

Apoptotic: *In-silico* and *In-vitro* Study on Alzheimer's Marker, and Bioactive Derived from *Benincasa hispida* using Gas Chromatography-Mass Spectroscopy

Pooja RC

Sri Adichunchanagiri College of Pharmacy <https://orcid.org/0000-0003-2625-3711>

Dr. Bharathi DR

rambha.eesh@gmail.com

Sri Adichunchanagiri College of Pharmacy <https://orcid.org/0000-0002-8153-2839>

Research Article

Keywords: Benincasa hispida, Preliminary screening, In-vitro assay, In-silico molecular docking simulation

Posted Date: June 6th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4531379/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Purpose: Molecular docking is a viable and useful method to develop new herbal therapies to fulfill different therapeutic needs. One of the biggest issues is the lack of understanding of the processes underlying the common use of herbs. **Method:** Potential binding sites between the different ligands and the target protein were loaded using Schrodinger software, which also predicts protein-ligand interactions. The forty (40) molecules of *Benincasa hispida* with Apoptosis and Acetylcholine targets were carried out so, as to evaluate their theoretical binding affinities. The chemical structure was predicted from gas-chromatography (GC-MS), National Institute of Standards and Technology (NIST) library (2014). Molecule 40 (M40) with the highest binding affinity of -10 kcal/mol was observed. In-silico ADME and drug-likeness prediction of the molecules showed good pharmacokinetic properties having high gastrointestinal absorption, orally bioavailable, and less toxic.

Results: Phytochemical screening, *In-vitro* analysis free radical scavenging assay (DPPH, ABTS, H₂O₂, and FRAP). Phytochemical screening showed the presence of flavonoids, terpenoids, Phenols, sterols, and glycosides was observed in the preliminary test. In the scavenging assay, Ascorbic acid showed regression values DPPH ($R^2=0.9746$), ABTS ($R^2=0.9865$), H₂O₂ ($R^2=0.988$), and FRAP ($R^2=0.9834$).

Conclusion: The outcome of the present research strengthens the relevance of these compounds as promising lead candidates for the treatment of multidrug-resistant Alzheimer's disease which could help medicinal chemists and pharmaceutical professionals in further designing and synthesis of more potent drug candidates. Moreover, the research also encouraged the *in-vivo* and *in-vitro* evaluation study for the proposed designed compounds to validate the computational findings.

1 Introduction

Alzheimer's disease is a degenerative neurological disorder that is commonly associated with cognitive impairment and memory loss worldwide. The sickness has a long and gradual course, starting with the pathophysiological features of AD [1]. AD, which includes extracellular amyloid-beta plaques, intracellular hyperphosphorylated tau protein tangles (NFTs), inflammation, mitochondrial dysfunction, oxidative stress, and excitotoxic cell death, also causes an abnormal apoptotic cascade in susceptible brain regions (cerebral cortex, hippocampus) and neurodegeneration, which may be caused by uncontrolled activation of microglia in the brain, resulting in the secretion of neurotoxins and inflammatory factors [2]. The epidemiology of Alzheimer's disease and its course, and the presence of amyloid plaques and neurofibrillary tangles is still necessary for a diagnostic diagnosis. As Alzheimer's disease advances, more neuropsychiatric symptoms may appear, such as confusion, disorientation, mood changes, aggression/agitation, and, in later stages, delusion/hallucination. [3]. It is frequently challenging to differentiate between cognitive decline brought on by AD and normal aging-related declines (such as reductions in processing speed and specific memory, language, visuospatial, and executive function abilities). This is because normal aging entails subtle cognitive deterioration [4]. Rising prevalence, incidence, and mortality are indicators of AD's global toll, as are low AD estimates resulting from

underdiagnosis. From early neurodegeneration to MCI, and from normal cognition without amyloid-beta (Ab) deposition, the probability of AD dementia rises. According to estimates, the lifetime risk of AD dementia for individuals with Ab accumulation and neurodegeneration is 33.6% for males and 41.9% for women [5]. When apoptosis operates in an abnormal and disorderly way, it can lead to several illnesses, including neurodegenerative symptoms. Apoptosis is a predefined and regulated process that governs various molecular processes [6]. At these sites, the apoptotic actors affect the mitochondria and endoplasmic reticulum, as well as trophic factors and several pathways, such as PI3K/AKT, JNK, MAPK, and mTOR signaling [7]. Abnormal neuronal loss is the final step in a dysregulated apoptotic cascade. This is a major event that may occur before other events of AD development and is closely correlated with the severity of dementia [8]. Natural substances with putative Alzheimer's disease features have recently attracted interest in the scientific community [9]. Known by many names as wax gourd, *Benincasa Hispida* is a popular vegetable that has some therapeutic uses. Cucurbitaceae is the family to which it belongs. The ability to resist oxidation varies throughout wax gourd components, including the peel, pulp, and core; fresh seeds seemed to have the most antioxidant activity [10]. Sanskrit texts claim that it is beneficial for a variety of conditions, including insanity, nerve problems, dyspepsia, and epilepsy. To find anti-inflammatory, antioxidant, and anti-convulsant qualities, several scientific studies have been carried out [11]. These seeds are mostly composed of triterpenoids, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, β -sitosterol, and uronic acid [12]. The work was specifically conducted to better grasp the clear outline, phytochemical screening, bioactive molecule characterization, and computerized in-silico docking simulations. The goal of this research is to improve food security and public health by learning more about *Benincasa hispida* so that it may be valued and used sustainably. As a result, there is still a need to investigate and confirm its nature in research and everyday traditional healthcare.

