



**INTERNATIONAL JOURNAL OF
PHARMACEUTICAL SCIENCES**
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: <https://www.ijpsjournal.com>



Research Article

DNA Barcoding, Phytochemical analysis, and Computational Insights into the Neuroprotective Potential of *Benincasa hispida*

Dr. Anuroopa Nadh*, Aswin Mohan, Rajesh Raju

Centre for Integrative Omics Data Science (CIODS), Yenepoya (Deemed to be University), Mangalore, India

ARTICLE INFO

Published: 14 Jan 2026

Keywords:

Benincasa hispida;
Alzheimer's disease;
Lipinski; Barcoding;
Docking; Network
pharmacology

DOI:

10.5281/zenodo.18245717

ABSTRACT

Benincasa hispida is a traditionally employed medicinal plant recognized for its diverse therapeutic benefits, including neuroprotective effects. The present study evaluated the binding affinity of phytochemicals derived from this plant against selected molecular targets of Alzheimer's disease (AD). Plant material was authenticated through morphological features and DNA barcoding. Phytochemical constituents were identified through literature survey and GC-MS analysis. Among 79 identified compounds, 74 satisfied Lipinski's criteria for oral bioavailability. These compounds were virtually screened against selected AD targets: acetylcholinesterase (AChE), β -secretase 1 (BACE1), gamma-aminobutyric acid receptor (GABABR), and glycogen synthase kinase-3 β (GSK3 β). Molecular docking results revealed that several compounds exhibit strong binding affinities across multiple targets than the controls. Notably, '9-octadecenoic acid' demonstrated the highest docking scores against AChE (133.87), BACE1 (159.36), and GABABR (154.74), whereas naringenin showed the strongest interaction with GSK3 β (88.57). Network pharmacology analysis revealed that BACE1 had the highest number of interacting phytochemicals (35), followed by GABABR with 21 compounds. AChE displayed the fewest interactions, involving 14 phytochemicals. Among the top-scoring compounds, 11 were found to interact with all three targets. Overall, these findings highlight *Benincasa hispida* as a promising source of neuroprotective agents that merit further exploration for the development of AD therapeutics.

INTRODUCTION

Benincasa hispida, commonly referred to as winter melon or ash gourd, is a traditionally employed medicinal plant with multitude of

therapeutic benefits known to be effective for many disorders, such as respiratory disease, neurological disorders, heart diseases, gastrointestinal problems, diabetes mellitus and

***Corresponding Author:** Dr. Anuroopa Nadh

Address: Centre for Integrative Omics Data Science (CIODS), Yenepoya (Deemed to be University), Mangalore, India

Email ✉: anuroopagnadh@yenepoya.edu.in

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.



urinary diseases. The phytopharmacological features of *Benincasa hispida* is listed in Table 1.

Table 1. Phytopharmacological information of *Benincasa hispida*

Name	<i>Benincasa hispida</i> (Thunb.) Cogn
Common Names	Ash gourd, Wax gourd
Family	Cucurbitaceae
Morphological features	Habit: Annual or perennial trailing herbaceous vine belonging to the family Cucurbitaceae. Stem: Soft, hairy, ridged, and branched Leaves: Large, simple, alternate, and broadly ovate to suborbicular. Tendrils: Slender, simple, unbranched, and spirally coiled, arising opposite the leaves. Flowers: Unisexual, solitary, axillary. Fruit: Large, fleshy, oblong to nearly spherical berry. Seeds: Numerous, obovate or compressed, pale yellowish to brownish.
Proven therapeutic activities	Antioxidant, Anti-Inflammatory, Antimicrobial, Antihelminthic, Anticancer, Gastroprotective, Antidiabetic, Antipyretic, Analgesic, Bronchodilator, Antihypertensive, Nephroprotective, Neuroprotective ^{1, 2}

Among the broad spectrum of therapeutic benefits, various parts of the plant, particularly the fruit have been specifically indicated for managing CNS disorders, including cognitive dysfunction, schizophrenia, epilepsy, and depressive disorders. In addition to these traditional claims, plant extracts have been scientifically validated for their neuroprotective effects to some extent. For instance, different solvent-based extracts confirmed anxiolytic activity². In addition to these findings, the fruit extract of *Benincasa hispida* has demonstrated protective effects in a colchicine-induced Alzheimer's disease (AD)³ and, the fruit

peel extract demonstrated anticonvulsant effect. Notably, treatment with the plant extract has been shown to restore acetylcholine, dopamine, and serotonin levels while reducing amyloid beta aggregation, which are considered key hallmarks of AD pathology⁴. Despite its well-documented ethnopharmacological relevance and demonstrated neuroprotective effects in experimental models, the capacity of its phytoconstituents to interact with critical molecular targets implicated in AD remains incompletely characterised. It has to be noted that, in silico methods are increasingly employed to elucidate potential interactions between disease-associated proteins and drug-like molecules^{5, 6}. In this context, the present study aimed to comprehensively evaluate the neuroprotective potential of phytochemicals derived from *Benincasa hispida* against AD targets using computational approaches.

Initially, the plant material was authenticated through morphological assessment and DNA barcoding to ensure accurate identification. Phytoconstituents were characterised via literature survey and gas chromatography–mass spectrometry (GC-MS) analysis. A combination of virtual screening, molecular docking, and network pharmacology approaches was subsequently applied to assess the binding affinities of these compounds against selected AD-related targets, including acetylcholinesterase (AChE), β -secretase 1 (BACE1), gamma-aminobutyric acid receptor (GABABR), and glycogen synthase kinase-3 β (GSK3 β).

