6/interaction score:-4.373 kcal/mol.), 3(e). Illustrates the 2D binding interactions observed with ESR1 (PDB: 6VPF/interaction score:-8.159 kcal/mol.)

In vitro studies using BSA as model protein

DSC results revealed that BSA undergoes an exothermic transition, marked by a higher negative enthalpy change (–215.25 mJ) and a major transition peak at 84.47 °C. In the presence of steviol glycoside, BSA still displayed an exothermic event. The enthalpy of unfolding (ΔH) of BSA was 19.09 J g^{-1}, which increased to 21.53 J g^{-1} in the presence of steviol glycoside, indicating a ligand-induced stabilization effect as shown by DSC. The corresponding DSC profiles were shown in fig. 4(a).

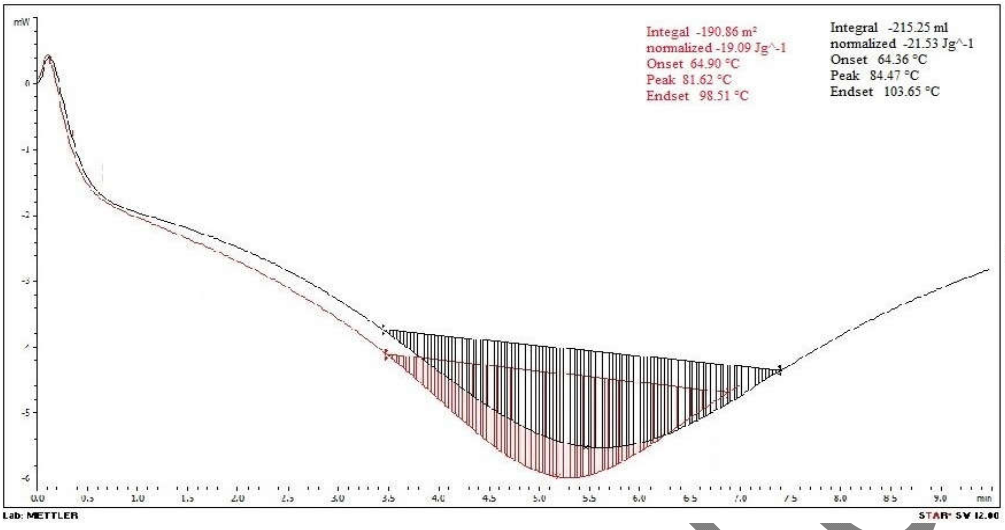


Fig. 4a: **DSC thermograms were obtained using a Bruker DSC instrument under nitrogen atmosphere at a heating rate of 10 °C min^{-1} over the temperature range of 20–100 °C and resolution of 4 with MMS1 sensor and 136 thermocouples. BSA depicted in black and BSA in the presence of steviol glycoside depicted in red**

FT-IR analysis revealed distinct peaks for BSA confirming their characteristic functional groups. BSA exhibited prominent Amide I and II bands indicating peptide structures, while in presence of steviol glycoside, FT-IR showed strong O-H, C-O-C, and C=O bands confirming the presence of sugar and diterpenoid functional groups of the glycoside. However, the overlay indicates lack of physical interaction between BSA and steviol glycoside. The FT-IR overlay spectra was mentioned in fig. 4(b).