2 Methodology

2.1 Material

Chemical and reagents Dilute iodine solution, Wagner's reagent, Dragendorff's reagent, zinc powder, hydrochloric acid, sodium hydroxide solution, ferric chloride, Molisch's reagent, chloroform, gelatine, 10% lead acetate solution, methanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis 3-Ethylbenzothiazoline-6- sulfonic acid (ABTS), Potassium persulfate, hydrogen peroxide solution, 50 mM phosphate buffer, Ascorbic acid (AA), 1% potassium ferricyanide, 10% Trichloro acetic acid (TCA), 0.1% ferric chloride, sigma Aldrich.

2.2 Authentication of specimen

Benincasa hispida fruit seed was collected in an area of the Nelamangala market in the month of May. The specimen was authenticated by a Pharmacognosiste professor Dr. Nandeesh, Sree Siddaganga College of Pharmacy, B.H. Road, Tumkur. specimen number SSCP/No/15/22.

2.3 Preparation of plant specimen

The fresh 200g fruit seed of *Benincasa hispida* was ground using a mixer. The collected homogenate paste was mixed with (70:20 v/v) Hydroethanolic solvent for 72 hours by cold maceration process. The homogenate was filtered via muslin cloth followed by Whatman No. 1 filter paper and lyophilized to obtain a dry powder of seed extract. The lyophilized powder extract was stored in air-tight amber-color bottles, Yield percentage (w/w) from the dried extracts was calculated [13].

2.4 Phytochemical screening test

The preliminary phytochemical analysis is done to verify the presence or absence of carbohydrates, protein, amino acids, alkaloids, glycosides, terpenoids, and Quantitation of total phenolic, flavonoid content, and tannic acid [14].

3 Methods

3.1 Qualitative and quantitative screening of phytochemicals

3.2 Estimation of Total Phenolic Content (TPC)

Briefly, 20 μ L of *Benincasa hispida* (10 mg/mL) was ethanol to 5.0 mL with distilled water, and 0.5 mL of 1:1 FC reagent was added to this solution. After 5 minutes of incubation, 5.0 mL of 7% sodium carbonate was added and the mixture was incubated for 30 minutes at room temperature and then the absorbance was measured at 765 nm. Gallic acid was used as a 3 standard. The results were expressed as μ g of gallic acid equivalents per milligram of plant material (μ g GAE/mg plant material) [15].

3.3 Estimation of Total Flavonoid Content (TFC)

Briefly, 20 μ L of *Benincasa hispida* (10 mg/mL) was diluted to 5.0 mL with distilled water and then 0.1 mL of 10% AlCl_3 and 0.1 mL of 1 M potassium acetate were added. This mixture was incubated at 37°C for 40 minutes and the absorbance was measured at 415 nm. Quercetin was used as a standard, and the flavonoid concentration in *Benincasa hispida* was calculated. The results were expressed as μ g of quercetin equivalents per milligram of plant material (μ g QE/mg plant material) [15].

3.4 Estimation of Total Tannin Content (TTC)

Briefly, 0.1 ml of the *Benincasa hispida* extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin- Ciocalteu phenol reagent, 1 ml of 35% sodium carbonate solution, and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 μ g/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 700 nm with a UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample [15].

4 In-vitro screening

4.1 DPPH Radical Scavenging

The reaction mixture consisted of 0.5 mL of *Benincasa hispida*, 3 mL of methanol, and 0.3 mL of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in ethanol. After incubation for 45 min, absorbance was determined in a spectrophotometer at 517 nm [16].

4.2 ABTS Radical Scavenging

ABTS assay, of equal amounts of 7.4 mM ABTS and 2.45 mM potassium persulfate was going to prepare a working solution that is put away in 12-13 h dark incubation to react and produce active ABTS radical cation which is the reaction solution with *Benincasa hispida* sample to estimate antioxidant activity. Ethanol was used for making the dilution of the ABTS solution. Then, 50 µL of samples and 1.9 ml of ABTS solution were transferred into a test tube and allowed to dark incubate for 6 mines. After incubation, absorbance was recorded at 734 nm [16].

4.3 H₂O₂ Radical Scavenging

H₂O₂ assay, twenty millimolars of hydrogen peroxide solution were prepared in 50 mM phosphate buffer (pH 7.4). 0.4 mL of *Benincasa hispida* or ascorbic acid with varying concentrations (0-10 µg/mL) were mixed with 0.6 mL of hydrogen peroxide solution. After incubation for 10 minutes at room temperature, the absorbance was measured against the blank at 240 nm. The blank consisted of phosphate buffer and test *Benincasa hispida* without hydrogen peroxide [17].