2. MATERIALS AND METHODS

2.1 Plant authentication

Accurate and scientific method of authentication is essential for the species identification and further



evaluation of medicinal plants for their medicinal properties.

2.1.1 Morphological identification

The plant was collected from the botanical garden of the Department of Botany, University of Kerala, Trivandrum, and was identified and authenticated by the taxonomist of the same department. The leaf specimen of the plant along with the flowers was labelled and preserved on an herbarium sheet, and a corresponding herbarium voucher/reference number was assigned.

2.1.2. DNA Barcoding

After the identification based on the morphological characteristics the plant was further subjected to species-level identification using DNA barcoding. In this process, a short, conserved region of the *rbcL* gene was sequenced to determine related species. Fresh leaf samples the plant was collected and submitted for sequencing at the Rajiv Gandhi Centre for Biotechnology (RGCB), Trivandrum. Universal forward and reverse primers targeting the *rbcL* gene, RBCL-AF(ATGTCACCACAAACAGAGACTAAAGC) [12] and RBCL-724R (TCGCATGTACCTGCAGTAGC) [13], respectively were used for amplification. DNA was isolated using the NucleoSpin® Plant II Kit, and the quality of the extracts was assessed by agarose gel electrophoresis. Gels were visualized with a UV transilluminator (Genei), and images were captured using a Gel documentation system (Bio-Rad). Sanger sequencing was performed in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) employing the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), based on the manufacturer's protocol. The quality of the resulting sequences was evaluated using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and

editing were carried out in Geneious Pro v5.1 to assemble consensus sequences for each plant. The consensus sequence was compared to existing records using the Basic Local Alignment Search Tool (BLAST) [<https://blast.ncbi.nlm.nih.gov/Blast.cgi>] to identify related species. Later, the sequence was deposited in GenBank [<https://www.ncbi.nlm.nih.gov/genbank/>].

2.2. Phytochemical analysis

After authentication, the plant's secondary metabolites were identified through literature review and GC-MS analysis.

2.2.1. Literature analysis

A comprehensive literature search was conducted to compile previously reported phytochemicals from *Benincasa hispida*. The search strategy used the keywords “plant scientific name + secondary metabolites/phytochemicals/phytocompounds” in the PubMed database. Results were then filtered to include only studies reporting phytochemicals present specifically in the fruit.

2.2.2. GC-MS analysis

The collected samples were thoroughly washed with distilled water and shade-dried at room temperature. The dried material was crushed, and 25 g of the sample was measured and extracted with 250 ml of water using the Soxhlet extraction method) for 24 hours. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. Subsequently, the extracts were freeze-dried in a lyophilizer and stored in sterile pre-weighed screw-cap bottles at 4 °C. The dried extract was subjected to GC-MS analysis using a Shimadzu GC-MS system (Model QP2020) equipped with an SH-Rxi-5Sil MS column. The



oven temperature was maintained at 60 °C for 8 minutes, and 1.0 µl of each sample was injected for analysis. High-purity helium gas (99.99%) served as the carrier and eluent, with a flow rate of 1 ml/min. The injector temperature was set at 250 °C, and the split ratio was maintained at 10 throughout the run. Ionization was performed at 70 eV, and mass spectra were recorded over a mass range of 10–20 m/z for approximately 8 minutes. As individual compounds eluted from the column, they were introduced into the electron ionization detector, where they were fragmented by electron bombardment into charged ions. The resulting mass-to-charge (m/z) ratios, unique to each compound, were used to generate molecular fingerprints. Identification of compounds was accomplished by comparing the acquired mass spectra with reference data from the National Institute of Standards and Technology (NIST) 17 Library.

2.3. Drug Likeness

The set of phytochemicals identified through both the literature survey and GC-MS analysis was subjected to 'Lipinski's Rule of Five filtering' to screen for compounds exhibiting drug-like properties, in terms of oral bioavailability. This rule determines the physicochemical properties of small molecules and assesses their pharmacological potential as orally active drugs in humans⁷. According to Lipinski's Rule of Five, an orally active drug candidate should meet the following criteria: no more than 5 hydrogen bond donors (HBD), no more than 10 hydrogen bond acceptors (HBA), a molecular weight (MW) of 500 daltons or less, and an octanol–water partition coefficient (AlogP) not exceeding 5.

2.4. Molecular Docking

To evaluate the binding affinity of the identified compounds toward molecular targets associated

with CNS dysfunction, four proteins with critical roles in neurological disorders, particularly Alzheimer's disease were selected for the study. The 3D structures of the selected molecular targets, AChE (PDB Id:6O4W), BACE1 (PDB Id:3UQU), GABABR (PDB Id:4MS3), and GSK3β (PDB Id:4J1R) were retrieved from the Protein Data Bank (PDB), a digital repository containing atomic coordinates of biological macromolecules (<https://www.rcsb.org/>). Non-mutated X-ray crystallographic structures were downloaded in PDB format. The control drugs, Donepezil (PubChem ID: 3152), LY2811376 (PubChem ID: 44251605), endogenous ligand, GABA (PubChem ID: 119), and drug, Cromolyn (PubChem ID: 2882) were taken as control for AChE, BACE1, GABABR, and GSK3β respectively. The 3D structures of all identified phytochemicals along with the reference drugs were downloaded from the PubChem database in SDF format. PubChem is a publicly accessible resource containing structural data for small molecules <https://pubchem.ncbi.nlm.nih.gov/>

Prior to docking, all target proteins and ligands were prepared following the recommended protocols of the *Discovery Studio* (DS) drug docking suite, client version⁸. The ligand's protonation states and tautomers were optimized using the 'prepare ligand' module. The bound ligand and water molecules of the selected targets were removed manually and the binding sites were defined based on PDB site records. Virtual screening of phytochemicals against the targets was performed using the LibDock site-feature docking algorithm in DS. LibDock is a rigid docking method that leverages the physicochemical properties of ligands to identify and dock them into protein binding sites by detecting and matching polar and apolar hot spots within the receptor cavity.



