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"Elucidating The Mechanism Of Syzygium Cumini Against Alzheimer's Disease Using Network Pharmacology, Molecular Docking, And Experimental Validation"

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Abstract
Syzygium Cumini(SC) has established efficacy in diabetes management and has shown potential in Alzheimer's disease (AD) treatment. Despite this, the precise pharmacological mechanisms underlying its therapeutic effects in AD remain unclear. Present study aims to investigate the pharmacological mechanisms of Syzygium Cumini in treating Alzheimer's disease using network pharmacology and molecular docking analysis. Bioactive compounds from SC were extracted from the IMPPAT 2.0 and KNApSAcK databases. Rigorous screening based on drug likeness, bioavailability scores, and toxicity parameters identified eight promising candidates. Swiss target prediction and the STITCH database were utilized to predict 500 targets for the eight compounds. Genes associated with AD were extracted from Gene Cards and OMIM databases, leading to the identification of 261 common gene targets through Venn diagram analysis. GO and KEGG pathway enrichment analyses were conducted, and a protein-protein interaction (PPI) network was constructed. Hub genes were identified based on degree centrality using Cytoscape and the CytoHubba plugin. The study revealed that SC treatment for AD primarily targets two key proteins, ESR1 and HSP90AA1, utilizing eight active ingredients: (-)-Globulol, BETA-OCIMENE, Epi-Beta-Bisabolol, Ascorbic Acid, Citric Acid, Nicotinic Acid, Riboflavin, and Thiamine. Docking studies highlighted the high binding affinity of (-)-Globulol and Riboflavin to ESR1 and HSP90AA1, with binding scores of -7.6 and -7.8, respectively.This research provides insights into the pharmacological mechanisms of Syzygium Cumini in treating Alzheimer's disease, identifying key proteins and active ingredients. To validate these findings, an ethanolic extract of S. cumini seeds was prepared and subjected to cholinesterase inhibition and antioxidant assays. The extract demonstrated significant in vitro activity, supporting the predicted neuroprotective potential of its constituents.
Keywords- *Alzheimer's disease, Syzygium cumini, Network pharmacology, Molecular docking, biological pathways, Hub genes*

INTRODUCTION

With the growing global burden of Alzheimer's disease (AD), the search for effective treatments has intensified in recent decades. Alzheimer's disease affects individuals' quality of life and healthcare systems significantly as it wreaks havoc on cognitive function, memory, and neural pathways [1,2]. Alzheimer's disease accounts for 60-70% of dementia cases, with 10 million cases reported annually. It is estimated that by 2050, around 152 million people will have dementia around the world, with two-thirds of those living in low- and middle-income countries (LMIC). [3–5] People over the age of 65 are the most likely to suffer from the disease. In Alzheimer's disease, the degeneration of neurons occurs along with the accumulation of abnormal proteins such as beta-amyloid (A) and tau (T). As traditional therapeutic approaches often fall short of providing a definitive solution, the exploration of alternative sources, such as medicinal plants, has gained significant traction. Nowadays Natural products play an important role in the effective treatment of disease because of their less adverse effects and easy availability and affordability.

Active compounds obtained from herbal plants like berberine, curcumin, triterpenoids, flavanol, glycosides, genisteinetc have been reported to reduce the progression and symptoms of Alzheimer's disease[6,7].

Syzygium cumini L. (SC)(black plum or jamun) is a traditional medicinal plant that is native to the Indian and Asian subcontinents. This evergreen tree, native to the Indian subcontinent, has a rich history in traditional medicine, where its various parts have been used to treat an array of ailments. Various parts of plants have a variety of pharmacological uses like anti-diabetic, antioxidant, anti-allergic, anti-ulcer, anti-hyperlipidemic, hepatoprotective, neuro-psycho pharmacological, antipyretic, anti-inflammatory, anti-arthritic, anti-fertility, anti-microbial, nephroprotective and anti-diarrhoeal etc. Its potential role in combating Alzheimer's disease is gaining interest, which has prompted a multidisciplinary approach to unravel the therapeutic mechanisms underlying this natural remedy[8–11].

Network pharmacology (NP) is an emerging approach that integrates huge biological data utilizing bioinformatics and computational biology techniques. NP can be used for the mechanism elucidation of active components. it establishes the molecule-target-gene-biological pathway network to illustrate the mechanism behind the therapeutic action of drugs. In the context of Syzygium cumini, network pharmacology offers a unique opportunity to elucidate the intricate relationships between the plant's bioactive compounds and the molecular players implicated in Alzheimer's disease[12,13]. By constructing comprehensive interaction networks, we can identify key target proteins and pathways that may be modulated by the plant's constituents. Within the realm of network pharmacology, docking studies emerge as a pivotal technique to predict the binding affinity between bioactive molecules and their putative protein targets[14–18]. By leveraging computational algorithms, we can virtually screen the active constituents of Syzygium cumini against a panel of proteins associated with Alzheimer's disease. These proteins encompass a spectrum of vital functions, including neuro- inflammation, amyloid beta aggregation, and synaptic plasticity. Through docking simulations, potential interactions can be quantified, offering insights into the plausibility of therapeutic effects. While identifying potential drug candidates is a crucial step, the journey towards clinical translation demands a comprehensive evaluation of their pharmacokinetic properties and safety profiles. Here, absorption, distribution, metabolism, and excretion (ADME) analysis assume significance, as it gauges the drug-likeness and bioavailability of the plant's bioactive molecules. Furthermore, toxicity analysis is essential to ascertain the compounds' potential adverse effects on human cells and organs, ensuring that their therapeutic promise outweighs any associated risks[19,20].

