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Functional profiling of Nardostachysin against Alzheimer's Disease via integrated chromatographic and in silico methods.

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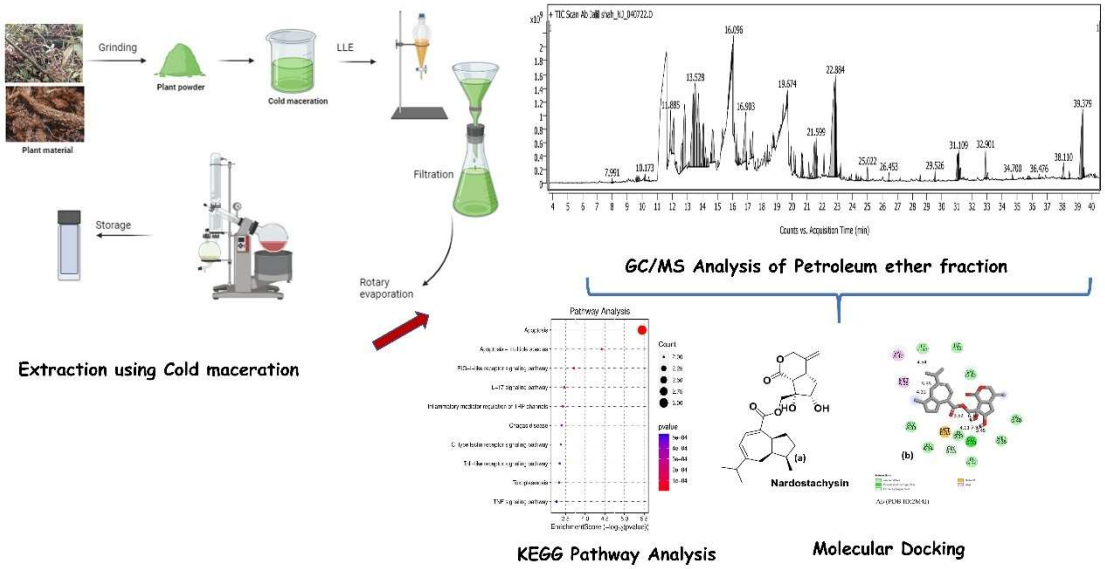
Graphical Abstract



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Abstract

Background

Alzheimer disease (AD) is a well-known brain disorder affecting memory, and in later stages causing severe cognitive deficits. At present, few effective drugs are available in the clinic to manage symptoms, however none of these alter or reverse the progression in AD cases. In this study, we have utilised nardostachysin, a terpenoid ester found in *Nardostachys jatamansi*, for its inhibition of AD pathological features.

Methods

We utilised the SwissTargetPrediction database to identify potential targets for nardostachysin, which we then cross-referenced with known Alzheimer's disease (AD) targets. The overlapping targets were used to create a protein-protein interaction network. Next, we focused on identifying how nardostachysin might target key AD-related pathologies, specifically tau and amyloid-beta (Aβ). To validate our findings, we performed molecular docking studies to assess the reliability and of these core target interactions.

Results

GC-MS analysis revealed presence of 15 different phytoconstituents. We identified 92 targets of nardostachysin, out of them 8 were found specific to AD. Bioinformatic analyses indicated that Aβ (PDB ID:2M4J), Tau (PDB ID:2MZ7), NTRK1 (PDB ID:4F01), MAPK10 (PDB ID:1MPU) CASP8 (PDB ID:2C2Z) have a good binding force with nardostachysin. The best docking score was found to be -9.24 for aβ.

Conclusions

In this study, nardostachysin was found to exert multichannel effects via modulating multiple AD related pathways.

Keywords: Alzheimer disease; GC-MS; Nardostachysin; Molecular docking.

Abbreviations: Aβ (Amyloid Beta), AD (Alzheimer's Disease), BP (Biological Process), CASP10 (Caspase 10), CASP3 (Caspase 3), CASP8 (Caspase 8), CC (Cellular Component), DRD3 (Dopamine Receptor D3), GO (Gene Ontology), h-tau (Hyperphosphorylated Tau), KEGG (Kyoto Encyclopedia of Genes and Genomes), MF (Molecular Function), PRKCD (Protein Kinase C Delta), TNF (Tumor Necrosis Factor).

1. Introduction

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative condition that leads to severe memory loss and cognitive decline. AD is a significant global public health challenge, placing substantial health, social, and economic burdens on society[1]. It is the leading cause of dementia worldwide, impairing individuals ability to perform daily activities[2]. Approximately 55 million people are living with dementia globally, with over 60% residing in low- and middle-income countries. Projections suggest that by 2030, this number will increase to 78 million, reaching 139 million by 2050 [3], driven by aging populations, especially in developing regions such as India, where 14.2% of the global elderly population resides [4]. Despite its widespread impact, there is currently no cure for AD, and existing treatments are largely symptomatic, offering limited therapeutic options. This has created an urgent need for innovative therapies. One promising medicinal plant, *Nardostachys jatamansi* has shown neuroprotective role in multiple in vitro and in vivo studies [5].