4.4 FRAP Radical Scavenging

FRAP assay, 1 mL of *Benincasa hispida* with different concentrations (0-10 ug/mL) was mixed with 2.0 mL of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The *Benincasa hispida* was incubated at 50°C for 20 minutes followed by the addition of 2.5 ml of 10% TCA. The reaction mixture was centrifuged at 3000 × g for 10 minutes 1.25 ml supernatant from each sample was mixed with 1.25 ml of distilled water and 0.5 ml of 0.1% ferric chloride. Absorbances were measured at 700 nm. Ascorbic acid (AA) was used as a standard [17].

4.5 Gas Chromatography-Mass Spectroscopy Analysis

The Clarus 680 GC was used in the analysis employing a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm), and 4 the components were separated using Helium as carrier gas at a constant flow of 2 mL/min. The injector temperature was set at 280°C during the chromatographic run. The 1 µL of extract sample was injected into the instrument the oven temperature was as follows: 100°C (2 min); followed by 200°C at the rate of 10 per min and 200°C, where it was held for 3min and then followed by 300°C at the rate of 25 per min; it was held for 10 min. The mass detector conditions were an Inlet line temperature of 250°C; an ion source temperature of 230°C; an ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan interval of 0.1 sec. The

fragments from 40 to 440 Da. The spectrums of the components were compared with the database of a spectrum of known components stored in the GC-MS NIST library (2014) [18].

4.6 *In-silico* molecular docking simulation

Preparation of Ligands: From the literature, we selected the set of forty phytoconstituents structures, known for their brain stimulant action. The phytoconstituents were extracted in their 2D conformation from PubChem and were in SDF format. Donepezil was used as the reference standard, as the first line of treatment for various neurological problems. Retrieval of Protein Structure and Preparation: The X-ray-co-crystallized structures of the protein molecules were chosen from the Protein data bank, and the receptor file was saved in “Mol2” format. The Schrodinger software was used to load the potential binding sites between the various ligands and the target protein. Prediction of protein-ligand interactions is performed. software. The docking results were then compared with the docked result of the reference ligand obtained from the corresponding PDB ID. The docking scores, 2D pose views, as well as the binding affinities of the selected natural compounds, and verifying ADME / Lipinski rule 5, were analyzed for further investigation. The best-docked phytoconstituent for neuroprotective action was identified based on binding energy and interaction with amino acid residues [19].

4.7 Statistical analysis

All the values are expressed as mean±SEM (n=6) statistical analysis by one-way ANOVA followed by Tukey’s test.

5 Results

5.1 Phytochemical screening test

Table: 1 Phytochemical screening of *Benincasa hispida*

Sl. No	Test for Phytochemicals	Hydroethanolic extract (70%)
1	Carbohydrates	
	(a) Molisch's test	Absence
	(b) Fehling's test	Absence
2	Reducing sugars	
	Benedict's test	Absence
3	Glycosides	
	(a) Liebermann's test	presence
	(b) Keller-Kiliani's test	presence
4	Terpenoids	
	(a) Acetic anhydride test	presence
5	Phenolic compounds	
	(a) Ferric chloride test	presence

5.2 Quantitative estimation of TPC, TFC, and TTC of extracts *Benincasa hispida*

Table 02: Quantitative estimation of TPC and TFC, TTC of extracts *Benincasa hispida*

<i>Benincasa hispida</i>	Total phenolic (mg/g)	Total flavonoids (mg/g)	Total Tannic acid (mg/g)
Hydroethanolic	32.97 ± 0.12	21.72 ± 0.06	31.82 ± 0.06

5.3 Quantitative estimation of TPC, TFC, and TTC of extracts *Benincasa hispida*

5.4 In-vitro radical scavenging activity Assay

Table 03: In-vitro radical scavenging activity Assay

<i>Benincasa hispida</i>	DPPH	ABTS	H ₂ O ₂	FRAP
Hydroethanolic	34.9 ± 0.32	24.68 ± 0.08	46.70 ± 0.13	17.74 0.10