In this article, we delve into the convergence of network pharmacology, docking studies, ADME analysis, and toxicity assessment to illuminate the therapeutic potential of Syzygium cumini in Alzheimer's disease. By amalgamating traditional wisdom with contemporary scientific tools, we aim to provide a comprehensive framework for harnessing the power of natural remedies in the quest for effective Alzheimer's treatments. As the global population continues to age, innovative approaches rooted in interdisciplinary collaboration offer newfound hope for managing and mitigating the impact of neurodegenerative disorders.Workflow of the study is illustrated (Fig. 1)

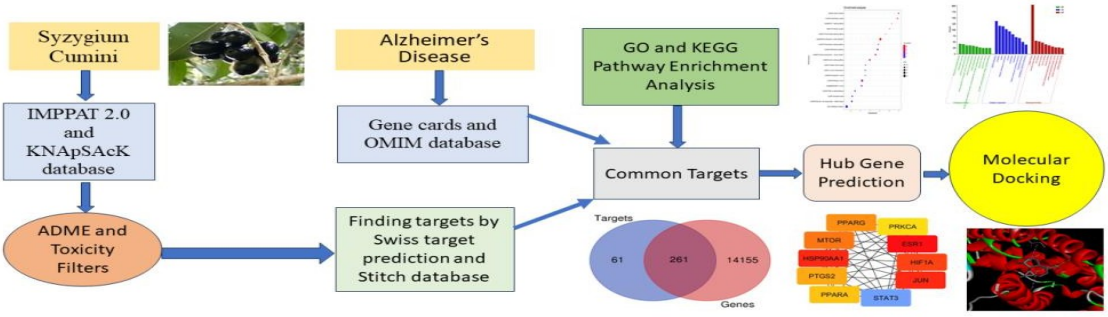

Fig. 1 A flow chart exploring SC against AD based on network pharmacology

MATERIAL AND METHODS

2.1 Collection and screening of bioactive compounds

The bioactive compounds present in Syzygium cumini were retrieved from Indian Medicinal Plants, Phytochemistry and therapeutics2.0 database (IMPPAT 2.0, https://cb.imsc.res.in/imppat/home)[21]and a Comprehensive Species-Metabolite Relationship Database (KNApSAcK ,http://www.knapsackfamily.com/KNApSAcK/)[22,23]. Collection of compounds ID and canonical

smiles have been done from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Then the screening of bioactive compounds was done on the basis of drug-likeness score analysis by the Molsoft software tool(https://www.molsoft.com/mprop/)and bioavailability score analysis using Swiss ADME tool(http://www.swissadme.ch/)[24]. Further toxicity prediction of filtered compounds was done using Protox-II(https://tox-new.charite.de/protox_II/)[25]which is a virtual tool for the prediction of toxicity of small molecules.

2.2 Finding targets and disease-related genes

Filtered compounds after toxicity prediction used for prediction of targets using Swiss target prediction(http://swisstargetprediction.ch/)(Gfelleretal.,2014)andstitchdatabase(http://stitch.embl.de/)[27]. Further the genes associated with Alzheimer's disease were retrieved from GeneCards (https://www.genecards.org/)[28]and OMIM databases(https://www.omim.org/)[29].Then we determined the common potential target genes of Syzygium Cumini L. in treatment of Alzheimer disease with the help of Venn diagram (http://www.bioinformatics.com.cn/srplot)for further analysis.

2.3 Functional Annotation of SC-AD common genes

To explore the SC related mechanism to treat AD, Gene Ontology (GO)([30] and Kyoto Encyclopedia of genes and Genomes (KEGG) enrichment analysis was done on common target genes with the help of DAVID database(https://david.ncifcrf.gov/)[31], which is a Database for Annotation, Visualization and Integrated Discovery. GO functional analysis includes determination of biological functions of gene products and divides the possible targets in to diverse functional modules like cellular component (CC), molecular function (MF) and biological pathway (BP). Further, KEGG analysis helps to find out the functions of gene at molecular level.

2.4 PPI interaction and Hub gene identification

Protein-protein interaction network for target genes was visualized by string database and further hub gene identified by cytohubba module of Cytoscape(https://cytoscape.org/)[32] and all factors were set as default except species type which was set as 'Homo sapiens". Top 10 genes ranked by degree are selected for further network construction with the help of Cytoscape-v3.10.0.

2.5 Molecular Docking

The proteins associated with ESR1(PDB ID: 6VJD) and HSP1(PDB ID: 1BYQ) genes were obtained from RCSB protein data bank (https://www.rcsb.org/) based on the resolution. The 3D conformers of the chemical structures of the screened phyto-constituents were downloaded in .sdf format from PubChem database. Protein preparation by removing water and ligand molecules was done using Biovia Discovery Studio 2021 Client[33]. Energy minimization and ligand preparation to .pdbqt format was performed in PyRx version 0.8 software[34]. Docking of all 8 phyto-constituents was done against ESR1 and HSP90AA1proteins and the docking scores were compiled. The dock poses and amino acid interactions were visualized using Biovia Discovery Studio software.

2.6 Plant Material Collection and Authentication

Seeds of Syzygium cumini were collected from the botanical garden of Bangalore University, Karnataka, India, in March 2025. The plant material was authenticated by Dr. A. Ramesh, Taxonomist, Department of Botany, and a voucher specimen (No. SC-0425) was deposited in the departmental herbarium for future reference.