Nardostachys jatamansi is a small, perennial, rhizomatous herb found in the mountainous regions of India, Nepal, China, and Bhutan, thriving at altitudes between 2,300 and 6,000 meters. Known for its wide-ranging pharmacological properties [6], it exhibits neuroprotective, tranquillizing, hepatoprotective, hypolipidemic, hypotensive, anti-ischemic, anticonvulsant, and antiarrhythmic activities [7]. Nardostachysin, a bioactive compound isolated from its rhizomes, has the molecular formula $C_{25}H_{34}O_6$ and a molecular weight of 430.5 g/mol [8]. To investigate its potential against AD, targets associated with nardostachysin were identified using the Swiss Target Prediction database and cross-referenced with AD-specific targets [9]. Protein-protein interaction (PPI) networks and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analyses were performed to identify key pathways [10]. Additionally, targets linked to

amyloid beta (Aβ) and tau proteins were also studied, as these are hallmark pathological features of AD. Molecular docking studies revealed strong binding affinities between nardostachysin and multiple targets [11]. The KEGG pathway analysis highlighted significant enrichment in AD-related pathways, identifying eight key targets for nardostachysin. Moreover, nardostachysin demonstrated interactions with multiple Aβ and tau-related targets, suggesting its multipotent therapeutic potential. These findings underscore the promise of nardostachysin as a novel, multitarget treatment for Alzheimer's disease, paving the way for further preclinical and clinical exploration.

2.0 METHODS

2.1 GCMS analysis of *N. jatamansi* oil.

The GC-MS analysis was performed using an Agilent GC-MS/MS-7000D system, equipped with an HP-5Ms column (15 m × 250 mm × 0.25 µm) from Agilent Technologies, Santa Clara, CA, USA [12]. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and the injector was set to 280 ºC in split-less mode. The oven temperature program started at 60 ºC, where it was held for 4 minutes, then increased to 150 ºC at a rate of 10 ºC per minute and maintained for 15 minutes. To optimize the mass spectra, the ion source temperature was kept at 280 ºC, and the transfer line was set at 150 ºC. The system included a solvent delay of 2 minutes, and the mass spectrometer scanned a range of 35–500 Da. The final oven temperature was raised to 310 ºC, bringing the total runtime of the GC analysis to 40.5 minutes. Compounds were identified by matching their mass spectra against the National Institute of Standards and Technology (NIST) library.

2.2 Swiss Target Prediction and Target identification using Genecard and OMIM databases.

The structural formula of nardostachysin was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). The SMILES representation of the compound was then input into SwissTargetPrediction to predict potential targets for nardostachysin. The gene symbols corresponding to these targets were obtained through searches in the UniProt database (http://www.uniprot.org/uniprot/) [13]. For Alzheimer's disease (AD)-related gene exploration, electronic databases such as GeneCards (https://www.genecards.org/) and OMIM (https://www.omim.org/) were utilized.

2.3 Protein-Protein Interaction

To explore the interaction between Alzheimer's disease (AD)-related targets and the potential targets of nardostachysin, we identified their intersection and visualized the results using an online Venn diagram tool (http://bioinformatics.psb.ugent.be/webtools/Venn/). Next, the identified anti-AD targets associated with nardostachysin were classified using the Panther Classification System (http://www.pantherdb.org/). For further analysis, a protein-protein interaction (PPI) network was constructed by inputting these therapeutic targets into the STRING database (https://string-db.org), with the species set to "Homo sapiens" and a minimum interaction confidence score of 0.4 (medium to high confidence) [14]. The resulting network was then imported into Cytoscape (Version 3.8.0) for enhanced visualization. Based on their degree of connectivity within the network, the top targets were identified as key candidates for further investigation.

2.4 Molecular Docking

Molecular docking in this study was conducted using AutoDockTools (v1.5.6). The crystal structure of the target protein was sourced from the Protein Data Bank (http://www.rcsb.org/pdb/), and each docking simulation was repeated three times to ensure consistency [14]. The structure of nardostachysin was obtained from the PubChem database for use in the docking experiments. Aβ (PDB ID:2M4J), Tau (PDB ID:2MZ7), NTRK1 (PDB ID:4F01), MAPK10 (PDB ID:1MPU) CASP8 (PDB ID:2C2Z) were obtained from Protein Data Bank. We modified the target proteins by removing water molecules and adding Kollman charges and polar hydrogen atoms. After that, both target and receptor molecules were saved in .pdbqt format. Molecular docking was performed within a grid box of particular dimensions and spacing.