5.5 In-vitro radical scavenging activity

5.6 Gas Chromatography-Mass Spectroscopy analysis of *Benincasa hispida*

5.7 IN-SILICO DOCKING SIMULATION

5.7.1 Bax Pro-Apoptotic

5.7.2 Acetylcholinesterase

5.7.3 The interaction of Pro-apoptosis BAX protein on *Benincasa hispida* ligands

5.7.4 The interaction of Acetylcholine protein on *Benincasa hispida* ligands

6 DISCUSSIONS

The collected plant samples were identified and standardized by using the pharmacological method and prepared Hydroethanolic extract of *Benincasa hispida* qualitative phytochemical and qualitative analysis like TPC, TFC, TTC, and antioxidant activity (DPPH, ABTS, H₂O₂, and FRAP). Qualitative assay of phytochemicals: Phytochemical extract of *Benincasa hispida* (hydroethanolic) reveals the presence of tannic, phenols, flavonoids, glycosides, and terpenoids, while free anthraquinone. The concentration of phytochemicals was determined visually by the intensity of the color. Phenolic, flavonoid, and tannic compounds, are well known for their antioxidant properties, these secondary metabolites neutralize free radicals by donating hydrogen and terminating the chain for the presence of polyphenolic in the leaves of *Benincasa hispida* the TPC of HEE *Benincasa hispida* was found to be $32.97 \pm 0.12 \mu\text{g GAE/g}$. The TPC of the extract was calculated from the regression equation of the calibration curve ($R^2=0.9743$). The TFC of the extract was calculated from the regression equation of the calibration curve ($R^2=0.9912$), and flavonoid content was determined to be $21.72 \pm 0.06 \text{ mg QE/g}$. and TTC of the extract was calculated from the regression equation of the calibration curve ($R^2=0.9903$), tannic content was determined to be $31.82 \pm 0.06 \text{ mg TAE/g}$. Determination of antioxidant potential: DPPH Radical: Antioxidant activity of the *Benincasa hispida* is measured by the standard curve of AA (fig: 02 & Table 03). The absorbance was observed with both HEE *Benincasa hispida* (34.9 ± 0.32) and AA ($R^2=0.9746$). the results were expressed by mmol AA E/g. Scavenging activity of ABTS Radical: The absorbance was observed with both HEE *Benincasa hispida* (24.68 ± 0.08) and AA ($R^2=0.9865$). which was comparable to that of standard AA. (fig: 02 & Table 03). the results were expressed by mmol AA E/g. H₂O₂ Assay: The effect of HEE on H₂O₂ was investigated, and AA served as standard. The absorbance was observed with both HEE *Benincasa hispida* (46.70 ± 0.13) and AA ($R^2=0.988$). which was comparable to that of standard AA. (fig: 02 & Table 03). the results were expressed by mmol AA E/g. FRAP Assay- antioxidants neutralize free radicals by donating an electron. The higher the reducing powder, the greater the absorbance of the reaction mixture. which was comparable to that of standard AA. (fig: 02 & Table 03). A concentration-dependent increase in absorbance was observed with both HEE *Benincasa hispida* (17.74 ± 0.11) and AA ($R^2=0.9834$). This observation reflects the HEE's ability to donate electrons, which may be a major contributor to neutralizing free radicals. Bioactive components identified in the Hydro-ethanol extract of *Benincasa hispida* by GC-MS: Glutaric acid, Sebacic acid, 3-methylbut-2-yl undecyl ester, 2-methylpent-3-yl undecyl ester, 4-methylhept-3-yl undecyl ester, 3-hexyl undecyl ester, 3,3-dimethylbut-2-yl undecyl ester, 4-methylpent-2-yl undecyl ester, Adipic acid, 3-heptyl undecyl ester, 2-(4-bromophenoxy) ethyl hexyl ester, Spiro-[4H-1,3-benzodioxin,2,1'-[2,5] cyclohexadiene]-2' carboxylic acid, 6,8-dichloro-5-hydroxy-6'-methoxy-7-methyl-4,4'-dioxo-, methyl ester, Phthalic acid, 4-Bromobenzoic acid, Propan-1-one, 3-(4-chlorophenyl)-3-(4-chlorophenylthio)-1-(3,4-dimethylphenyl), α -Mono-n-butylaminomethyl-2,6-di[p-chlorophenyl]-4-pyridinemethanol, Trans-1-Benzyl-2,4-diphenyl-5-benzoyl-2-imidazoline, Benzoic acid

(silver (1+) salt), Triphosphoric acid, 3-Bromobenzoic acid, Isopentendrone, 7,8-Diazabicyclo[4.2.2]deca-2,4,7-trien-7-oxide, 2H-[1,2,4]Triazole-3-sulfonic acid benzyl-methyl-amide, 2-Aminohydratropic acid, 1-Propanol, 3-(ethylthio), 2-[3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl] aniline, Benzenemethanamine, 3-Amino-1-chloro-4-phenyl-2-butanone, N-(2-Phenylpropan-2-yl) acetamide, Propane, 1-[(1-methyl ethyl)thio], Benzamidine, Hydrazine carboxamide, 2-(phenyl methylene), 3-Pyridinecarbonitrile, 1,4-dihydro-1-methyl, 2-Aminohydratropic acid, p-Toluic acid, 2-phenylethyl ester, Hydrazine carboxamide, 2-(phenylethylene), 1,2-Naphthalenediol, 1,2,3,4-tetrahydro, Pyridine, 5-ethenyl-2-methyl, m-Toluic acid, 2-phenylethyl ester, o-Toluic acid, 2-phenylethyl ester. Interaction between pro-apoptotic and acetylcholine protein: Interaction between pro-apoptotic and acetylcholine protein/In-silico ADME/Pharmacokinetic Predictions: 2-amino benzamide: ASP113;(2S,3S,4S,5R)-2,3,4,5-Tetrahydroxy-6-Oxohexanamide: THR14, PRO13, GLY11, GLY10, PRO8, Isopropenylpyrazine: Gly11, Gly10; 2-Aminobenzamide: Gly10, Gly11, Pro13; Tetrahydropalmatine: Typ86, Typ337, Phe338, Tyr341. 4-[Bis(4-chlorophenyl) methylsulfanyl]-4-phenylbutan-2-one: Asp74, Gly121, Tyr341, Phe295; Thiadiazole derivative: Phe295, Trp286.