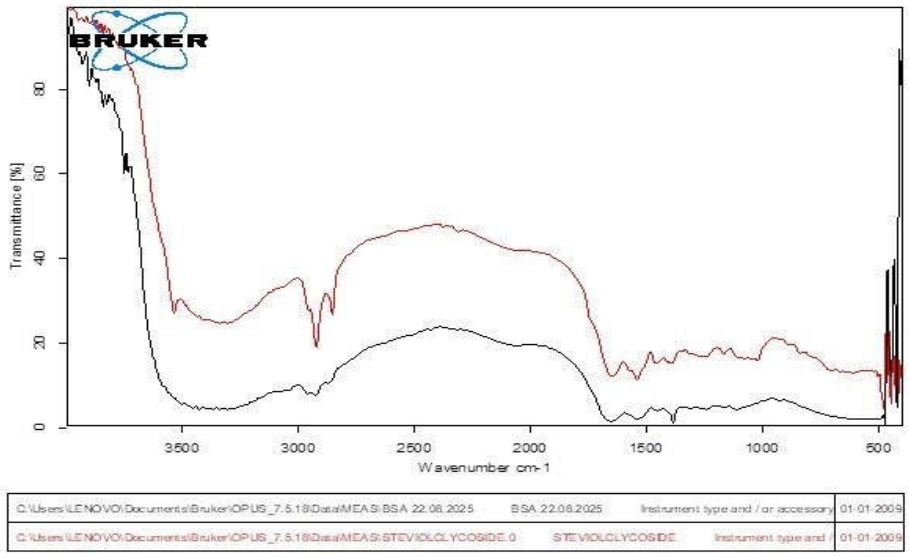


Fig. 4b: Overlay of FT-IR spectra of BSA (black) and BSA in presence of steviol glycoside (red). FT-IR spectra were recorded using a Bruker FT-IR spectrometer in ATR mode over 400–4000 cm^{-1} at 4 cm^{-1} resolution with 32 scans

The effect of steviol glycoside on protein carbonyl contents

The addition of hydrogen peroxide markedly increased protein carbonyls, confirming oxidative stress to BSA. However, steviol glycoside (10-160 µg/ml) caused a dose-dependent decrease in carbonyl content compared to control BSA. These levels remained slightly lower than the BSA control group, indicating milder oxidative modification. The results were tabulated in table 1.

Table 1: The effect of steviol glycoside on protein carbonyl contents

S. No.	Compound	nM carbonyl content/mg protein
1	BSA	0
2	BSA+H_2O_2	14.54±0.3256*
3	BSA+SG (10 µg/ml)	1.18±0.0057#
4	BSA+SG (20 µg/ml)	0±0.0015#
5	BSA+SG (40 µg/ml)	0.56±0.0039#
6	BSA+SG (80 µg/ml)	0.47±0.0018#
7	BSA+SG (160 µg/ml)	0.35±0.0020#

All the values were given as mean±SD of multiple observations (n=3). BSA-bovine serum albumin; SG-steviol glycoside. The carbonyl content below detectable levels were given as "0";* p<0.05 on comparison between BSA Vs BSA+H_2O_2; #p<0.05 on comparison between BSA+H_2O_2Vs BSA+SG (One way ANOVA followed by tukey's multiple comparison test)

Effect of steviol glycoside on advanced glycation end-product assay

The UV absorbance spectra exhibited a marked increase in intensity (hyperchromic effect) upon the addition of glucose and steviol glycoside to BSA, indicating potential structural perturbations. A slight blue shift (hypsochromic shift) in the absorption maximum was also observed relative to BSA alone, suggesting alterations in the protein's microenvironment. These spectral deviations, collectively, reflect interaction-induced conformational modifications in the BSA structure, as illustrated in fig. 4C.