3.0 RESULTS

3.1 GC-MS analysis of petroleum ether fraction *Nardostachys jatamansi* rhizome

Compounds identified using GC-MS analysis revealed identification of 15 compounds, representing about 31.57 % of total extract as shown in **Figure 1,2 and Table 1**. The major components of the extract were found to be 1-[3-(2,6,6-Trimethyl-cyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone (11.38 %), followed by Spirojatamol (4.67%), cyclopentanel, 2-ethyl-6,7-dihydro-2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl) propan-2-ol (2.81%), Spiro [cyclopenta[d]-1,3,2-dioxaborin-4(5H), 1'-cyclopentane (3.81%), Undeca-3,4-diene-2,10-dione, 5,6,6-trimethyl (1.29%) and Calerene (1.21%).

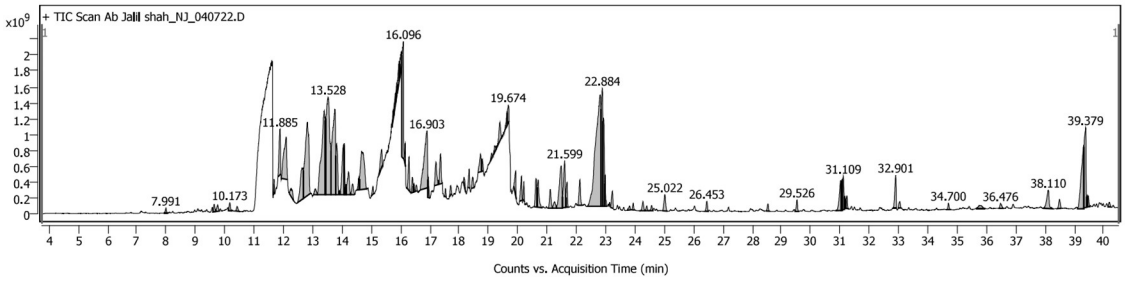
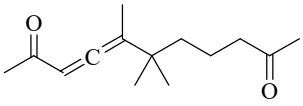


Figure 1: GCMS chromatogram of *N. jatamansi* petroleum ether fraction

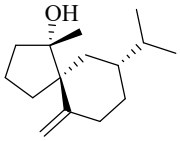
Table 1: Phytochemical constituents identified in the petroleum ether extract of *N. jatamansi* using gas chromatography-mass spectrometry.

S.No	Compound	Molecular Formula	Molecular Weight (g/mol)	RT (min)	Area Sum %
1	Undeca-3,4-diene-2,10-dione, 5,6,6-trimethyl-	$C_{14}H_{22}O_2$	222.32	11.88	1.29
2	Spirojatamol	$C_{15}H_{26}O$	222.37	13.528	4.67
3	Spiro[cyclopenta[d]-1,3,2-dioxaborin-4(5H),1'-cyclopentane	$C_{12}H_{19}BO_2$	206.09	16.096	3.38
4	Cyclopentanel, 2-ethyl-6,7-dihydro-2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl) propan-2-ol	$C_{15}H_{26}O$	222.36	19.674	2.81
5	Calarene	$C_{15}H_{24}$	204.35	21.599	1.21
6	1-[3-(2,6,6-Trimethyl-cyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone	$C_{14}H_{22}N_2O$	234.34	22.884	11.38
7	Patchouli alcohol	$C_{15}H_{26}O$	222.37	25.022	0.54
8	Jatamansone	$C_{15}H_{26}O$	222.37	29.526	0.28
9	Curcumenol	C15 H22 O	234.33	31.109	0.81

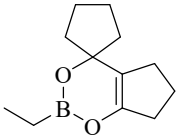
10	Cedryl methyl ether	$C_{16}H_{28}O$	236.4	14.575	0.86
11	Curcumenol	$C_{15}H_{22}O_2$	218.34	32.901	1.01
13	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	36.646	0.78
14	Methyl 9,10-octadecadienoate	$C_{19}H_{34}O_2$	294.47	38.11	0.29
15	(R,Z)-2-Methyl-6-(4-methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol	$C_{15}H_{24}O$	220.35	39.379	2.26
	Total				31.57



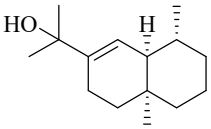
Undeca-3,4-diene-2,10-dione, 5,6,6-trimethyl-



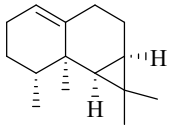
Spirojatamol



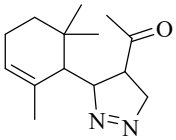
Spiro[cyclopenta[d]-1,3,2-dioxaborin-4(5H),1'-cyclopentane], 2-ethyl-6,7-dihydro-