2.5. Network pharmacology

The target-ligand interaction network was generated to understand the interactions of the top-scored phytochemicals of *Benincasa hispida* against multiple targets of AD. The network was constructed based on the plant-phytochemical-target interaction using Cytoscape (<https://cytoscape.org/>).

3. RESULTS

3.1. Plant Authentication

3.1.1. Based on Morphological Features

The leaf specimen of *Benincasa hispida* identified based on the morphological features was preserved in an herbarium sheet with voucher number 'KUBH11054' (Fig. 1).



Fig 1. Herbarium specimen of leaf of *Benincasa hispida*

3.1.2. DNA Barcoding

The annotated genomic sequence of *Benincasa hispida*, with a length of 1120 base pairs, has been submitted to GenBank with the accession number 'ON032715.1'. The identified sequence is provided below in FASTA format.

>ON032715.1 *Benincasa hispida* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast

```
TTATTATACTCCTGAATATGAAACCAAAGATACTGATAT
CTTGGCAGCATTCCGAGTAACTCCTCAACCGGGAGTTCC
ACCTGAGGAAGCAGGGGCCGCTGTAGCTGCTGAATCTTC
TACTGGTACATGGACAACCTGTGTGGACCGATGGGCTTAC
CAGTCTTGATCGTTACAAAGGACGATGCTATGGAATCGA
GCCTGTTCTCGGAGAAGAAAATCAATATATTGCTTATGT
AGCTTATCCCCTAGACCTTTTTGAAGAAGGTTCTGTACT
AACATGTTTACTTCCATTGTCTGGTAATGTATTTGGATTCA
AGGCTCTACGTGCTCTACGTCTGGAGGATTGCGAATCC
CTACTGCTTATATTAATAAACTTTCCAAGGCCCGCCTCATG
GTATCCAGGTTGAAAGAGATAAATTGAACAAGTATGGT
CGCCCTCTATTGGGATGTACTATTAAACCAAAATTGGGA
TTATCCGCTAAGAATTATGGTAGAGCAGTTTATGAATGT
CTACGCGTGGACTTGATTTTACCAAAGATGATGAAAAC
GTGAATTCCCAACCATTATGCGTTGGAGAGACCGTTTC
CTATTTTGTGCGGAAGCTATTTATAAATCACAGGCTGAA
ACAGGTGAAATCAAGGGACATTACTTGAATGCTACTGC
AGGTACATGCGAAGAAATGATCAAAAGGGCTGTATTTG
CCCGAGAATTGGGAGTTCCTATCGTAATGCATGACTACT
TAACAGGTGGATTCACTGCANATACTAGCTTGGCTCATT
ATTGCCGAGATAATGGTCTACTTCTTCACATTCACCGTG
CAATGCATGCCGTTATTGATAGACAGAAGAATCATGGTA
TGCACTTCCGTGTACTAGCTAAAGCGTTACGTATGTCTG
GTGGAGACCATATTCACGCTGGTACCGTAGTAGGTAAAC
TTGAAGGGGAAAGAGAAATCACTTAGGCTTTGTTGATT
TACTACGTGATGATTTTATTGAAAAAGACCGAAGCCGCG
GTATTTATTTCACTCAAGATTGGGTCTCTTTACCAGGTGT
TCTACCAGTGGCTTCCGGTGGTATTACGTTTGGCATAT
GCCTGCTCTAACCGAGATTTTGGAGAT
```

3.2. Phytochemical analysis

3.2.1. Literature survey

From the analysis of PubMed data, 78 bioactive compounds were identified for different solvent-based extracts for *Benincasa hispida*. The compounds with their references are listed in Supplementary Table. S1.

3.2.2. GC-MS analysis

GC-MS analysis of the water extract of *Benincasa hispida* fruit revealed the presence of 26 compounds. GC-MS chromatograms of these compounds are depicted in Fig. 2 and the list of identified compounds with their name, retention

time (RT), percentage of area, are presented in Table 2.

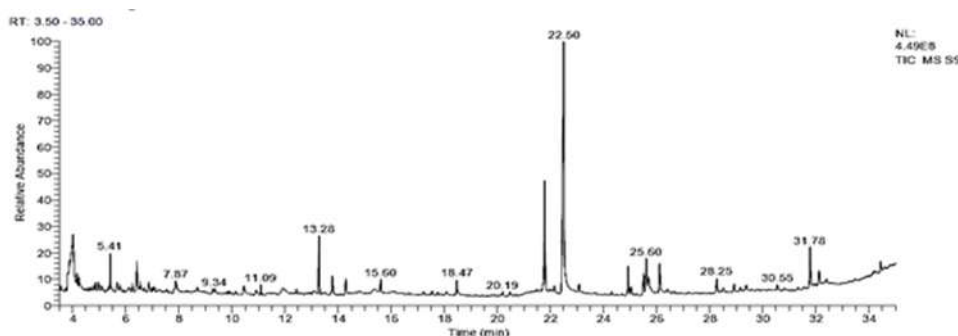


Fig 2. GC-MS Chromatogram of water extract of *Benincasa hispida*. The compounds detected were represented as peaks. The X-axis represents retention Time (RT) and Y-axis represents the relative abundance of the compounds.