7 CONCLUSIONS

In the present research, Bioactive compounds play an important role in blocking free radical generation. Mainly phenolic compounds are involved in antioxidant properties. Not only phenolic compounds but other phytochemicals also influence antioxidant activity. Like glycoside has therapeutic value in the treatment of inherited deficiency in man. This study reveals that polyphenolic compounds can influence antioxidants but, other phytochemicals are also important in antioxidant activity. Furthermore, we have observed in a phytochemical screening of HEE in *Benincasa hispida* TPC, TFC, and TTC among them TPC & and TTC values of these plants fall in the vicinity of the highest value. On the other hand, through the DPPH, ABTS, FRAP, and H₂O₂ among these antioxidant assays, we have observed from the results that H₂O₂ and DPPH have the highest antioxidant activity, and ABTS, FRAP has the modest anti-oxidative activity. Additionally, a GC-MS investigation of a hydro-ethanolic extract from *Benincasa hispida* found 40 distinct chemicals that may have useful medicinal qualities. Therefore, increasing future activities on this plant is imperative.

ABBREVIATIONS

BH: *Benincasa hispida*; **HEE:** Hydroethanolic extract; **TPC:** Total phenolic content; **TFC:** Total flavonoid content; **TTC:** Total tannic acid content; **FRS:** Free radical scavenging activity; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** (2,2'-azino-bis 3-Ethylbenzothiazoline-6- sulfonic acid); **H₂O₂:** Hydrogen peroxide scavenging; **TCA:** Trichloro acetic acid, **NaOH:** Sodium hydroxide; **FC:** Folin– Ciocalteu; **GC-MS:** Gas chromatography-mass spectroscopy; **PDB:** Protein data bank; **NIST:** National institute of standard and technology; **MF:** Molecular formula; **MW:** Molecular weight; **RB:** Rotatable bond; **DS:** Docking Score; **Logp:** Lipophilicity; **RMSD:** Rotatable Mean Square Deviation.

Declarations

ACKNOWLEDGMENT

My Humble Pranamias to the honorable chancellor of Adichunchanagiri university for providing the best facility for research. My deep to all officials of Adichunchanagiri university and the faculty of pharmacy

UNIQUENESS OF THE RESEARCH: Although there is limited proof, previous study suggests that this plant possesses anti-inflammatory and antioxidant properties. Moreover, the ongoing research effort is yet unfinished. Thus, after giving it some thought, we have decided to concentrate on this species.

CONFLICT OF INTEREST: The authors declare that we have no competing financial investments or personal relationships. Which can influence the results of the study and do not have any conflict with any other research work.

REFERENCE

1. Jicha, G. A., & Carr, S. A. (2010). Conceptual evolution in Alzheimer's disease: implications for understanding the clinical phenotype of progressive neurodegenerative disease. *Journal of Alzheimer's disease : JAD*, 19(1), 253–272. <https://doi.org/10.3233/JAD-2010-1237>
2. Sharma, V. K., Singh, T. G., Singh, S., Garg, N., & Dhiman, S. (2021). Apoptotic Pathways and Alzheimer's Disease: Probing Therapeutic Potential. *Neurochemical research*, 46(12), 3103–3122. <https://doi.org/10.1007/s11064-021-03418-7>
3. Tahami Monfared, A. A., Byrnes, M. J., White, L. A., & Zhang, Q. (2022). Alzheimer's Disease: Epidemiology and Clinical Progression. *Neurology and therapy*, 11(2), 553–569. <https://doi.org/10.1007/s40120-022-00338-8>
4. Haller, S., Montandon, M. L., Rodriguez, C., Ackermann, M., Herrmann, F. R., & Giannakopoulos, P. (2017). *APOE*E4* Is Associated with Gray Matter Loss in the Posterior Cingulate Cortex in Healthy Elderly Controls Subsequently Developing Subtle Cognitive Decline. *AJNR. American journal of neuroradiology*, 38(7), 1335–1342. <https://doi.org/10.3174/ajnr.A5184>
5. Kivisäkk, P., Carlyle, B. C., Sweeney, T., Trombetta, B. A., LaCasse, K., El-Mufti, L., Tuncali, I., Chibnik, L. B., Das, S., Scherzer, C. R., Johnson, K. A., Dickerson, B. C., Gomez-Isla, T., Blacker, D., Oakley, D. H., Frosch, M. P., Hyman, B. T., Aghvanyan, A., Bathala, P., Campbell, C., ... Arnold, S. E. (2023). Plasma biomarkers for diagnosis of Alzheimer's disease and prediction of cognitive decline in individuals with mild cognitive impairment. *Frontiers in neurology*, 14, 1069411. <https://doi.org/10.3389/fneur.2023.1069411>
6. Xu, X., Lai, Y., & Hua, Z. C. (2019). Apoptosis and apoptotic body: disease message and therapeutic target potentials. *Bioscience reports*, 39(1), BSR20180992. <https://doi.org/10.1042/BSR20180992>
7. Sharma, V. K., Singh, T. G., Singh, S., Garg, N., & Dhiman, S. (2021). Apoptotic Pathways and Alzheimer's Disease: Probing Therapeutic Potential. *Neurochemical research*, 46(12), 3103–3122. <https://doi.org/10.1007/s11064-021-03418-7>