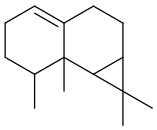
2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol



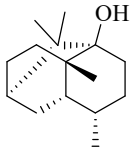
Jatamansone



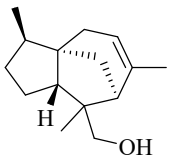
1-[3-(2,6,6-Trimethyl-cyclohex-2-enyl)-4,5-dihydro-3H-pyrazol-4-yl]-ethanone



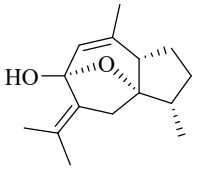
Calarene



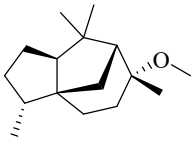
Patchouli alcohol



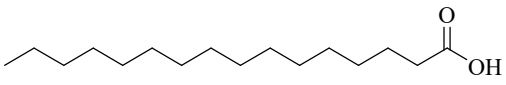
Cedr-8-en-13-ol



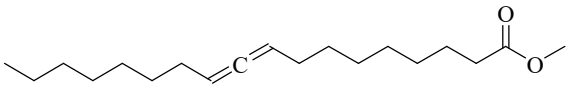
Curcumenol



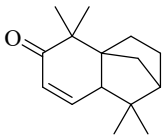
Cedryl methyl ether



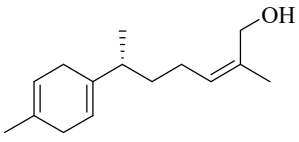
n-Hexadecanoic acid



Methyl 9 10-octadecadienoate



2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one



(R,Z)-2-Methyl-6-(4-methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol

Figure 2: Structure of identified molecules from *N. jatamansi* using GC-MS

3.2 AD-related targets and potential targets of Nardostachysin

In order to determine the targets related with nardostachysin, a total of 92 targets were gathered from Swiss Targets database. Furthermore, a comprehensive set of 13004 targets associated with diseases were acquired from GeneCard, and OMIM databases. Furthermore, the 92 genes were categorised into 7 separate groups using the Panther Classification System (**Figure 3**). The PPI network was constructed using the Cytoscape based on the obtained PPI relationships. The top 10 targets were chosen as core targets along with primary pathogenic proteins in AD. Aβ, Tau, NTRK1, MAPK10, CASP3, CASP6, CASP9, TNF, DRD3 and PRKCD were the top 10 targets (**Figure 4a and 4b**).

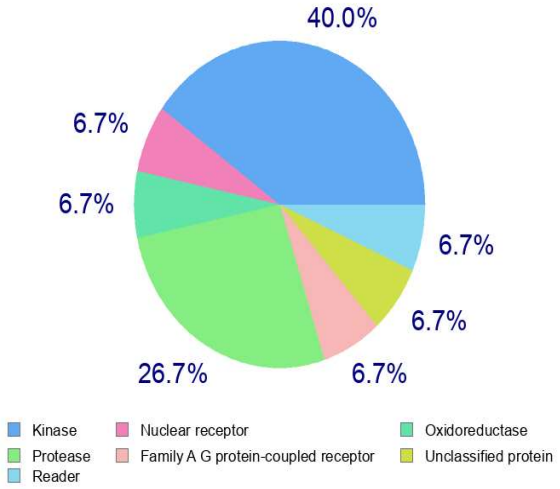


Figure 3: The protein classification of targets of Nardostachysin against AD using the PANTHER classification system.

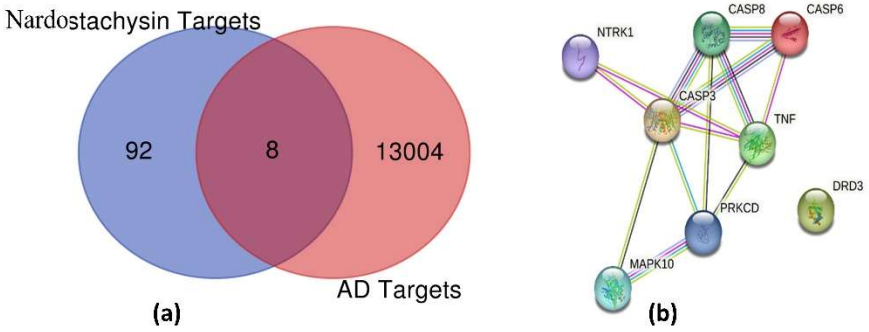


Figure 4: a) Venn Diagram of possible intersection of Nardostachysin targets specific to AD b) Protein-protein interaction network.