Table 2. Phytochemical Compounds Identified in *Benincasa hispida* Extract via GC-MS

Sr. No	RT (min)	Compound Name	Relative Abundance (Area %)
1.	3.81	Tetraacetyl-D-xylonic nitrile	1.25
2.	3.89	Tetraacetyl-D-xylonic nitrile	3.16
3.	4.01	1,2,3-Butanetriol	11.77
4.	4.16	1,2-Propanediol, 3-methoxy-	1.76
5.	4.97	Benzeneethanamine	0.93
6.	5.41	2,5-Difluoro- α ,3,4-trihydroxy-N-methyl-	0.82
7.	5.67	5-O-Methyl-D-gluconic acid dimethylamide	2.26
8.	6.41	Glycerin	1.19
9.	6.87	Erythritol	2.27
10.	7.88	Diglycerol	0.95
11.	10.45	3,4-Furandiol, tetrahydro-, trans-	1.36
12.	13.28	1,2,3,4-Butanetetrol, [S-(R*,R*)]-	0.87
13.	13.79	Tetraacetyl-D-xylonic nitrile	3.87
14.	14.29	L-Gala-L-ido-octose	1.40
15.	21.77	N-methyl-N-[4-[4-fluoro-1-hexahydropyridyl]-	1.47
16.	22.14	12,15-Octadecadiynoic acid, methyl ester	9.02
17.	22.5	9-octadecenoic acid	0.88
18.	24.92	Tetraacetyl-D-xylonic nitrile	30.73
19.	25.03	L-Gala-L-ido-octose	2.28
20.	25.5	Paromomycin	0.86
21.	25.6	Acetamide	1.69
22.	25.69	2,3-Dimethylfumaric acid	3.50
23.	26.11	Acetic acid	2.12
24.	28.26	6-morpholin-4-yl-9-oxobicyclo [3.3.1] non-3-yl ester	2.74
25.	31.78	Unspecified ester	1.65
26.	32.12	4,5-Diamino-6-hydroxypyrimidine	3.54

3.3 Drug likeness

The drug-likeness probability of 79 compounds, obtained after removing duplicates from the initial list of 104 identified small molecules (78 from the



literature and 26 from GC-MS analysis) was evaluated with respect to oral bioavailability. The molecular descriptors used for Lipinski's filtering criteria for these compounds are summarized in Table 3.

Table 3. Phytochemicals of *Benincasa Hispida* that passed Lipinski filtering

Sr. No	PubChem ID	H-Bond acceptor	H-Bond donor	Molecular weight (Dalton)	AlogP
1	176	2	1	60.052	-0.205
2	178	2	2	59.0672	-0.833
3	753	3	3	92.0938	-1.409
4	985	2	1	256.424	6.393
5	1001	1	2	121.18	1.257
6	6184	1	0	100.159	1.853
7	6212	0	0	119.378	1.617
8	6525	1	1	124.18	2.548
9	7938	2	0	108.141	0.093
10	7976	2	0	94.1145	-0.189
11	8222	0	0	282.547	9.495
12	8343	4	0	390.556	7.574
13	8998	4	4	122.12	-1.919
14	9064	6	5	290.268	2.021
15	12388	0	0	184.361	6.302
16	12389	0	0	198.388	6.758
17	12408	0	0	394.76	13.145
18	17218	1	1	256.467	6.764
19	20497	3	3	106.12	-1.031
20	25835	0	0	198.388	6.553
21	25913	0	0	210.399	6.823
22	25915	2	0	122.168	0.76
23	26808	2	0	122.168	0.376
24	29075	0	0	266.505	8.648
25	31252	2	0	108.141	0.093
26	42953	5	4	166.172	-2.05
27	61177	2	0	130.185	2.183
28	66340	2	1	307.267	6.042
29	90803	3	2	104.105	-1.153
30	92822	3	2	106.12	-1
31	98210	2	2	87.1204	-0.846
32	102296	2	0	336.552	7.97
33	115250	1	1	426.717	7.403
34	138824	1	1	228.414	5.375
35	219659	8	7	240.208	-3.812
36	222285	4	4	122.12	-1.919
37	228944	1	1	256.383	4.08
38	259846	1	1	426.717	7.403
39	439246	5	3	272.253	2.373
40	535560	7	4	484.692	4.702
41	536940	0	0	280.532	8.715
42	538453	2	0	290.44	6.887
43	541568	10	0	343.286	-0.661
44	542382	7	4	237.25	-2.62
45	545687	3	0	386.684	8.459

46	550072	4	1	326.433	3.449
47	556975	1	1	168.276	3.031
48	558102	1	0	183.849	2.481
49	580937	5	0	281.347	0.73
50	600682	3	1	366.282	6.147
51	609687	1	0	424.702	7.598
52	618607	1	0	424.702	7.449
53	5280343	7	5	302.236	1.63
54	5280450	2	1	280.445	6.416
55	5282761	2	1	282.461	6.86
56	5283468	4	2	356.54	6.036
57	5283663	1	1	412.691	7.639
58	5364412	1	1	280.489	6.787
59	5364413	1	1	280.489	6.787
60	5364599	0	0	252.478	8.138
61	5365774	0	0	278.516	8.66
62	5371506	4	2	144.125	0.931
63	6161490	2	1	282.461	6.86
64	6436630	4	2	354.524	5.591
65	6452096	5	3	436.625	3.487
66	9949088	2	0	322.525	7.514
67	10931630	1	1	152.233	1.824
68	12314300	0	0	138.25	3.122
69	21160048	4	0	444.647	8.455
70	40468165	4	0	370.566	7.037
71	57507578	1	1	412.691	7.834
72	91692471	2	1	379.544	8.633
73	129857151	0	0	292.373	4.597
74	135436550	5	5	126.117	-2.243

The result showed that, out of 79 screened compounds, 74 passed the filtering criteria to be act as orally bioavailable drug. All these compounds which passed the criteria was further taken for evaluating their binding affinity towards selected molecular targets, AChE, BACE1, GABABR, and GSK3 β .