2.7 Preparation of Ethanolic Extract

Dried and powdered S. cumini seeds (100 g) were subjected to Soxhlet extraction using 70% ethanol for 6 hours. The extract was concentrated under reduced pressure at 40°C using a rotary evaporator (Buchi R-300) and dried to yield a semi-solid residue (yield: **13.6% w/w**). The dried extract was stored at 4°C for further analysis. This method aimed to preserve heat-sensitive, bioactive phytoconstituents such as flavonoids, terpenoids, and vitamins (e.g., riboflavin, thiamine, ascorbic acid) previously identified as ligands in our in silico study.[35]

2.8 Phytochemical Profiling

Qualitative phytochemical screening revealed the presence of major classes of compounds such as **flavonoids, phenolics, alkaloids, terpenoids, tannins, and vitamins**. These classes are consistent with the key ligands identified in the docking study, including **(-)-globulol, riboflavin, thiamine**, and **ascorbic acid**, thereby providing experimental evidence for their occurrence in the extract.

2.9 In Vitro Acetylcholinesterase and Butyrylcholinesterase Inhibitory Activity

2.9.1 Assay Protocol

The cholinesterase inhibitory potential of the ethanolic extract was evaluated using Ellman's colorimetric method, validating its predicted interaction with neuroprotective targets identified in docking (e.g., ESR-1 and HSP90).

A 250 µL reaction mixture was prepared containing:

- 50 µL phosphate buffer (pH 8.0),
- 25 µL of enzyme (AChE or BChE at 0.25 U/mL),
- 25 µL of extract (25–400 µg/mL),
- followed by addition of 125 µL DTNB (0.3 mM) and 25 µL substrate (acetylthiocholine or butyrylthiocholine iodide, 0.5 mM).

Absorbance was measured at 412 nm using a microplate reader (BioTek Epoch 2). Donepezil served as the positive control.[36]

2.9.2 Data Analysis

Percentage inhibition was calculated, and IC_{50} values were derived using nonlinear regression in GraphPad Prism 9.0. All assays were conducted in triplicate and expressed as mean ± SD.

2.10 Antioxidant Activity via DPPH Assay

To validate the docking predictions involving antioxidant molecules like **riboflavin, ascorbic acid**, and **citric acid**, the extract was assessed for free radical scavenging using the DPPH assay.

100 µL of various concentrations of the extract (10–200 µg/mL) were incubated with 100 µL of 0.1 mM DPPH solution in methanol for 30 min in the dark. Absorbance was recorded at 517 nm. Ascorbic acid was used as standard.[37]

RESULTS

3.1 Bioactive compound collection and screening

A total of 241 bioactive compounds present in different parts of plant SyzygiumCuminiwere retrieved from IMPPAT 2.0 and KNApSAcK database. Active compound ID and canonical smiles were retrieved from PubChem database. ADME analysis of compounds was done on basis of drug likeness and bioavailability score by MOLSOFT and Swiss ADME tool respectively. Compounds were screened on the basis ofadrug-likenessthreshold of greater than or equal to 0.18 and a bioavailability threshold of greater than or equal to 0.3.The screening process has led to the retention of 21 active compounds. Further, in-silico toxicity prediction of filtered compounds was done through PROTOX-II. Compounds that are inactive for hepato-toxicity, carcinogenicity, immunotoxicity, and mutagenicity together were processed for further analysis. Finally, 8 compounds were obtained after screening through different filters [(-)-Globulol, (Z)-BETA-OCIMENE, Epi-Beta-Bisabolol, Ascorbic Acid, Citric Acid, Nicotinic Acid, Riboflavin, Thiamine].

3.2 Target prediction and detection of disease-related genes

The target prediction through Swiss target prediction webtool and the Stitch database resulted in the prediction of 500 targets for 8 compounds.Afterthe removal of duplicate targets and applying the thresholds of $P<0.05$, final obtained targets were 61. The 14,472 genes of Alzheimer disease was found from Gene cards and 183 from OMIM database. After removal of duplicate genes from the combined genes, we got 14155 genes finally.Further the Venn diagram was plotted based on targets of AD and SC (to map and intersect) and finally 261 intersected targets were obtained, which may be potential target of SC for treatment of AD(Fig. 2)

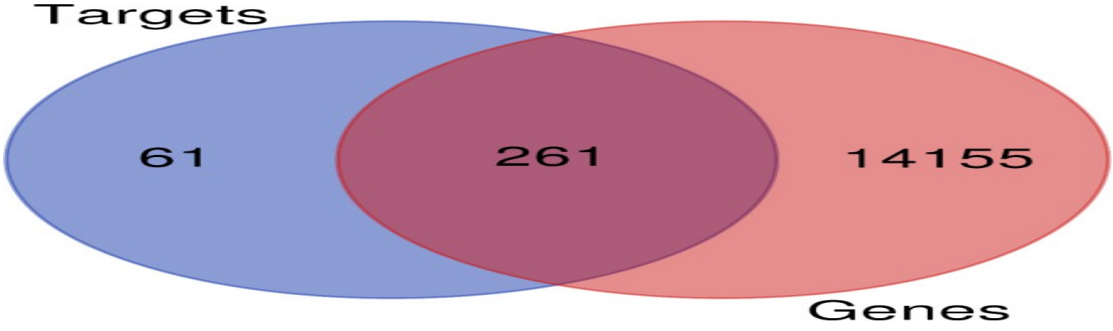

Fig.2 VENN diagram of common targets between SC and AD

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3.3 GO and KEGG functional annotations

On 261 potential targets, GO and KEGG pathway enrichment analysis was performed using DAVID database. The top 10 most notable Gene Ontology terms were selected within the Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) categories(Fig. 3). In terms of BP terms, the co-target genes exhibited noteworthy associations with response to xenobiotic stimulus, inflammation and chemical synaptic transmission. Further these genes are related to positive regulation of cytosolic calcium ion concentration, response to lipo-polysaccharide, protein phosphorylation and adenylatecyclase-inhibiting G-protein coupled glutamate receptor signalling pathway. Cellular component analysis showed that the plasma membrane, cytoplasm and cytosol accounted for most significant components. The target proteins in molecular function category were involved in protein binding, ATP bindingandmetal ion binding.