3.3 GO and KEGG analysis

Within the three categories (top 10), for Biological Process (BP) include: response to water (GO:0009415), response to acid chemical (GO:0001101), response to mechanical stimulus (GO:0009612), circadian rhythm (GO:0007623), response to temperature stimulus (GO:0009266), positive regulation of MAP kinase activity (GO:0043406), rhythmic process (GO:0048511), regulation of neuron death (GO:1901214), and B cell activation (GO:0042113). The Cellular Component (CC) terms are: recycling endosome membrane (GO:0055038), late endosome membrane (GO:0031902), perikaryon (GO:0043204), early endosome membrane (GO:0031901), recycling endosome (GO:0055037),

mitochondrial outer membrane (GO:0005741), organelle outer membrane (GO:0031968), outer membrane (GO:0019867), late endosome (GO:0005770), and membrane raft (GO:0045121). The Molecular Function (MF) terms include: cytokine receptor binding (GO:0005126), cysteine-type endopeptidase activity involved in apoptotic signaling pathway (GO:0097199), neurotrophin receptor binding (GO:0043121), cysteine-type endopeptidase activity involved in apoptotic process (GO:0004197), MAP kinase kinase activity (GO:0004708), ephrin receptor activity (GO:0005003), MAP kinase activity (GO:0004707), death receptor binding (GO:0005123), tumor necrosis factor receptor binding (GO:0005164), and protein serine/threonine/tyrosine kinase activity (GO:0004712).These results illustrate that nardostachysin against AD involved a variety of targets (**Figure 5**). After GO analysis, the top 10 enriched KEGG pathways **(Figure 6)** were obtained as shown in Fig. 8B. The pathways include: Apoptosis (hsa04210), Apoptosis – multiple species (ko04210), RIG-I-like receptor signaling pathway (hsa04622), IL-17 signaling pathway (hsa04657), Inflammatory mediator regulation of TRP channels (hsa04750), Chagas disease (hsa05142), C-type lectin receptor signaling pathway (hsa04625), Toll-like receptor signaling pathway (hsa04620), Toxoplasmosis (hsa05145), and TNF signaling pathway (hsa04668). These results indicate that targets of nardostachysin might play a significant role in ameliorating AD pathogenesis by interacting with multiple pathways.