3.4. Molecular Docking

Based on the docking results, 15, 36, and 22 compounds demonstrated higher binding affinity towards AChE, BACE1, and GABABR, respectively, compared to their corresponding reference molecules. In contrast, none of the compounds showed a higher docking score against GSK3 β than the reference drug, cromolyn. Several

compounds were found to interact with multiple targets, exhibiting greater binding affinity than the respective control molecules. The docking scores of the selected phytochemicals are represented in supplementary Table 2. It was observed that the compound, '9-Octadecenoic acid' (PubChem ID: 21160048) demonstrated highest docking score against three targets, AChE (133.87), BACE1 (159.36), and GABABR (154.74) than the reference drugs. For GSK3 β , the compound naringenin (PubChem ID: 439246) showed highest docking score (88.57). The interactions of top scored phytochemicals which showed highest affinity towards the selected targets are depicted in Fig. 3 and the binding mode of the ligands in the binding cleft of target proteins are shown in Fig. 4



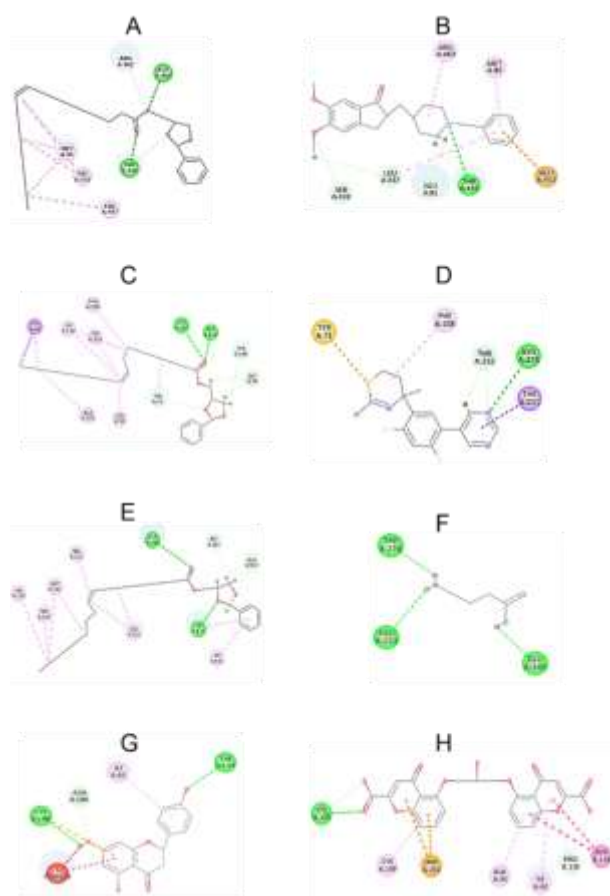


Fig 3. 2D representation of the molecular interactions between top scored phytochemicals of *Benincasa hispida* against selected targets of Alzheimer's disease. A: AChE-21160048; B: AChE-Donepezil (Control); C: BACE1-21160048; D: BACE1-LY2811376 (Control); E: GABABR-21160048; F: GABABR-GABA (Control); G: GSK3 β -439246; H: GSK3 β -romolyn (Control). Different colours are used to represent the types of interactions as detailed below.

	Conventional hydrogen bond		Alkyl bond
	Carbon hydrogen bond		Pi-alkyl bond
	Pi-Sigma bond		Pi-Pi T shaped
	Unfavourable bump		