8. Hadian, K., & Stockwell, B. R. (2023). The therapeutic potential of targeting regulated non-apoptotic cell death. *Nature reviews. Drug discovery*, 22(9), 723–742. <https://doi.org/10.1038/s41573-023-00749-8>
9. Kumari, N., Anand, S., Shah, K., Chauhan, N. S., Sethiya, N. K., & Singhal, M. (2023). Emerging Role of Plant-Based Bioactive Compounds as Therapeutics in Parkinson's Disease. *Molecules (Basel, Switzerland)*, 28(22), 7588. <https://doi.org/10.3390/molecules28227588>
10. Islam, M. T., Quispe, C., El-Kersh, D. M., Shill, M. C., Bhardwaj, K., Bhardwaj, P., Sharifi-Rad, J., Martorell, M., Hossain, R., Al-Harrasi, A., Al-Rawahi, A., Butnariu, M., Rotariu, L. S., Suleria, H. A. R., Taheri, Y., Docea, A. O., Calina, D., & Cho, W. C. (2021). A Literature-Based Update on *Benincasa hispida* (Thunb.) Cogn.: Traditional Uses, Nutraceutical, and Phytopharmacological Profiles. *Oxidative medicine and cellular longevity*, 2021, 6349041. <https://doi.org/10.1155/2021/6349041>
11. Wang, Q., Yang, X., Zhu, C., Liu, G., Han, W., Sun, Y., & Qian, L. (2022). Valorization of Polysaccharides From *Benincasa hispida*: Physicochemical, Moisturizing, and Antioxidant Skincare Properties. *Frontiers in pharmacology*, 13, 912382. <https://doi.org/10.3389/fphar.2022.912382>
12. Doshi, G. M., Nalawade, V. V., Mukadam, A. S., Chaskar, P. K., Zine, S. P., Somani, R. R., & Une, H. D. (2015). Structural elucidation of chemical constituents from *Benincasa hispida* seeds and *Carissa congesta* roots by gas chromatography: Mass spectroscopy. *Pharmacognosy research*, 7(3), 282–293. <https://doi.org/10.4103/0974-8490.157179>
13. Bimakr, M., Rahman, R. A., Taip, F. S., Adzahan, N. M., Sarker, M. Z., & Ganjloo, A. (2013). Supercritical carbon dioxide extraction of seed oil from winter melon (*Benincasa hispida*) and its antioxidant activity and fatty acid composition. *Molecules (Basel, Switzerland)*, 18(1), 997–1014. <https://doi.org/10.3390/molecules18010997>
14. Babu, S. C., Ilavarasan, R., Refai, M. S. T., Thameemul-Ansari, L. H., & Kumar, D. A. (2003). Preliminary pharmacological screening of *Benincasa hispida* Cogn. *Journal of Natural Remedies*, 143–147. <https://doi.org/10.18311/jnr/2003/154>
15. Phumat, P., Chaichit, S., Potprommanee, S., Preedalikit, W., Sainakham, M., Poomanee, W., Chaiyana, W., & Kiattisin, K. (2023). Influence of *Benincasa hispida* Peel Extracts on Antioxidant and Anti-Aging Activities, including Molecular Docking Simulation. *Foods (Basel, Switzerland)*, 12(19), 3555. <https://doi.org/10.3390/foods12193555>
16. Chaves, N., Santiago, A., & Alías, J. C. (2020). Quantification of the Antioxidant Activity of Plant Extracts: Analysis of Sensitivity and Hierarchization Based on the Method Used. *Antioxidants (Basel, Switzerland)*, 9(1), 76. <https://doi.org/10.3390/antiox9010076>
17. Doshi, G. M., Nalawade, V. V., Mukadam, A. S., Chaskar, P. K., Zine, S. P., Somani, R. R., & Une, H. D. (2015). Structural elucidation of chemical constituents from *Benincasa hispida* seeds and *Carissa congesta* roots by gas chromatography: Mass spectroscopy. *Pharmacognosy research*, 7(3), 282–293. <https://doi.org/10.4103/0974-8490.157179>
18. Thandivel, S., Rajan, P., Gunasekar, T., Arjunan, A., Khute, S., Kareti, S. R., & Paranthaman, S. (2024). *In silico* molecular docking and dynamic simulation of anti-cholinesterase compounds from the extract