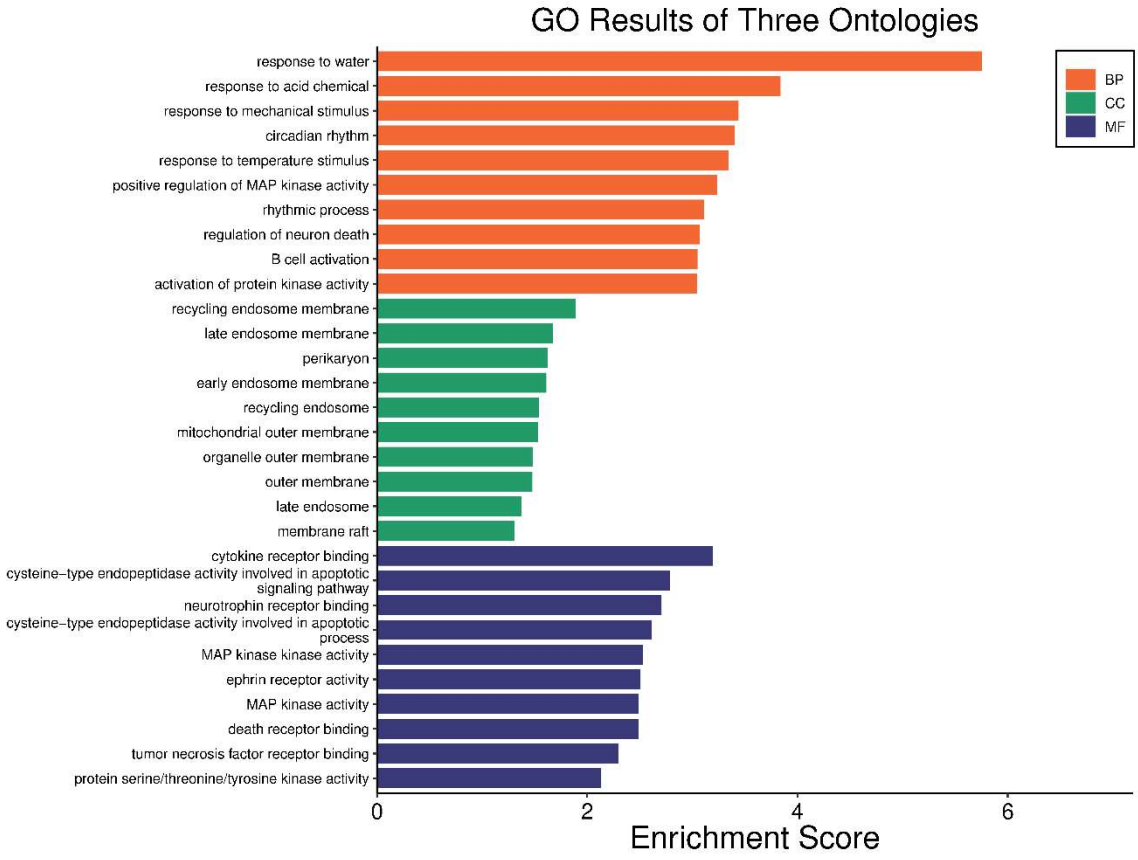


Figure 5: Gene ontologies of Biological process, Cellular component and Molecular function.

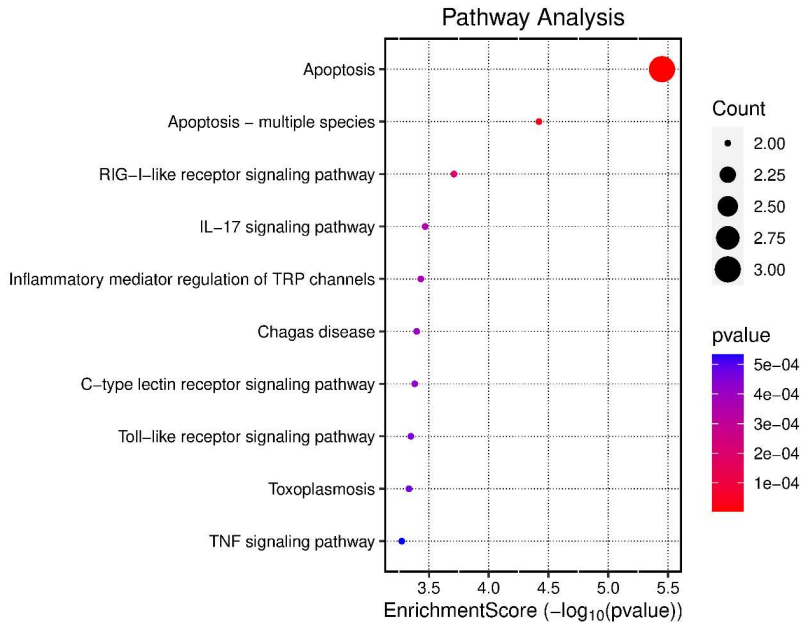
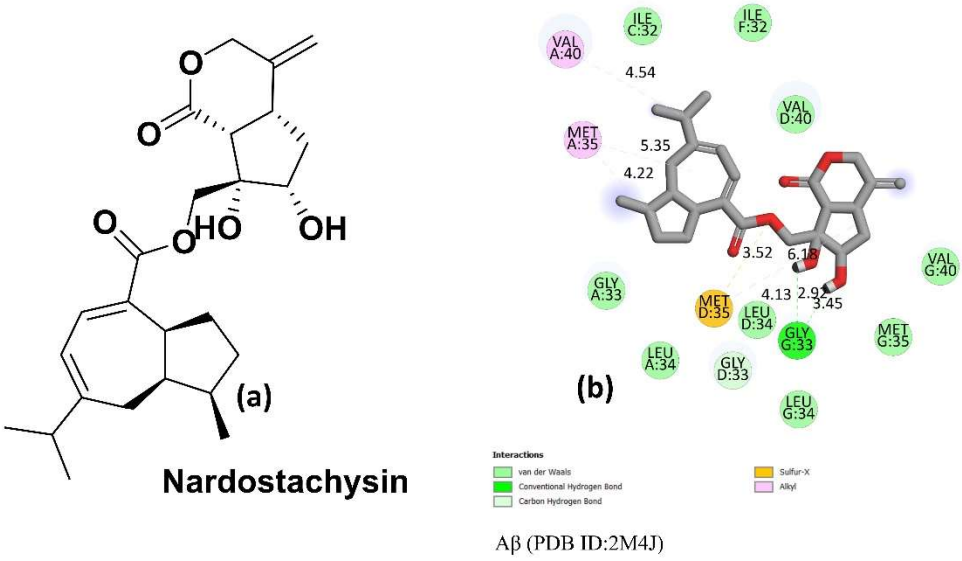
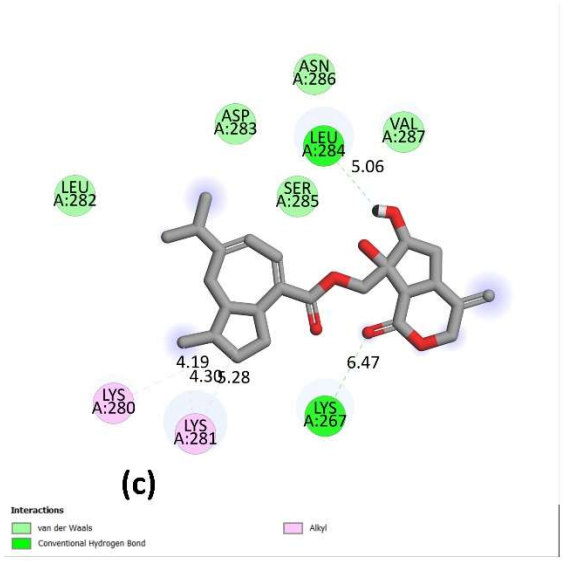