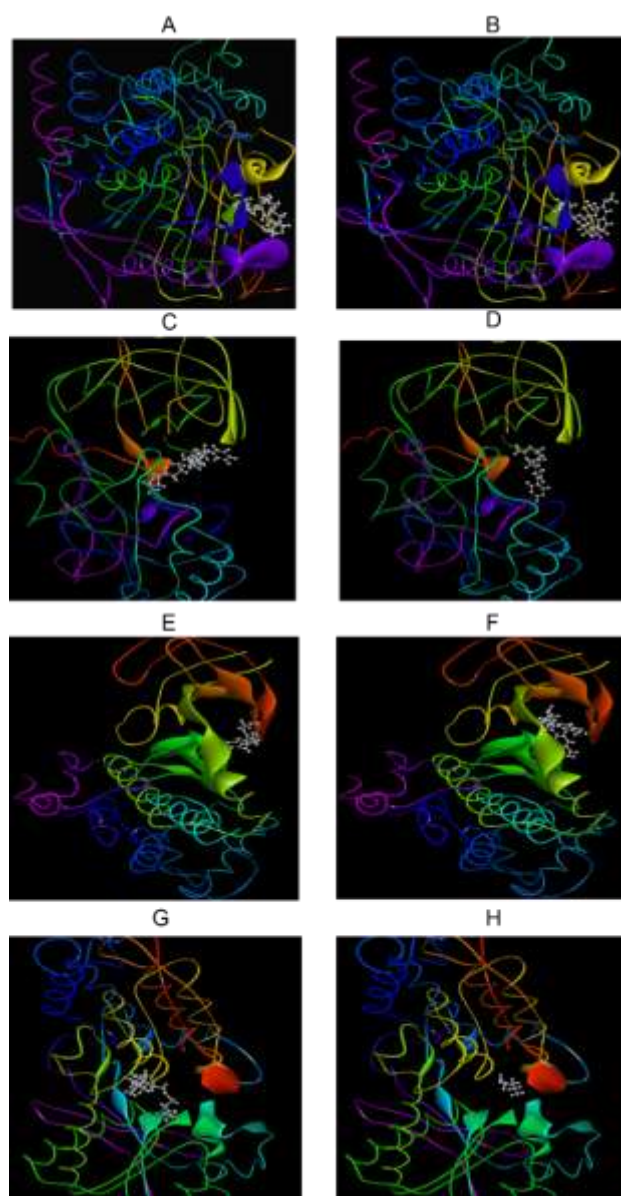


Fig 4. Binding mode of top-scoring phytochemicals of *Benincasa hispida* within the selected targets of Alzheimer's disease. A: AChE-21160048; B: AChE-Donepezil (Control); C: BACE1-21160048; D: BACE1-LY2811376 (Control); E: GABABR-21160048; F: GABABR-GABA (Control); G: GSK3 β -439246; H: GSK3 β -Cromolyn (Control). Targets are shown in ribbon and ligands are shown in ball and stick representation

The binding mode of the compound within the respective targets demonstrates that it adopts an orientation similar to that of the reference molecule, suggesting a potential regulatory effect.

3.5. Network pharmacology analysis

The target-ligand interaction network was generated to understand the interactions of the phytochemicals of *Benincasa hispida* against selected targets of AD. From the docking results,

the top-scoring phytochemicals against each target than the reference drugs were included for the network analysis to demonstrate the interaction with multiple targets (Fig 5).

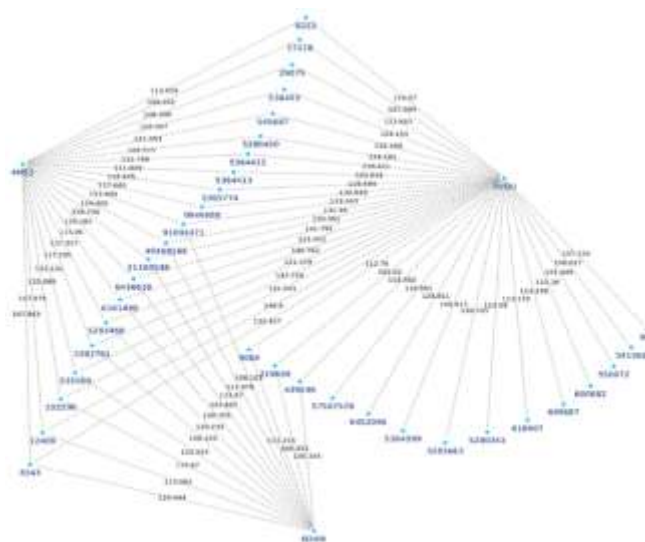


Fig 5. Target- phytochemical interaction network showing docking scores of phytochemicals of *Benincasa hispida* against selected molecular targets of Alzheimer's disease. The network diagram illustrates the binding affinities of selected phytochemicals that demonstrated higher docking score than the reference drugs, against three key molecular targets AChE (604W), GABABR (4MS3), and BACE1 (3UQU). Nodes represents targets and phytochemicals, where phytochemicals are represented with their PubChem IDs. Edges represent compound–target interactions, with labels indicating docking scores. Higher scores suggest stronger binding affinity.

As illustrated in Fig 5. the network consists of 38 nodes (3targets, and 35 phytochemicals) and 70 edges, where the edges encoded interaction and the nodes represented potential targets and phytochemicals (blue colour). The top scoring phytochemicals against each target were selected for the analysis and calculated the degree centrality using cytoNCA plugin in cytoscape. The degree centrality of each compound is represented in Table 4.

Table 4. The degree of centrality of phytochemicals of *Benincasa hispida*

Compounds with degree: 3	Compounds with degree: 2	Compounds with degree: 1
91692471	9949088	57507578
40468165	5365774	6452096
21160048	5364413	5364599
6436630	5364412	5283663
6161490	5280450	5280343

5283468	545687	618607
5282761	538453	609687
535560	439246	600682
102296	219659	550072
12408	29075	541568
8343	17218	985
	9064	
	8222	

The degree centralities of top scored phytochemicals in docking analysis are calculated using cytoscape. The phytochemicals are represented with their PubChem IDs.

The results revealed that BACE1 (3UQU) exhibited the highest number of interacting phytochemicals (35), followed by GABAR (4MS3), which showed substantial connectivity with 21 phytochemicals. In contrast, AChE (604W) displayed the fewest interactions,

involving 14 compounds. Notably, among the 35 top-scoring phytochemicals, 11 were identified as interacting simultaneously with all three selected targets.

4. DISCUSSION

The current study provides a qualitative assessment on the phytoconstituents of *Benincasa hispida* and their potential neuroprotective properties through multi-target interactions relevant to AD pathogenesis. The plant authentication was confirmed via morphological characterization and DNA barcoding, ensuring taxonomic accuracy of the investigated material. The *rbcL* gene sequence submitted to GenBank (accession number ON032715.1) contributes valuable reference data for future molecular identification of the plant since misidentification remains a major limitation in ethnopharmacological research that could affect pharmacological findings.