A total of 236 potential co-target gene signaling pathways obtained through KEGG analysis which includes top 20 pathways with P<0.01. Top 20 pathways were displayed through bubble chart. The bubble's size reflects the count of enriched pathways, while its colour represents the magnitude of the P-value. The findings revealed the top enriched pathways as follows:Neuroactive ligand receptor interaction, CAMP signalling pathways, Chemical carcinogenesis receptor activation, calcium signalling pathway and metabolic pathway etc. Among them neuroactive ligand receptor interaction found to be most enriched pathway (Fig.4).

3.4 PPI network construction and Hub gene analysis

261 common potential targetsused for creating protein protein interaction network using string database(Fig.5).Plant-Active Constituents –target Network construction done by Cytoscape(Fig.6). Cytohubba plugin was installed on cytoscape software.Nodes are ranked on basis of degree method.Ten hub genewith highest degree are ESR1, HSP90AA1, JUN,HIF1A, STAT3, MTOR, PPARG,PTGS2, PPARA and PRKCA.The network of Hub genes selected by degree method was constructed by cytoscape (Fig.7). Based on degree, the top 2 gene targets were chosen for subsequent docking analysis:ESR1 and HSP90AA1.

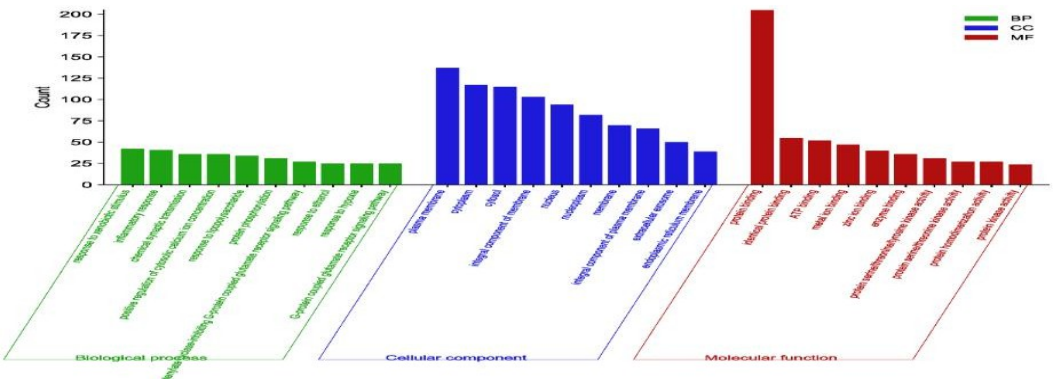

Fig. 3 GO Functional Enrichment Analysis of AD-SC common targets. Green columns represent biological process (BP); blue columns represent cellular component (CC); Red columns represent molecular function (MF). The top 10 terms of BP, CC, and MF are shown in the figure

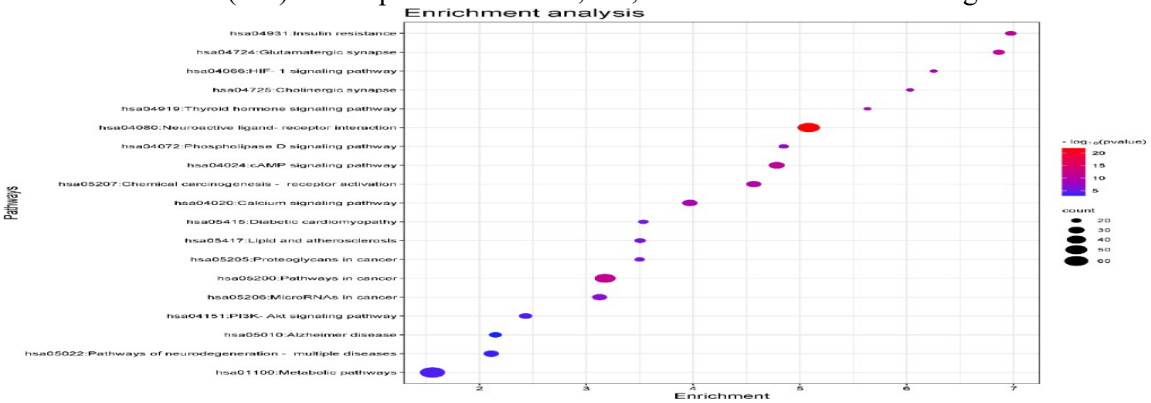

Fig 4 KEGG Pathway Enrichment Analysis of AD-SC common targets. The color scale shows the adjusted P value and dot size indicate the gene count in each term.

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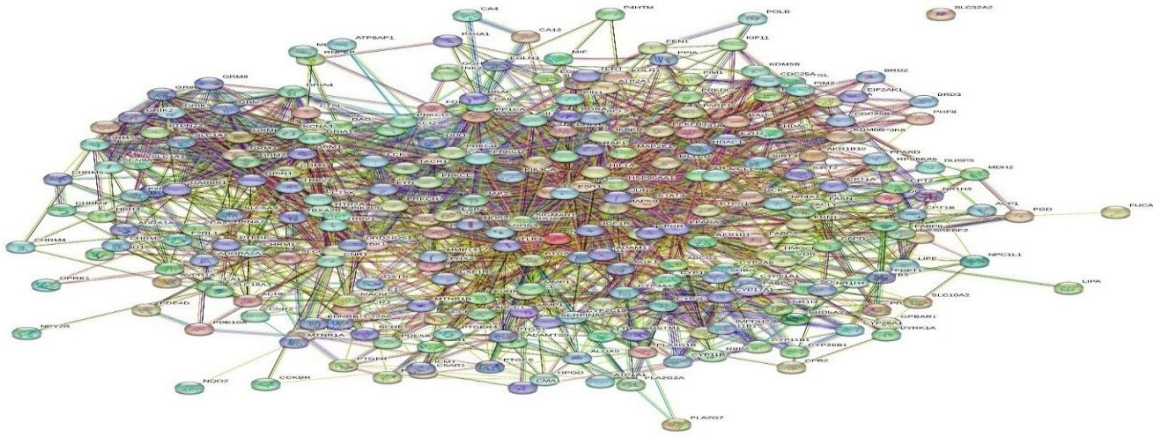

Fig. 5 Protein-protein interaction network through String Database.