Figure 6: Top 10 KEGG pathway analysis

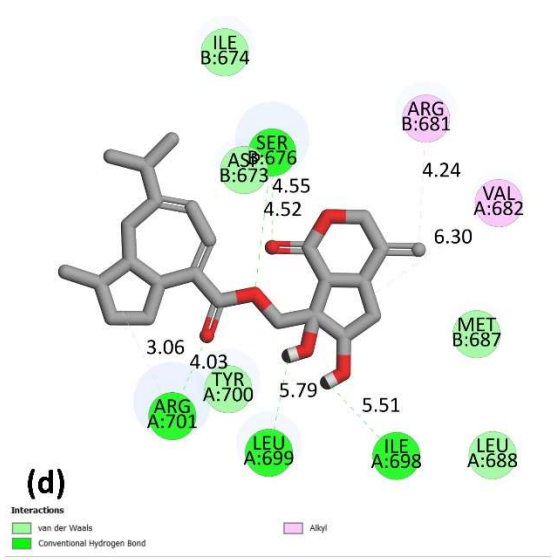
3.4 Molecular Docking analysis.

To understand how nardostachysin interacts with targets involved in Alzheimer's disease (AD) pathology, molecular docking studies were conducted. The results are summarized in **Table 2**. Among the docked targets, Aβ exhibited the lowest binding energy with a docking score of −9.24 kcal/mol (**Figure 7a-f and Table 2**). The analysis revealed that hydrogen bonds played a key role in the interaction between nardostachysin and the target proteins.

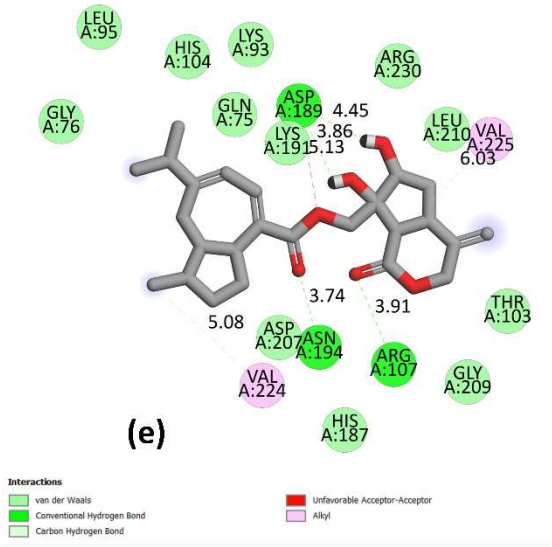




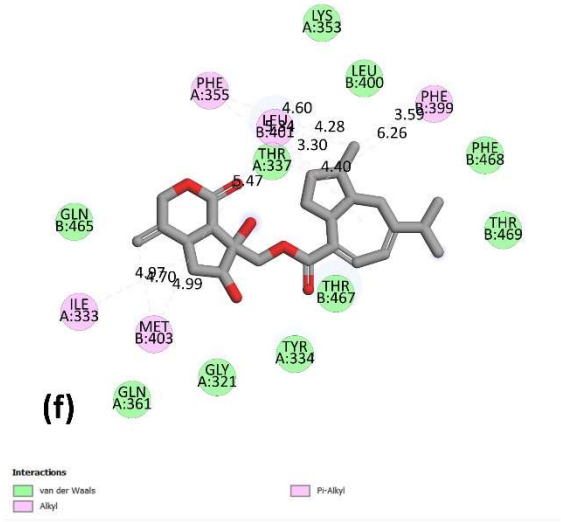
Tau (PDB ID:2MZ7)



NTRK1 (PDB ID:4F01)



MAPK10 (PDB ID:1MPU)



CASP8 (PDB ID:2C2Z)

Figure 7: a) Nardostachysin b) Aβ c) h-tau d) NTRK1 e) MAPK10 f) CASP8

Table 2: Protein with their energy binding score and amino acid residue involved.