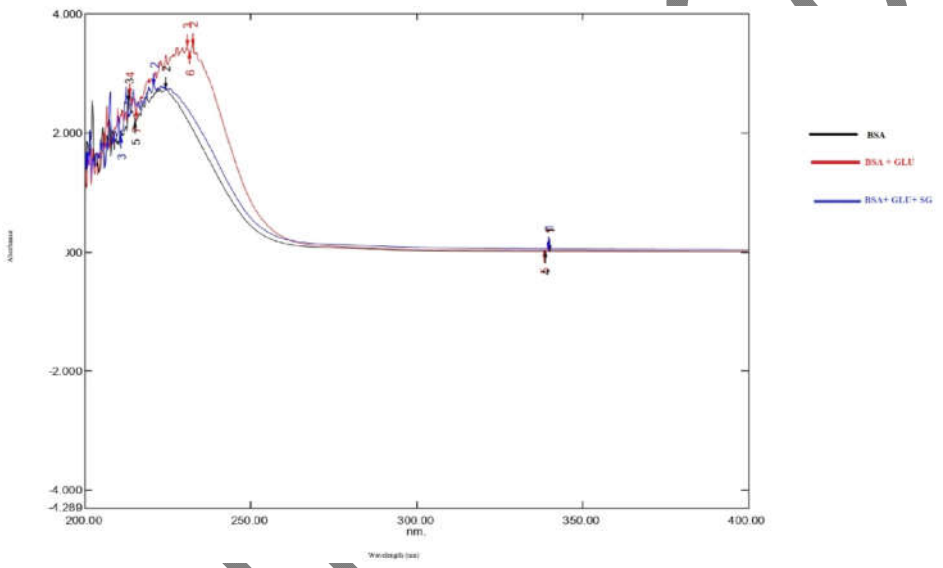


Fig. 4c: UV–Vis absorption spectra were obtained using a Shimadzu UV–Vis spectrophotometer in the range of 200–400 nm at room temperature. Overlay spectrum of BSA alone (black) and presence of glucose (red) and steviol glycoside (blue)

Assessment of amadori product formation showed that BSA generated negligible fructosamine levels. However, in the presence of glucose and steviol glycoside, a gradual and time-dependent increase in fructosamine content was observed. Notably, steviol glycoside induced a higher formation of fructosamine compared with BSA incubated with glucose alone (table 2).

Table 2: Effect of steviol glycoside on production of amadori products and thiol content in BSA

S. No.	Groups	% Fructosamine content				Thiol content (nmol/mg protein)			
		0th d	7th d	14th d	21st d	0th d	7th d	14th d	21st d
1	BSA	0	0	0	0	0.0083±0.0004	0.0083±0.0004	0.0083±0.0004	0.0083±0.0004
2	BSA+GLU	65.57±0.0038*	71.93±0.0004*	75.53±0.0010*	93.66±0.0013*	85.54±0.0001*	94.69±0.0047*	95.18±0.0001*	96.50±0.0009*
3	BSA+GLU+SG	48.96±0.0022ns	54.36±.0008ns	69.25±0.0002ns	96.12±0.0010ns	92.77±0.0001ns	95.68±0.0015ns	96.38±0.0001ns	97.66±0.0012ns

All the values were given as mean±SD of multiple observations (n=3). BSA-bovine serum albumin; GLU-glucose; SG-steviol glycoside. The fructosamine content below detectable levels were represented as "0"; The thiol content was absolute measurement of thiol group using DTNB reagent. * p<0.05 on comparison between BSA Vs BSA+GLU; nsp ≥ 0.05 on comparison between BSA+GLU Vs BSA+GLU+SG (Two-way ANOVA followed by tukey's multiple comparison test).

Table 2 also summarizes the effects of steviol glycoside on BSA thiol groups. While only BSA displayed minimal free thiol content, glucose exposure resulted in a slight, time-dependent increase. In contrast, steviol glycoside treatment led to a significantly higher percentage of free thiol groups compared with the glucose-treated BSA sample, indicating a pronounced influence on protein thiol status.

Determination of MAO enzyme activity

Steviol glycoside demonstrated a concentration-dependent reduction in measured MAO content, which contrasted with the expected inhibitory effect produced by the reference MAO inhibitor, selegiline. This opposing trend suggests that steviol glycoside may exert a stimulatory influence on MAO activity or promote conditions that enhance MAO-mediated oxidative turnover. These findings imply that, unlike selegiline, steviol glycoside does not suppress MAO and may instead facilitate pathways that elevate oxidative stress (table 3).

Table 3: The effect of steviol glycoside on MAO enzyme activity

Concentrations	% Inhibition of MAO enzyme activity	
	Steviol glycoside	Selegiline
10 µg/ml	-404.74±0.0001	47.96 ±3.18
20 µg/ml	-484.72±0.0003	63.68±0.62
40 µg/ml	-516.94±0.0003	64.94±0.56
80 µg/ml	-611.94±0.0003	71.02±4.18
160 µg/ml	-614.16±0.0001	73.36±5.77

All the values were given as mean±SD of multiple observations (n=3). Enzyme activity was expressed as a percentage relative to the control, with negative values indicating the absence of enzyme inhibition (Two-way ANOVA followed by tukey's multiple comparison test).

DISCUSSION

This study was conducted to address growing concerns regarding the excessive consumption of both artificial and natural sweeteners and their potential neurological and behavioral effects, particularly depression. Many health reports indicated that daily sweetener intake, especially among children and vulnerable populations, often surpass acceptable daily intake limits, raising significant safety concerns.