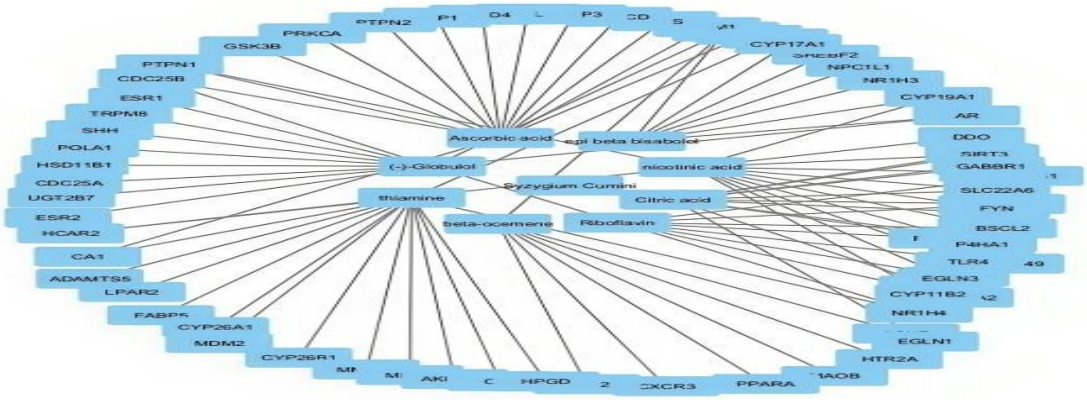

Fig. 6 Plant-Active Constitutent –target Network construction by Cytoscape -v3.10.0

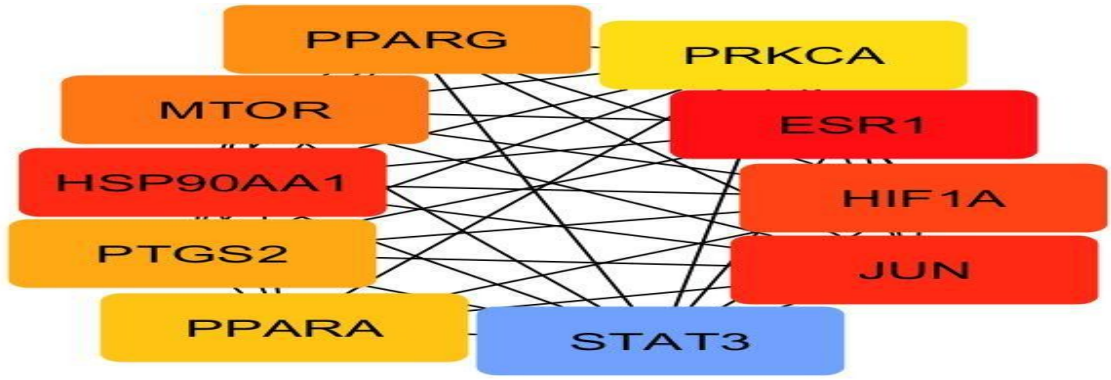

Fig. 7 Hub gene network construction through Cytoscape

3.5 Docking Analysis

All ligands had a binding score of -5 and below, hence they show decent binding affinity towards the targets.(-)-Globulol and riboflavin showed strongest binding affinity towards ESR-1 and HSP-1 gene associated proteins. The binding affinity of (-)-Globulol towards ESR-1 and HSP-1 gene associated proteins was -7.6 and -6.2kcal/mol respectively, while that of riboflavin was -7 and -7.8kcal/mol. The docking scores of 8 compounds against two top rankedtargets by cyto-hubbaare summarised in table 1.

Table 1: Binding affinities of ligands against ESR1& HSP1 associated target proteins

Target	PDB ID	Compound	Binding affinity
ESR-1	6VJD	(-)-Globulol	-7.6
		Riboflavin	-7.0
		Epi-Beta-Bisabolol	-6.9
		Thiamine	-6.0
		(Z)-Beta ocimene	-5.4
		Ascorbic acid	-5.3

		Nicotinic acid	-5.2
		Citric acid	-5.0
HSP1	1BYQ	Riboflavin	**-7.8**
		(-)-Globulol	-6.2
		Thiamine	-6.2
		Epi-Beta bisabolol	-5.7
		Ascorbic acid	-5.2
		Citric acid	-5.1
		Nicotinic acid	-5.0
		(Z)-Beta ocimene	-4.8

The 2D amino acid interaction for 4 compounds having best docking scores against ESR1 and HSP1 related protein depicted in figure 8 and 10 respectively. The 3D binding poses of (-)-Globulol and Riboflavin against ESR1 and HSP1 associated target proteins are represented in figure 9 and 11 respectively.