Tables

Tables 4 to 5 are available in the Supplementary Files section

Figures

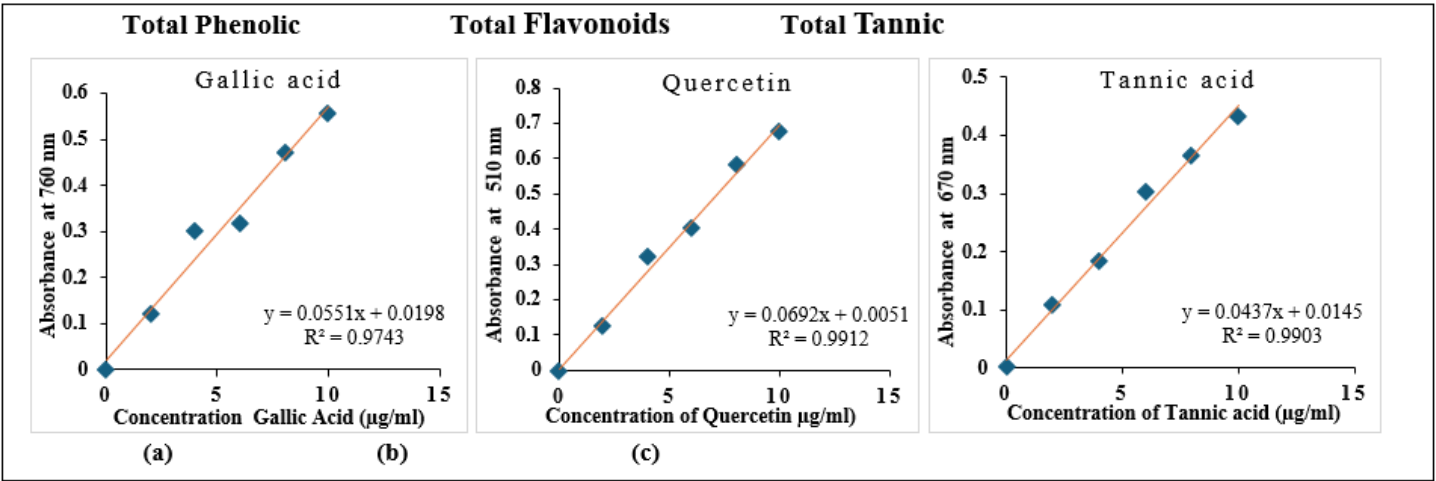


Figure 1

Standard Curve of Gallic acid, Quercetin, and Tannic acid

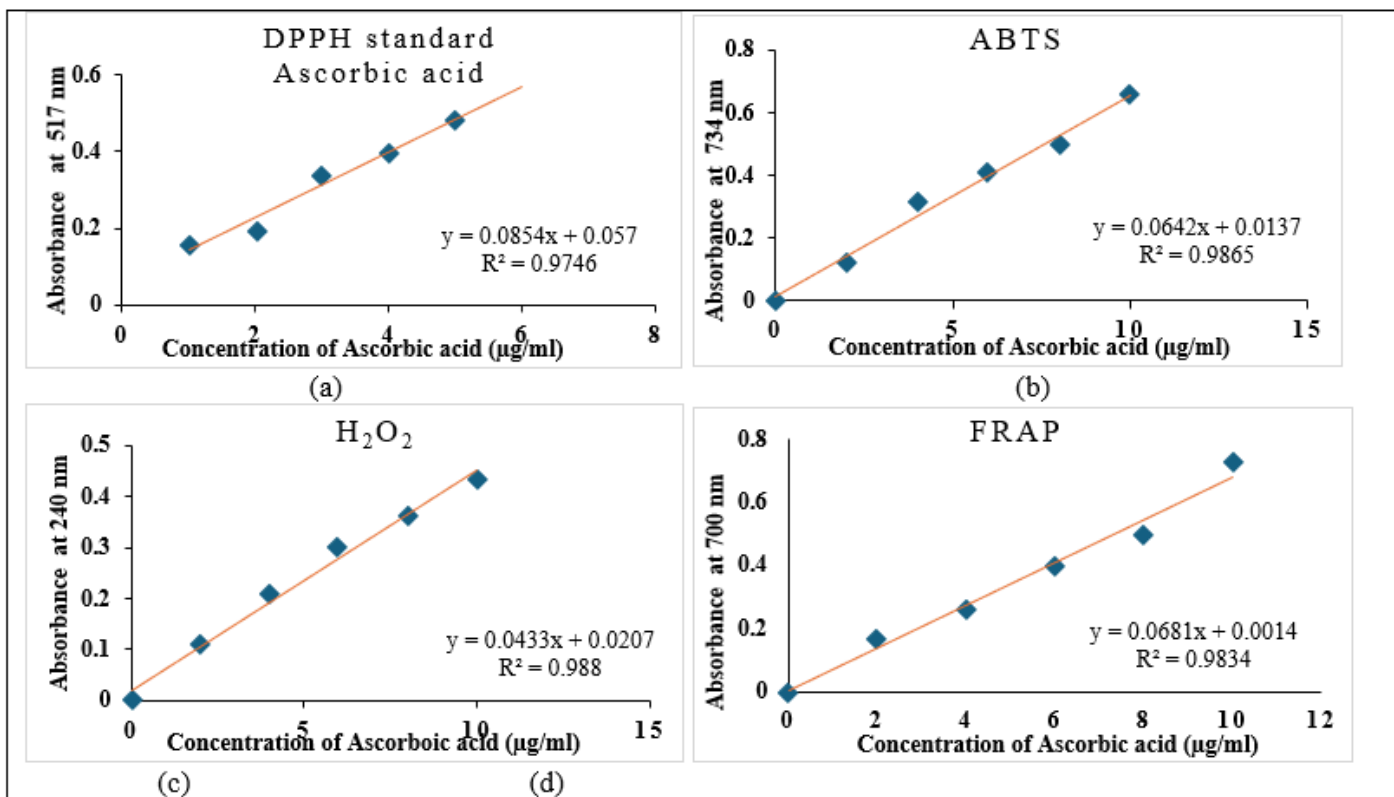


Figure 2