By focusing on EFSA-approved sweeteners, the study ensured the inclusion of compounds with established regulatory profiles and comprehensive toxicological documentation. Global agencies such as EFSA (2023) and WHO (2022) have also underscored the possible neurobehavioral effects of dietary additives, thereby reinforcing the relevance of this investigation.

Advances in computational toxicology now facilitate comprehensive assessments of dietary chemical risks, supporting the integrated approach employed in this study, which combined network toxicology, molecular docking, and biochemical validation. Similar methodologies have been widely applied in food additive research, but such studies are generally predictions and used primarily for generation of hypothesis [19]. Recently, bioinformatic tools such as SwissTargetPrediction, GeneCards, DisGeNET, STRING, Cytoscape, ADMETlab 3.0, and KEGG have become essential for predicting molecular targets, analyzing toxicity profiles, identifying disease-associated networks, and modeling protein interactions. Their extensive validation in nutraceutical and food toxicology research further supports their use in this context.

Network toxicology analysis revealed that sweeteners were associated with depression, exhibiting overlapping gene targets, making it the central focus of this study. The shared molecular nodes identified key hub proteins such as AKT1, SRC, TP53, ALB, and ESR1—proteins frequently implicated in neuroplasticity, inflammatory signaling, emotional regulation, and stress response [26]. To improve robustness, we incorporated two target screening tools such as SwissTargetPrediction and PharmMapper, then prioritized the overlapping targets. This comparative approach strengthens confidence while such *in silico* predictions need further validation.

Gene Ontology enrichment also validated that sweetener exposure predominantly influenced biological processes related to oxidative stress responses, chemical stimulus perception, receptor-mediated signaling, and neurotransmission, suggesting a potential role in synaptic regulation and mood-related mechanisms [19].

KEGG pathway analysis revealed significant involvement of neuroactive ligand–receptor interaction, which was reported to play a central role in neurotransmission, neuronal excitability, and behavior, thereby strengthening the mechanistic link between sweetener exposure and mood alteration [27].

Molecular docking studies provided further mechanistic insight by revealing strong binding affinities between steviol glycosides and CNS-related proteins. TP53 and ESR1 exhibited especially favorable interactions, suggesting sweetener-induced modulation of apoptotic regulation, hormonal receptor signaling, and neurobehavioral pathways. These findings align with earlier research on diterpenoid glycosides and their effects on metabolic and neuronal functions [28]. In the molecular docking studies, it was interesting to observe that steviol glycosides exhibited better docking score than other sweeteners (isomalt-E953, thaumatin-E957, lactitol-E966 etc.,) which can be because of the flexible, large, complex, polar structure with many hydrogen bonding sites as donors/acceptors. It was quite interesting to observe better docking scores for steviol glycoside (E960A) than steviol (E960D) which justifies that the glycosidic moiety was responsible for better docking score. But the docking model may introduce some kind of bias in favor of large molecules with many hydroxyl groups, even though they may not bind so specifically to the target or be relevant *in vivo*. This indicates need for further studies using suitable *in vitro* or *in vivo* studies.

Emami *et al.* reported that sodium benzoate, potassium sorbate, and sodium dihydrogen citrate, whether individually or in combination, exhibit strong interactions with BSA, resulting in enhanced glycation, structural alterations, and oxidative damage [25]. These interactions may exacerbate their detrimental effects on protein conformation and cellular function. To ascertain whether steviol glycoside induces similar effects, bovine serum albumin (BSA) was employed as the model protein due to its structural stability and relevance to human serum albumin.

Amyloid-β (Aβ) is transported in blood and CSF predominantly as an Aβ–albumin complex. Albumin, the most abundant protein in plasma and CSF, actively interacts with neurons and astrocytes, regulates brain metabolism, and supports neuronal survival. Its central role in CNS protein binding, transport, and peripheral clearance justifies the use of BSA as a relevant *in vitro* model [29].