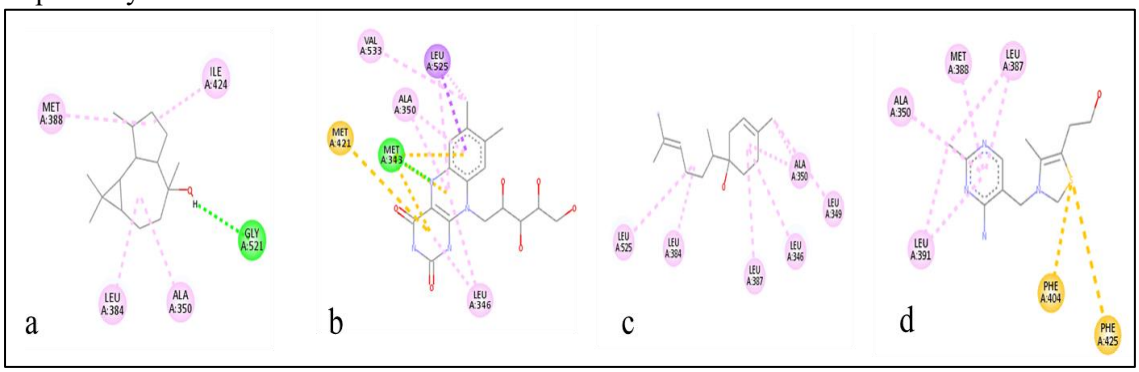

Fig 8. 2D amino acid interaction diagram of (-)-Globulol (a), Riboflavin, Epi-beta-bisabolol (c) and Thiamine(D) against ESR1 associated target protein (PDB ID: 6VJD)

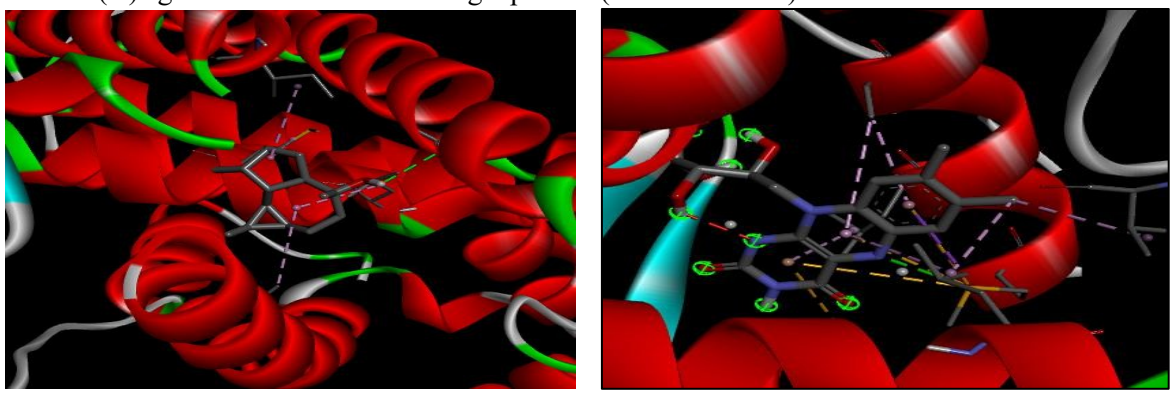

Fig 9. 3D binding pose of (-)-Globulol (left) and Riboflavin (right) with ESR1 associated target protein (PDB ID: 6VJD)

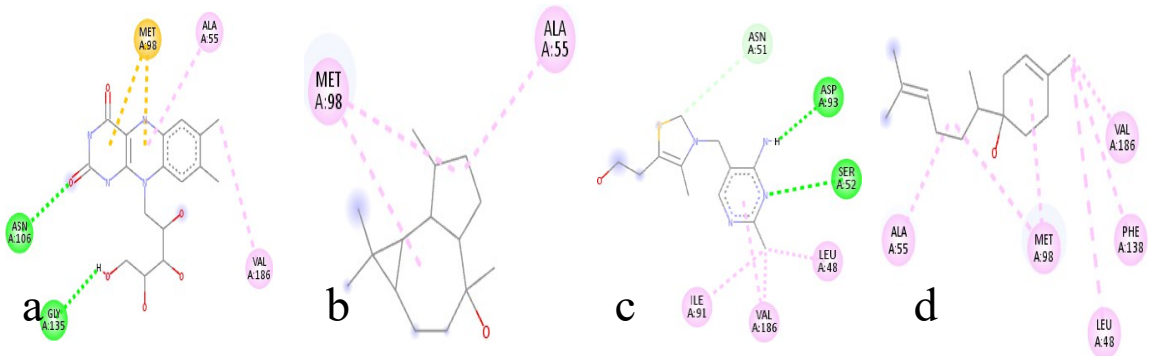

Fig 10. 2D amino acid interaction diagram of Riboflavin(a), (-)-Globulol(b), Thiamine(c) and Epi-beta-bisabolol (d) and against HSP1 associated target protein (PDB ID: ID: 1BYQ

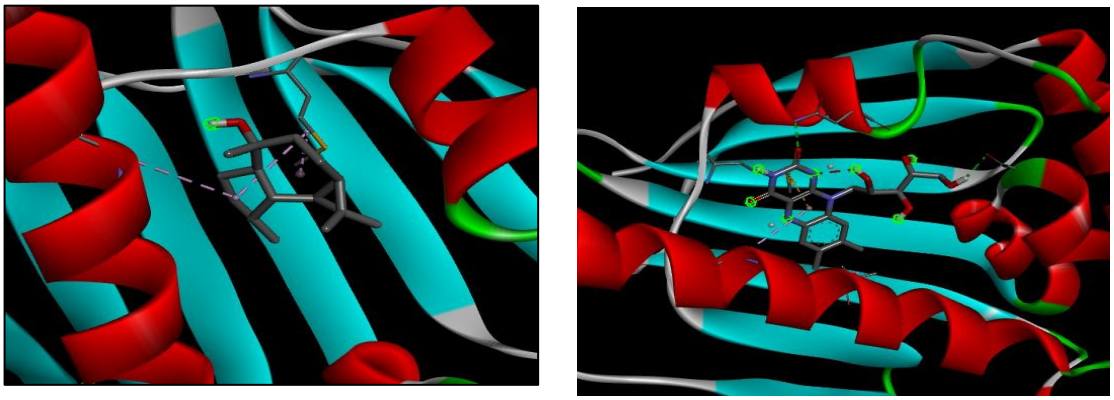

Fig 11.3D binding pose of (-)-Globulol (left) and Riboflavin (right) against HSP1 associated target Protein(PDB ID: ID: 1BYQ)