Radical scavenging activity Standard Curve of Ascorbic acids a, b, c, and d.

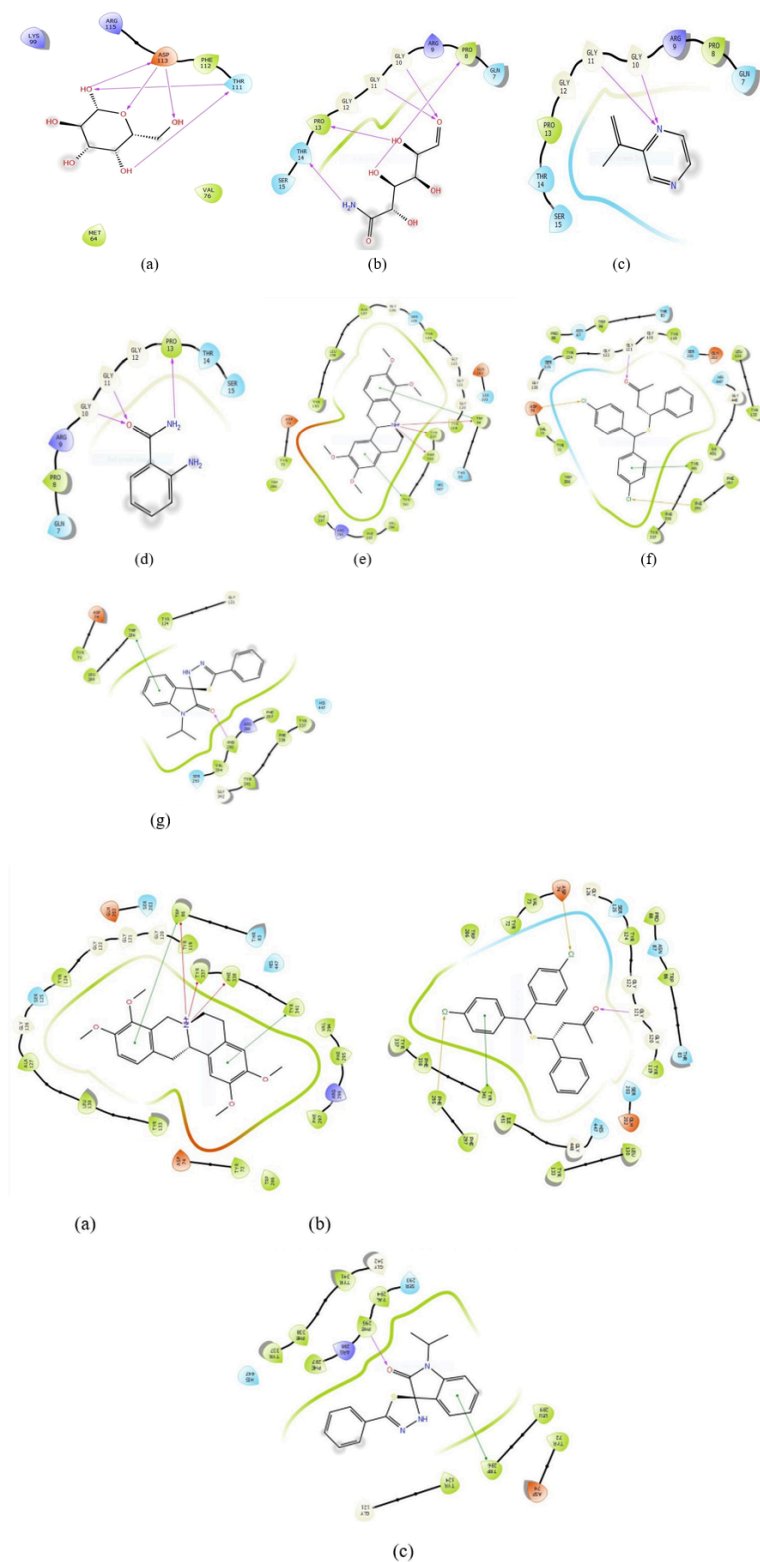


Figure 3

In-silico molecular docking stimulation on apoptotic and Acetylcholine targets.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Tables.docx](#)