BSA as test system has limitations, but it is worth noting that albumin plays a key role in redox homeostasis and xenobiotic transport, and oxidative modified albumin has been shown to influence endothelial function and BBB integrity, thereby indirectly contributing to neuroinflammatory and

neurotoxic outcomes [30, 31]. Protein glycation occurs through a non-enzymatic process whereby reducing sugars react with free amino groups of proteins, resulting in permanent changes to the protein structure. The initially formed, unstable Schiff bases subsequently undergo rearrangement to generate more stable amadori products (APs), which serve as early intermediates in glycation and contribute to progressive protein damage.

Dankowska *et al.* used bovine serum albumin (BSA) model to study the antiglycation properties of various substances, BSA in the presence of reducing sugars undergoes protein oxidation and protein glycation products resulting in alteration of thiol and protein carbonyl content followed by production advanced oxidation protein products (AOPPs), amadori products, advanced glycation end products (AGEs), and β-amyloid (βA) [32]. Thus, though simplified, the BSA model provides initial mechanistically relevant insight into systemic protein damage associated with potential CNS vulnerability.

Differential scanning calorimetry (DSC) thermograms demonstrated a reduction in transition temperature and enthalpy, suggesting increased thermal stability and stabilisation of BSA in presence of steviol glycoside. The FT-IR spectra confirmed the expected amide bands for BSA and characteristic functional groups of steviol glycoside, with no significant peak overlap, indicating primarily non-covalent interactions. However, broadening of peaks, new peaks at 1700 cm-1 indicates the decreased structural order and carbonyl formation in BSA due to steviol glycoside, respectively as per the earlier reports [33].

Overall, the results from DSC and FTIR spectra suggest that steviol glycoside binds to BSA mainly through weak interactions, which result in localized conformational changes, decreased structural order, and carbonyl formation. These protein modifications associated with redox reactions have been shown to occur due to oxidative stress-induced protein dysfunction, which has recently emerged as an important mechanism of depression pathophysiology. These data thus suggest that prolonged exposure to steviol glycosides could alter the regulation of mood through modulation of protein structure and redox-sensitive mechanisms. These results were further validated by the protein carbonyl content assay, amadori product formation and thiol content assay.

Biochemical assays showed that steviol glycoside reduced protein carbonyl content, indicating that steviol will protect BSA against H_2O_2 induced damage. However, in the glycation model, steviol glycoside did not exhibited any significant changes of UV-Vis spectrum of BSA but significantly increased the amadori product formation and elevated free thiol levels, implying enhanced early glycation, corroborating with the spectral studies on BSA.

Several studies in major depressive disorder have reported increased monoamine oxidase levels in the brains of affected individuals [34, 35]. Beyond its role in reducing catecholamine availability, recent evidence suggests that monoamine oxidase contributes to the initiation and progression of several chronic conditions by triggering mitochondrial damage-associated molecular patterns (mito-DAMPs). This process promotes the release of pro-inflammatory mediators, including IL-1β, IL-6, TNF-α, and the reactive oxygen species hydrogen peroxide (H_2O_2), thereby enhancing inflammatory responses [36]. Moreover, converging clinical and preclinical data indicate that neuroinflammation is a pivotal mechanism in depression, interacting with key neurobiological abnormalities such as diminished brain serotonin levels and dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis [37].

Based on existing literature, an MAO inhibition assay was conducted in the present study to assess the potential interaction of steviol glycoside with MAO. The use of liver MAO was intended to provide an initial, mechanistic indication of MAO interaction under controlled conditions rather than to model region-specific neuronal processes. The current study findings suggest interference with MAO enzymatic activity; however, confirmation of whether steviol glycoside upregulates MAO activity or expression would require mRNA expression studies in animal models. Overall, these findings suggest that steviol glycoside can induce measurable structural modifications in proteins, reduce oxidative protein damage, but enhance glycation processes, and alter enzyme activity relevant to neuronal signalling. Although network analysis indicated potential interactions with signalling and stress-response pathways, the absence of MAO inhibition indicates that monoaminergic catabolism is not a direct mechanism linking the additive to neurobehavioral outcomes.