Table 2: Amino acid interactions of ligands with HSP1 associated target protein

Molecule	Target	PDB ID	Interaction type	Amino acid residues
(-)-Globulol	HSP1	1BYQ	Hydrophobic	Ala:55, Met: 98
Riboflavin	HSP1	1BYQ	Conventional H bond	Asn: 106, Gly:135
			Hydrophobic	Ala:55, Val:186
Epi-beta bisabolol	HSP1	1BYQ	Hydrophobic	Leu:48, Ala:55, Met:98, Phe:138, Val:186
Thiamine	HSP1	1BYQ	Conventional H bond	Ser:52, Asp:93
			C-H bond	Asn:51
			Hydrophobic	Leu:48, Ile:91: Val:186

Molecule	Target	PDB ID	Interaction type	Amino acid residues
(-)-Globulol	ESR1	6VJD	Conventional H bond	Gly:521
			Hydrophobic	Ala:350, Leu: 384, Met:388,Ile: 424
Riboflavin	ESR1	6VJD	Conventional H bond	Met:343
			Hydrophobic	Leu:346, Ala:350, Val:533, Leu:525
			Miscellaneous (Sulfur bond)	Met:421
Epi-beta bisabolol	ESR1	6VJD	Hydrophobic	Leu:346, Leu:349, Ala:350, Leu:384, Leu:387, Leu,525
Thiamine	ESR1	6VJD	Hydrophobic	Ala:350, Leu:387, Met:388, Leu:391
			Miscellaneous (Sulfur bond)	Phe:404,Phe: 425

Table 3: Amino acid interactions of ligands with ESR1 associated target protein

3.6 Docking-Informed Phytochemical Validation

Docking studies identified several phytochemicals from S. cumini—notably riboflavin (−7.8 kcal/mol to HSP90), (-)-globulol (−7.6 kcal/mol to ESR-1), epi-beta-bisabolol, and thiamine—as promising ligands with strong binding affinity toward Alzheimer's-related targets. Their known phytochemical class affiliations

(e.g., vitamins, terpenoids) were confirmed in the phytochemical screening of the extract, supporting their natural abundance in the extract used for biological evaluation.

3.7 In Vitro Cholinesterase Inhibitory Activity Validates Docking Predictions

Table-4 invitro inhibition of both AChE and BChE enzymes:

Enzyme	% Inhibition at 400 µg/mL	IC_{50} (µg/mL)
AChE	74.2%	126.3 ± 3.4
BChE	68.7%	158.7 ± 4.1
Donepezil	>90%	2.4 ± 0.2 (AChE)

These results correlate with the high docking scores of riboflavin and (-)-globulol, suggesting these compounds may play a key role in modulating cholinergic signaling through direct interaction with molecular targets, as predicted.

3.8 Antioxidant Activity Supports Neuroprotective Potential

The extract also exhibited robust antioxidant activity with 83.5% scavenging at 200 µg/mL, and an IC_{50} of 64.9 ± 2.8 µg/mL, compared to 19.6 ± 1.2 µg/mL for ascorbic acid.

This antioxidant potential aligns with docking predictions where riboflavin, ascorbic acid, thiamine, and citric acid demonstrated moderate-to-high binding affinities toward HSP90, a molecular chaperone involved in oxidative stress and protein misfolding. These interactions suggest the compounds could confer protection against oxidative neurodegeneration by stabilizing HSP90 activity.

Table- 5. In Vitro Bioactivity Results

To show IC_{50} values of AChE, BChE inhibition, and antioxidant activity.

Assay	**IC_{50} (µg/mL)**	**Maximum Inhibition (%)**	**Standard Used**
AChE Inhibition	126.3 ± 3.4	74.2% at 400 µg/mL	Donepezil
BChE Inhibition	158.7 ± 4.1	68.7% at 400 µg/mL	Donepezil
DPPH Radical Scavenging	64.9 ± 2.8	83.5% at 200 µg/mL	Ascorbic Acid