The phytochemical profiling through GC-MS analysis of the water extract revealed a diverse array of small molecules, characterized by a range of polar and moderately nonpolar compounds eluting between 3.8 and 32 minutes. Tetraacetyl-D-xylonic nitrile was detected as the dominant constituent, appearing at multiple retention times (3.81, 3.89, 13.79, and most prominently at 24.92 min), with the peak at 24.92 min alone accounting for over 30% of the total ion current area. This high relative abundance indicates that this compound, though less frequently reported in prior studies of the plant, is a major component of the water-soluble fraction. The compound, 1,2,3-Butanetriol (RT 4.01 min) emerged as the second most abundant molecule (11.77%), reflecting the extract's richness in polyols and sugar alcohols commonly found in polar solvent extracts. Similarly, other compounds, including erythritol, glycerin, and diglycerol, were detected in

moderate quantities, consistent with the plant's known carbohydrate composition. The identification of 12,15-Octadecadiynoic acid, methyl ester (9.02%) and 9-octadecenoic acid (0.88%) demonstrates the presence of fatty acid derivatives, which are often associated with anti-inflammatory and neuroprotective activities. Several lower-abundance peaks corresponded to nitrogen-containing compounds, such as benzeneethanamine and 4,5-diamino-6-hydroxypyrimidine, were observed. The integration of these GC-MS data with literature-mined compounds enabled a robust composite library of 79 unique phytochemicals for virtual screening.

Drug-likeness assessment based on Lipinski's rule of five further narrowed this library to 74 compounds with favorable predicted oral bioavailability. This high proportion underscores the promising physicochemical properties of the identified phytoconstituents for potential development as orally administered agents. usually, only a small fraction of natural products often meets such criteria, highlighting the value of this plant as a reservoir of drug-like metabolites.

Molecular docking results demonstrated the potential neuroprotective properties of the plant, supporting its traditional use and providing a scientific rationale for its activity through interactions with multiple molecular targets of AD. From the docking results, it was observed that many compounds showed affinity to the selected multiple molecular targets. For instance, the compound, Naringenin (PubChem ID: 439246) was found to be interacting with here targets except GABABR. Naringenin is a flavonoid that has been extensively studied for its neuroprotective properties. Experimental evidence supports its ability to attenuate oxidative stress, neuroinflammation, and amyloid-beta toxicity,



which are central to AD pathology. Also, experimental studies have shown that Naringenin can improve cognitive deficits by modulating antioxidant enzymes, suppressing pro-inflammatory cytokines, and inhibiting AChE activity^{9, 10}. Another remarkable observation was for the compound, 9-Octadecenoic acid, which demonstrated higher binding affinity against AChE, BACE1, and GABABR, than the reference inhibitors, which suggests this fatty acid may exert polypharmacological modulation of cholinergic, amyloidogenic, and GABAergic pathways in AD. Previous studies which support the neuroprotective potential of this compound compliment these findings. Experimental studies indicate that this compound can protect neurons by reducing oxidative stress, attenuating inflammation, and preserving membrane integrity. Also, its ability to decrease pro-inflammatory cytokine production and to modulate neurotrophic factors support neuronal survival. In cellular models, 9-Octadecenoic acid was found to be promoting neurogenesis and enhanced anti-apoptotic activity, which collectively contribute to its protective role against neurodegenerative disorders¹¹.

From the molecular interaction analysis (Fig 3), it was observed that, both 9-Octadecenoic acid and the reference drug, Donepezil shares key interactions with conserved residues THR A:436, LEU A:437, MET A:85, and ARG A:463, which are critical for ligand anchoring within the active pocket. However, the phytochemical demonstrates a superior interaction profile by engaging additional residues such as GLU A:81, GLU A:452, and SER A:438, forming strong hydrogen bonds and electrostatic. This extended interaction network and higher docking score not only enhances binding stability but also indicates a better complementarity with the active site environment. In contrast, the reference compound

shows a more limited interaction pattern, relying primarily on hydrophobic contacts and lacking involvement with key acidic or polar residues. These findings support the identified natural compound's potential as a more effective and selective candidate as AChE inhibitor.

For BACE1, the interaction analysis revealed that the 9-Octadecenoic acid establishes a broader and more diverse network of contacts within the active site of BACE1 compared to the reference compound. While both compounds share conserved interactions with critical residues such as TYR A:71, PHE A:108, and THR A:231/232, the phytochemical further engages additional residues including TYR A:114, TRP A:115, ILE A:110, ALA A:335, LEU A:30, and GLY A:34, as well as forming hydrogen bonds with ASP A:232. This expanded interaction profile combines hydrophobic, aromatic, and polar contacts, potentially conferring enhanced stabilization and stronger binding affinity within the target site.

In the case of GABABR, 9-Octadecenoic acid demonstrates a promising profile compared to the endogenous ligand, GABA. While both ligands share a conserved hydrogen bond with TRP A:278, the phytochemical additionally engages multiple polar residues such as GLN A:348, GLU A:423, ASP A:281, and GLU A:434, which collectively enhance electrostatic stabilization. Also, the phytochemical forms extensive hydrophobic and aromatic interactions with residues including HIS A:170, MET A:312, VAL A:311, LEU A:313, and LYS A:431, suggesting improved complementarity with the binding pocket

While numerous compounds displayed promising interactions with these three targets, no tested phytochemicals exceeded the affinity of the reference drug, Cromolyn against GSK3 β , indicating that this kinase may remain a more challenging target. This observation aligns with



previous reports that potent GSK3 β inhibition often requires more rigid heterocyclic scaffolds¹².