CONCLUSION

In conclusion, the integrated computational and experimental findings from this study are preliminary first evidence, and hypothesis-generating in nature. The study suggests that habitual intake of sweeteners, particularly steviol glycosides, may be associated with neurobiological pathways relevant to mood regulation. Observed alterations in thiol and carbonyl levels, along with the formation of amadori products, indicate potential interactions with redox-sensitive biochemical processes that have been implicated in mood-related mechanisms. Network toxicology analysis further highlighted the AKT1 signaling pathway as a pathway of interest; however, these results do not imply direct modulation of central signaling cascades. Rather, they identify molecular nodes that warrant further investigation. Future studies employing physiologically relevant models, well-defined dose–response designs, and brain-specific systems are required to validate these associations, examine targets such as ESR and TP53, and assess whether long-term sweetener exposure poses any biologically meaningful risk.

ACKNOWLEDGEMENT

The authors sincerely acknowledge the financial support provided by Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, which contributed to the research, writing, and/or publication of this article.

FUNDING

This study was funded by the PM-USHA (MERU) research grant under component 12, provided by Sri Padmavati Mahila Visvavidyalayam (Women's University), Tirupati, Andhra Pradesh, India with number R. O. C. No. SPMVV/UGC/F1/PM-USHA/2024 dated 31/12/2024.

AUTHORS CONTRIBUTIONS

Conception and design, interpretation of data, drafting of paper and critical review; Sujatha Dodoala, interpretation of data, and drafting of paper; Latha Pujari, Statistical analysis; Madhavi Duvuru, Sai Suneel Adem, Experimental work; Swetha Petlu, Mohitha Pilla and Chandna shrinivaasiny Thathappagari.

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest associated with this work.

REFERENCES

1. Pozdnyakova Y, Murzatayeva A. Neuroprotective potential of *Stevia rebaudiana* and *Stachys sieboldii*: effects on oxidative stress and locomotor activity in male rats fed a high-fat, high-sucrose diet. Biology (Basel). 2025;14(4):359. doi: 10.3390/biology14040359, PMID 40282224.