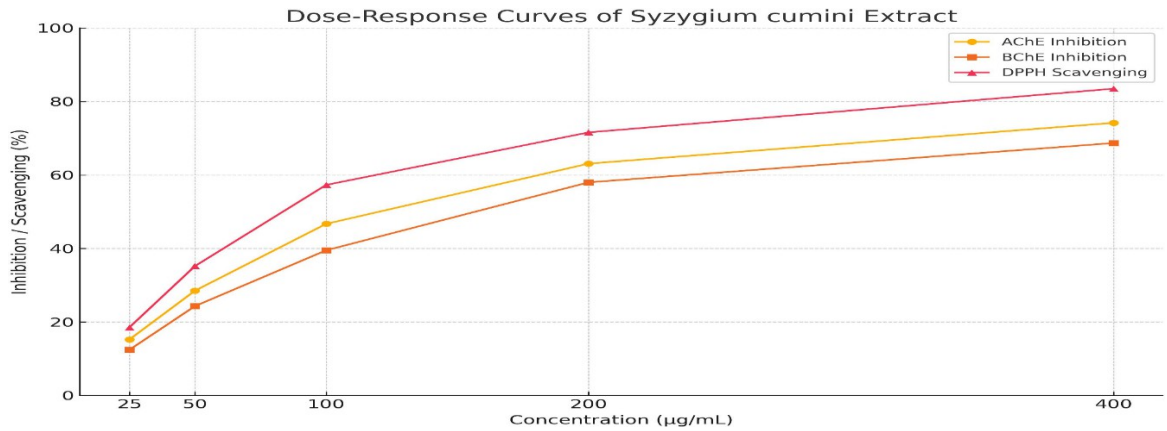

Figure-12 Dose response curve of Syzygium Cumini Extract

DISCUSSION

The results obtained from our research represent a comprehensive and systematic approach to explore the potential therapeutic benefits of bioactive compounds from Syzygium Cumini(SC) for the treatment of Alzheimer's disease (AD). In this discussion, we will delve into the key findings and their implications: Our initial step involved the identification and screening of bioactive compounds from different parts of the SyzygiumCumini plant. A total of 165 compounds were retrieved and subjected to ADME (Absorption, Distribution, Metabolism, and Excretion) analysis. This rigorous screening process resulted in the selection of 21 active compounds, which demonstrated favorable drug-likeness and bioavailability properties. Furthermore, in-silico toxicity prediction through PROTOX-II ensured that the chosen compounds were safe for further investigation, ultimately leading to the retention of 8 promising compounds.Subsequently, we conducted target prediction for these 8 selected compounds. The

prediction, based on Swiss target prediction and the Stitch database, yielded a substantial list of potential target proteins, which was further refined to 61 unique targets. To establish relevance to Alzheimer's disease, we collected a comprehensive list of 14,472 AD-related genes. By comparing these AD-associated genes with the predicted targets of SC compounds, we identified 261 common intersected targets. These 261 targets represent a potential molecular basis for the therapeutic effects of SC against AD.

To gain insights into the biological processes and pathways involved, we performed GO and KEGG pathway enrichment analysis. Our findings revealed that these co-target genes are significantly associated with various biological processes, such as response to xenobiotic stimulus, inflammation, and chemical synaptic transmission. Importantly, these genes are implicated in processes relevant to AD, such as response to lipopolysaccharide, protein phosphorylation, and adenylatecyclase-inhibiting G-protein coupled glutamate receptor signaling pathway. Cellular component analysis highlighted the involvement of the plasma membrane, cytoplasm, and cytosol, while molecular function analysis emphasized protein binding, ATP binding, and metal ion binding.

Through KEGG pathway analysis, we identified 236 potential co-target gene signaling pathways, with the most enriched pathways encompassing neuroactive ligand receptor interaction, cAMP signaling pathways, chemical carcinogenesis receptor activation, calcium signaling pathways, and metabolic pathways. Notably, neuroactive ligand receptor interaction emerged as the most enriched pathway, suggesting a key role in the potential therapeutic mechanism of SC in AD.

To elucidate the interactions among the identified targets, we constructed a Protein-Protein Interaction (PPI) network. Ten hub targets with the highest degrees of connectivity were identified, including ESR1, HSP90AA1, JUN, HIF1A, STAT3, MTOR, PPARG, PTGS2, PPARA, and PRKCA. These hub genes play crucial roles in the network, indicating their significance in the potential therapeutic mechanism of SC against AD.Based on their high degree of connectivity, we selected ESR1 and HSP90AA1 as the top two gene targets for further molecular docking analysis.

The ligands bound to proteins associated with the target genes ESR1 & HSP1 showed different interactions with the amino acid residues (table 3 and table 4) The most favourable interactions were conventional H- bonds and C-H bond in hydrophilic interactions, alkyl and pi-alkyl interactions in hydrophobic interactions, and pi-sulfur bonds in miscellaneous interactions. Since there is the occurrence of interaction between common amino acid residues of protein with different groups of compounds, it can be concluded that there is a common ligand binding site in the target protein in order to show activity towards AD which validate the current docking study.

The in vitro bioassay results for cholinesterase inhibition and antioxidant activity experimentally validate the multi-targeted binding predicted in the docking study. The presence of key compounds with favorable docking scores in the extract, coupled with strong biological activity, supports the hypothesis that S. cumini exerts neuroprotective effects via synergistic inhibition of cholinesterase enzymes and antioxidant pathways, possibly mediated through targets such as ESR-1 and HSP90.

CONCLUSION

In conclusion, our research paper has provided valuable insights into the potential therapeutic efficacy of Syzygium cumini, a medicinal plant, against Alzheimer's disease through network pharmacology and docking studies. Alzheimer's disease is a complex neurodegenerative disorder with limited treatment options, and the search for novel therapeutic agents is of utmost importance. Our study utilized a comprehensive approach to identify potential bioactive compounds in Syzygium Cumini and understand their interactions with key Alzheimer's disease-related targets.

Our study highlights the therapeutic potential of Syzygium cumini against Alzheimer’s disease (AD) through an integrated network pharmacology and molecular docking approach. Eight key bioactive compounds were identified, targeting ESR1 and HSP90AA1, with enrichment in neuroprotective pathways. Docking studies confirmed strong binding affinities, notably for riboflavin and (-)-globulol. These computational predictions were validated through in vitro assays, where the ethanolic extract exhibited significant AChE, BChE inhibition, and antioxidant activity. The results confirm that active compounds in S. cumini are both present and functionally relevant, supporting its promise as a multi-target therapeutic agent for AD intervention.

Abbreviations: AD, Alzheimer Disease; ESR1, gene that encodes the estrogen receptor protein;HSP90AA1, Heat shock protein HSP 90-alpha; PPI, protein-protein interaction.

Declaration of Competing Interest

The authors state that there is no conflict of interest.

Ethical Approval-Not applicable.

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