Protein	Energy binding Score (Kcal/mol)	Amino acid Residue

Aβ	-9.24	GLY33, LEU34, MET35, VAL40, ILE32
h-tau	-8.4	LEU284, LYS267, SER285, VAL287, LEU282, LYS280, LYS281
NTRK1	-9.1	ARG701, LEU699, ILE698, SER676, LEU688, TRY700, MET687, ASP673
MAPK10	-7.7	ASP189, ASN194, ARG107, GLY209, GLN75, LYS93, HIS104, LEU95, GLY76
CASP8	6.93	GLN361, GLY321, TRY334, THR467, THR469, PHE468, THR337, GLN465, LEU400

Discussion

Alzheimer's disease (AD), the most common form of dementia in the elderly, poses significant challenges for those affected[15]. Many plant-derived compounds have shown potential in combating AD[16]. This study aimed to explore the therapeutic potential of nardostachysin against AD using a integrated in silico approach. Our findings suggest that nardostachysin may mitigate pathological processes by modulating key pathways, including apoptosis, RIG-I-like receptor signaling, IL-17 signaling, inflammatory mediator regulation of TRP channels, C-type lectin receptor signaling, Toll-like receptor signaling, and TNF signaling. The top proteins found with help of network pharmacology indicate complex interplay and crosstalk at molecular level suggesting evidence in favor of further research. The interplay among several critical proteins—Aβ, tau, NTRK1, MAPK10, CASP3, CASP6, CASP9, TNF, DRD3, and PRKCD—drives the progression of this condition in complex ways. At the heart of the disease, Aβ oligomers trigger the abnormal hyperphosphorylation of tau through kinases like GSK-3β and MAPK. This interaction sets off a damaging cycle of synaptic failure and neuronal death, which becomes a hallmark of AD pathology[17]. Adding to this, the decline in NTRK1 signaling, induces mouse cognitive impairment and hippocampal neuronal damage through mitophagy suppression via inactivating the AMPK/ULK1/FUNDC1[18]. MAPK10, exacerbates the problem by responding to cellular stress. Its overactivation promotes both tau hyperphosphorylation and amyloid formation, creating a bridge between oxidative stress and neuronal damage[19]. Simultaneously, the activation of caspases, especially CASP3, CASP6, and CASP9 leads to widespread neuronal apoptosis [20]. CASP6 plays a particularly damaging role by cleaving tau, producing toxic fragments that worsen the disease [21]. Aβ further aggravates this by disrupting mitochondria, activating CASP9, which sets off a cascade involving CASP3 and CASP6, leading to programmed cell death [22].

Neuroinflammation adds another layer to this intricate network. Elevated levels of tumor necrosis factor-alpha (TNF) fuel this inflammatory response, amplifying Aβ production and tau pathology through the activation of pathways like NF-κB and MAPK [23]. Dopaminergic signaling, critical for cognitive health, is also impaired in AD. Aβ disrupts the function of DRD3, a dopamine receptor involved in synaptic plasticity, resulting in cognitive decline and reduced neuroprotection [24]. Meanwhile, PRKCD, a protein kinase activated by oxidative stress, contributes to neuronal loss by promoting both apoptosis and Aβ toxicity, forging yet another link between inflammation and cell death [25]. Together, these proteins form a complex web of interactions where Aβ and tau emerge as central players, linking inflammation, apoptosis, and synaptic dysfunction [26]. Tackling this disease requires a multidisciplinary approach. Enhancing NTRK1 signaling could help protect neurons [27], while targeting caspases or modulating TNF activity may disrupt the destructive cycles of inflammation and cell death [28]. A comprehensive understanding of these molecular relationships holds the key to developing effective therapies that address the root causes of Alzheimer's disease.

Conclusion

In conclusion, we explored the potential anti-AD mechanism of nardostachysin by network pharmacology and molecular docking. Through network pharmacology we identified Aβ, h-tau, NTRK, MAPK10, CASP3, CASP6, CASP8, PRKCD, TNF, DRD3 were the main AD molecular targets. Moreover, molecular docking studies showed that nardostachysin possess the highest binding against aβ, followed by other molecular targets. Therefore, the present study provides a basic understanding of the suggested computational mechanism of nardostachysin against AD.

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Author contributions

AJS. writing, editing and conceptualization; SM. writing and editing; MYD. Writing, editing and resources; NB. writing and editing; RHM. Reviewing, editing and data curation; RG. Writing and reviewing; RM. Reviewing and resources; MHM. Writing, editing and conceptualization.

Declaration of Conflicts of Interests

Authors declare that they have no conflict of interest.

Availability of data and materials.

All data supporting the findings of this study are available within the article. For access to materials and resources, reasonable requests may be directed to the corresponding authors.

Use of Artificial Intelligence

Not applicable

Declarations

Authors declare that all works are original and this manuscript has not been published in any other journal

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