2. Harismah K, Fazeli F, Amini I, Dai M, Mirzaei M. *In silico* effects of steviol on depression, inflammation and cancer biomarkers. Biointerface Res Appl Chem. 2021;12(6):8385-93. doi: 10.33263/BRIAC126.83858393.
3. Straits Research. Sweeteners market report [Internet]. Com/Report/sweeteners-market. [cited 13/3/2026] Available from: https://straitsresearch.
4. Lindseth GN, Coolahan SE, Petros TV, Lindseth PD. Neurobehavioral effects of aspartame consumption. Res Nurs Health. 2014;37(3):185-93. doi: 10.1002/nur.21595, PMID 24700203.
5. EBSCO research starters. Sweetener [Internet]. Available from: https://www. EBSCO. com/research-starters/health-and-medicine/sweetener.
6. Sardesai V. Natural and synthetic intense sweeteners. J Nutr Biochem. 1991;2(5):236-44. doi: 10.1016/0955-2863(91)90081-F.
7. Palomar-Cros A, Straif K, Romaguera D, Aragonés N, Castaño-Vinyals G, Martin V et al. Consumption of aspartame and other artificial sweeteners and risk of cancer in the Spanish multicase-control study (MCC-Spain). Int J Cancer. 2023;153(5):979-93. doi: 10.1002/ijc.34577, PMID 37323037.
8. Zhang Y, Tang Z, Shi Y, Li L. Associations between artificial sweetener intake from cereals, coffee, and tea and the risk of type 2 diabetes mellitus: a genetic correlation, mediation, and mendelian randomization analysis. PLOS One. 2024;19(2):e0287496. doi: 10.1371/journal.pone.0287496. PMID 38324548.
9. López-Meza MS, Otero-Ojeda G, Estrada JA, Esquivel-Hernández FJ, Contreras I. The impact of nutritive and non-nutritive sweeteners on the central nervous system: preliminary study [The impact of nutritive and non-nutritive sweeteners on the central nervous system: preliminary study] [preliminary study]. Nutr Neurosci. 2022;25(8):1623-32. doi: 10.1080/1028415X.2021.1885239, PMID 33641634.
10. Xiong J, Wang L, Huang H, Xiong S, Zhang S, Fu Q et al. Association of sugar consumption with risk of depression and anxiety: a systematic review and meta-analysis. Front Nutr. 2024;11:1472612. doi: 10.3389/fnut.2024.1472612, PMID 39479195.
11. Said Elshama SS. Synthetic and natural food additives: toxicological hazards and health benefits. OAJT. 2020 Oct 1;4(4):555643. doi: 10.19080/OAJT.2020.04.555643.
12. Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, et al. PubChem substance and compound databases. Nucleic Acids Res. 2016;44(D1):D1202-13. doi: 10.1093/nar/gkv951, PMID 26400175.
13. Ahmed SR, Al-Sanea MM, Mostafa EM, Qasim S, Abelyan N, Mokhtar FA. A network pharmacology analysis of cytotoxic triterpenes isolated from *Euphorbia abyssinica* latex supported by drug-likeness and ADMET studies. ACS Omega. 2022;7(21):17713-22. doi: 10.1021/acsomega.2c00750, PMID 35664578.
14. Daina A, Michielin O, Zoete V. SwissTargetPrediction. SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. Nucleic Acids Res. 2019 Jul 2;47(W1):W357-64. doi: 10.1093/nar/gkz382, PMID 31106366.
15. Tipugade O, Sawale JA, Jadhav N. Network pharmacology and molecular docking based exploration of Rubiaceous plants for breast cancer: phytochemicals, preclinical studies, and regulatory perspectives. Asian J Pharm Clin Res. 2025;18(7):52-71. doi: 10.22159/ajpcr.2025v18i7.54934.
16. Bhosale VB, Akshada Amit Koparde, Vandana M Thorat, Mayuri V Bhosale. Network pharmacology and molecular docking based investigation of *Calotropis gigantea Linn*. Leaf extract against vulvovaginal candidiasis. Asian J Pharm Clin Res. 2025;18(12):54-66. doi: 10.22159/ajpcr.2025v18i12.56803.
17. Adhish M, Madhan B, Manjubala I. Exploring capsaicin as a multi-target agent for osteoporosis through computational insights. In Silico Pharmacol. 2025;13(2):112. doi: 10.1007/s40203-025-00400-x, PMID 40761847.
18. Bibi M, Baboo I, Majeed H, Kumar S, Lackner M. Molecular docking of key compounds from *Acacia* honey and *Nigella sativa* oil and experimental validation for colitis treatment in albino mice. Biology (Basel). 2024;13(12):1035. doi: 10.3390/biology13121035, PMID 39765702.
19. Li Y, Yang JM, Cui WH, Wang JK, Chen X, Zhang C et al. Prediction of active ingredients and mechanism of Siwei Jianbu decoction in the treatment of atherosclerosis by network pharmacology. Eur Rev Med Pharmacol Sci. 2022;26(15):5436-46. doi: 10.26355/eurrev_202208_29412, PMID 35993639.