The network pharmacology analysis further demonstrated the multi-target interaction landscape, revealing BACE1 as the hub protein with the highest degree of phytochemical connectivity. The presence of 11 compounds predicted to interact concurrently with all three targets supports the potential neuroprotective effect of the plant which may be attained by multi-target directed modulatory effect which may be essential to achieving disease-modifying efficacy in chronic multifactorial CNS disorders such as AD. Also, the degree centrality analysis highlighted many key hub compounds including 9-octadecenoic acid and several mid-polar constituents, as high-priority candidates for further experimental validation.

Overall, these findings support the ethnomedicinal claims of *Benincasa hispida* as neuroprotective agent which provide a rational basis for prioritizing its phytochemicals as leads for the development of multitarget therapeutics against AD. However, it is important to recognize that in silico docking predictions, while highly informative, require further validation through different experimental assays to fully establish their pharmacological relevance and safety profiles.

5. CONCLUSION

Based on the traditional indication and scientific evidence on neuroprotection, phytochemicals of the medicinal plant, *Benincasa hispida* was assessed for its multi-target directed molecular interaction against selected key molecules of AD pathogenesis. By combining morphological characterization and DNA barcoding, a systematic scientific authentication of this medicinal plant was established. Most of the compounds,

identified both from literature and GC-MS profiling, demonstrated favorable drug-like properties and significant binding affinities toward key AD-related targets, including AChE, BACE1, GABABR, and GSK3 β based on in silico assessment. In conclusion, the present study provides supportive evidence for the neuroprotective potential of *Benincasa hispida* through in-silico investigation, targeting multiple molecular targets of AD. Among the screened compounds, 9-octadecenoic acid showed consistently high docking scores across multiple targets of AD, underscoring its promise as a natural compound with multitarget regulatory effect. These findings highlight *Benincasa hispida* as a rich source of neuroprotective compounds which may further be considered for experimental validation.

REFERENCES

1. Singh S, Gohil KJ and Singh MP: Pharmacological update on *Benincasa hispida* (Thunb.): A review, Pharmacological Research – Modern Chinese Medicine (2024), 12:100478.
2. Islam MT, Quispe C, El-Kersh DM et al.: A literature-based update on *Benincasa hispida* (Thunb.) Cogn.: Traditional uses, nutraceutical and phytopharmacological profiles, Oxidative Medicine and Cellular Longevity (2021), Article ID 6349041.
3. Satish M, Dattatraya KA: Therapeutic potential of *Benincasa hispida* for the treatment of Alzheimer disease, Journal of Advanced Zoology (2024), 45.
4. Acharya T, Nanda A and Swain SS: Exploration of bioactive compounds in *Benincasa hispida* seeds: Insight into their therapeutic potential for Alzheimer's disease management using computer-aided drug



- design platform, *Chemistry & Biodiversity* (2025).
5. Nadh AG, Kunhikrishnan MJ, Ravi V, Ramakrishnan K, Rehman N, Adithya KSB, Revikumar A, Sudhakaran PR and Raju R: Convolidine as a potent BACE1 inhibitor for Alzheimer's disease: In silico coupled with in vitro assessment, *Journal of Computer-Aided Molecular Design* (2025), 39:13.
 6. Deepthi A, Krishnan D and Sanju A: Semisynthesis of ursolic acid-2-(2-thienylidene)-oxadiazole hybrid molecule and evaluation of its COX inhibition property, *Journal of Heterocyclic Chemistry* (2020), 57:2048–2055.
 7. Lipinski CA: Drug-like properties and the causes of poor solubility and poor permeability, *Journal of Pharmacological and Toxicological Methods* (2000), 44:235–249.
 8. Dassault Systèmes: BIOVIA Discovery Studio Client, Version 18.01.100.18065, Dassault Systèmes (2018).
 9. Goyal A, Verma A, Dubey N, Raghav J and Agrawal A: Naringenin: A prospective therapeutic agent for Alzheimer's and Parkinson's disease, *Journal of Food Biochemistry* (2022).
 10. Nouri Z, Fakhri S, El-Senduny FF, Sanadgol N, Abd-ElGhani GE, Farzaei MH and Chen J-T: On the neuroprotective effects of naringenin: Pharmacological targets, signaling pathways, molecular mechanisms and clinical perspective, *Biomolecules* (2019), 9:690.
 11. Kim H, Youn K, Yun E-Y, Hwang J-S, Jeong W-S, Ho C-T and Jun M: Oleic acid ameliorates A β -induced inflammation by downregulation of COX-2 and iNOS via NF- κ B signaling pathway, *Journal of Functional Foods* (2015), 14:1–11.
 12. Arciniegas Ruiz SM and Eldar-Finkelman H: Glycogen synthase kinase-3 inhibitors: Preclinical and clinical focus on CNS—A decade onward, *Frontiers in Molecular Neuroscience* (2022), 14.

HOW TO CITE: Dr. Anuroopa Nadh, Aswin Mohan, Rajesh Raju, DNA Barcoding, Phytochemical analysis, and Computational Insights into the Neuroprotective Potential of *Benincasa hispida*, *Int. J. of Pharm. Sci.*, 2026, Vol 4, Issue 1, 1433-1446. <https://doi.org/10.5281/zenodo.18245717>