20. Giordano D, Biancaniello C, Argenio MA, Facchiano A. Drug design by pharmacophore and virtual screening approach. Pharmaceuticals (Basel). 2022 May 23;15(5):646. doi: 10.3390/ph15050646, PMID 35631472.
21. Chaturvedi SK, Ahmad E, Khan JM, Alam P, Ishtikhar M, Khan RH. Elucidating the interaction of limonene with bovine serum albumin: a multi-technique approach. Mol Biosyst. 2015;11(1):307-16. doi: 10.1039/c4mb00548a, PMID 25382435.
22. Alhazmi HA, Al Bratty M, Meraya AM, Najmi A, Alam MS, Javed SA et al. Spectroscopic characterization of the interactions of bovine serum albumin with medicinally important metal ions: platinum (IV), iridium (III) and iron (II). Acta Biochim Pol. 2021;68(1):99-107. doi: 10.18388/abp.2020_5462, PMID 33596034.
23. Colombo G, Clerici M, Garavaglia ME, Giustarini D, Rossi R, Milzani A et al. A step-by-step protocol for assaying protein carbonylation in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci. 2016;1019:178-90. doi: 10.1016/j.jchromb.2015.11.052, PMID 26706659. jchromb.2015.11.052.
24. Coligan JE, Dunn BM, Speicher DW, Wingfield PT, editors. Current protocols in protein science. Hoboken, NJ, USA: John Wiley & Sons, Inc. 2001. doi: 10.1002/0471140864.
25. Emami L, Khodarahimi E, Mardaneh P, Khoshnoud MJ, Rashedinia M. Binding interaction of sodium benzoate, potassium sorbate and sodium dihydrogen citrate with BSA as food preservatives: *in vitro* analysis and computational studies. Sci Rep. 2024;14(1):29237. doi: 10.1038/s41598-024-80642-5, PMID 39587338.
26. Huang G, Zhu F, Chen Y, Chen S, Liu Z, Li X, et al. A spectrophotometric assay for monoamine oxidase activity with 2, 4-dinitrophenylhydrazine as a derivatized reagent. Anal Biochem. 2016;512:18-25. doi: 10.1016/j.ab.2016.06.020, PMID 27503749. ab.2016.06.020.
27. Mayank JV, Jaitak V. Interaction model of steviol glycosides from *Stevia rebaudiana* (Bertoni) with sweet taste receptors: a computational approach. Phytochemistry. 2015;116:12-20. doi: 10.1016/j.phytochem.2015.05.006. PMID 26021732.
28. Raj B, Thomas SM, Varghese S, Ruba Priya MG, John S, Ramakrishna P et al. A Mini-Review on Molecular Docking Studies and Pharmacological Activities of Stevia rebaudiana. Asian J Chem. 2021;33(12):2919-23. doi: 10.14233/ajchem.2021.23393.
29. Tabernero A, Granda B, Medina A, Sánchez-Abarca LI, Lavado E, Medina JM. Albumin promotes neuronal survival by increasing the synthesis and release of glutamate. J Neurochem. 2002;81(4):881-91. doi: 10.1046/j.1471-4159.2002.00843.x. PMID 12065647.
30. Tanti GK, Goswami SK. SG2NA recruits DJ-1 and Akt into the mitochondria and membrane to protect cells from oxidative damage. Free Radic Biol Med. 2014;75:1-13. doi: 10.1016/j.freeradbiomed.2014.07.009, PMID 25035075.
31. Dańkowska K, Nesterowicz M, Lauko KK, Trocka D, Żendzian-Piotrowska M, Ładny JR et al. *In vitro* and *in silico* studies and a systematic literature review of antiglycation properties of amlodipine. Sci Rep. 2025;15(1):33277. doi: 10.1038/s41598-025-18925-8, PMID 41006534.
32. Barca-Mayo O, Lu QR. Fine-tuning oligodendrocyte development by microRNAs. Front Neurosci. 2012;6:13. doi: 10.3389/fnins.2012.00013, PMID 22347159.
33. Peng X, Yao D, Pan Y, Yu Q, Ni S, Bian H et al. Study on the structural changes of bovine serum albumin with effects on polydatin binding by a multitechnique approach. Spectrochim Acta A Mol Biomol Spectrosc. 2011;81(1):209-14. doi: 10.1016/j.saa.2011.06.003, PMID 21723188.
34. Uzbekov MG. Monoamine oxidase as a potential biomarker of the efficacy of treatment of mental disorders. Biochemistry (Mosc). 2021 Jun;86(6):773-83. doi: 10.1134/S0006297921060146, PMID 34225599.

35. Meyer JH, Ginovart N, Boovariwala A, Sagrati S, Hussey D, Garcia A et al. Elevated monoamine oxidase A levels in the brain: an explanation for the monoamine imbalance of major depression. Arch Gen Psychiatry. 2006;63(11):1209-16. doi: 10.1001/archpsyc.63.11.1209, PMID 17088501.
36. Beucher L, Gabillard-Lefort C, Baris OR, Mialet-Perez J. Monoamine oxidases: a missing link between mitochondria and inflammation in chronic diseases? Redox Biol. 2024 Nov;77:103393. doi: 10.1016/j.redox.2024.103393. PMID 39405979.
37. Troubat R, Barone P, Leman S, Desmidt T, Cressant A, Atanasova B et al. Neuroinflammation and depression: a review. Eur J Neurosci. 2021 Jan;53(1):151-71. doi: 10.1111/ejn.14720, PMID 32150310.