International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 1s, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Network Pharmacology for Identifying Bioactive Compounds in Syzygium cumini with Multi-Targeting Potential for Diabetes and Cardiovascular Disease

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Received: 17.05.2024 Revised: 12.06.2024 Accepted: 25.07.2024

ABSTRACT
Plant-based medicinal product usage has increased throughout the world for various chronic disorders. Syzygium cumini, a traditional medicinal plant, shows promise in managing diabetes mellitus and cardiovascular disease. This study employs an integrated network pharmacology approach to explore its bioactive compounds' potential. Disease-related genes for diabetes and cardiovascular disease were identified. Functional enrichment analysis and network construction were performed to understand the molecular mechanisms. Six bioactive compounds (Sitosterol, Betulinic acid, crategolic acid, quercitin, kaempferol) were identified, with 211 key target genes associated with diabetes and cardiovascular disease. Crategolic acid, Sitosterol, and kaempferol showed high connectivity. Protein-protein interaction analysis revealed IL6, SRC, ESR, and MAPK3 as key targets. Molecular docking analysis supported potential interactions. It demonstrates the utility of network pharmacology for identifying active compounds and key target genes in traditional medicine. These findings offer insights for drug discovery and further validate the historical use of Syzygium cumini for diabetes-related conditions.

Keywords: Diabetes, Docking, Genes, Network Pharmacology, Syzygium cumini

INTRODUCTION

Herbal medicine use has a long history in various chronic illnesses. Recent advancements in contemporary therapeutics have encouraged the use of natural products for a variety of diseases and disorders all over the world. [1, 2]The therapeutic benefits of herbs are of great interest to the educated public and the medical community. Still, there is much uncertainty regarding their identification, efficacy, therapeutic dosage, toxicity, standardization, and regulation.[3] Traditional medicine is widespread worldwide, and its usage is increasing even in affluent nations, according to the WHO.[4, 5]Patients with chronic difficulties have received many medication prescriptions, leading to polypharmacy.[6]About 60% of people utilize traditional medicines made from medicinal plants. Herbal drugs and plants are used to treat Diabetes since Diabetes is a severe multi-factorial illness that affects many people worldwide from all areas of life.[7-9] It is proven to be a severe health issue all over the world. However, several ways exist to lessen Diabetes's adverse effects and subsequent complications.[10] Plant-based medicines and herbal are preferred since they have less side effects and are less expensive. These are the common plants used to treat diabetes:Syzygium cumini Allium sativum, Eugenia jambolana, Momordica charantia, Ocimum sanctum, Phyllanthus amarus,Pterocarpus marsupium, Tinosporacordifolia, Trigonellafoenum graecum, and Withaniasomnifera.[11]Syzygiumcumini (S. cumini) (L.) Skeels (jambolan) are widely used to treat diabetes since jambolan is rich in anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferolmyrecetin, etc.[12, 13]Hence the present study is aimed to estimate the list of bioactive compounds and impact in the management of Diabetes mellitus and cardiovascular disease through integrated network pharmacology approach.

METHODOLOGY

Active compounds of Syzygium cumini were retrieved from both the literature and the Indian Medicinal Plants, Phytochemistry Additionally, Therapeutics (IMPPAT) database of biologically active phytochemicals. All bioactive compounds of Syzygium cumini were subjected to virtual screening using the SwissTargetPrediction database. GeneCard was employed to identify diabetes and cardiovascular disease-related targets. Pathway and Functional Enrichment Analysis Database for annotation, visualization, and integrated discovery (DAVID) was utilized to conduct functional annotation and enrichment analysis.Key targets were subjected to DAVID to predict function at three levels: biological process (BP), molecular function (MF), and cellular component (CC).Network analysis was performed using Cytoscape 3.8.0 to understand the mechanism of Syzygium cumini in diabetes mellitus and cardiovascular disease. This freely available graphical user interface enabled importing, visual exploration, and analysis of biomolecular interaction networks.Protein-protein interaction (PPI) networks were constructed using the STRING database for key targets with a combined score exceeding 0.4. The

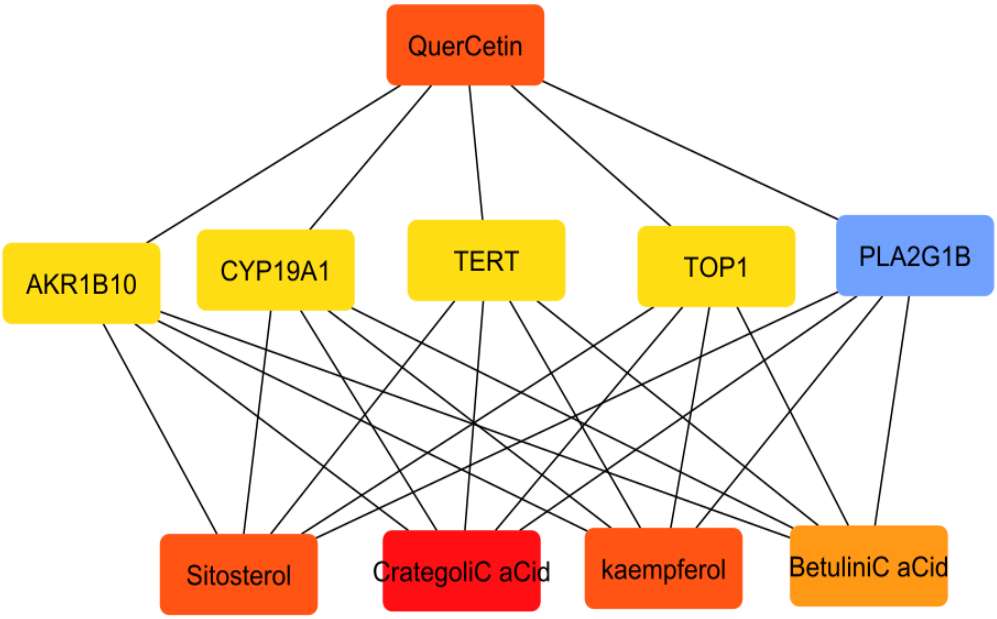

Figure 1. Top ranked genes ranked by degree method for Phytoconstituents.

resulting PPI network was analyzed using the CytoHubba plugin in Cytoscape to identify core regulatory genes and key targets.Major targets of all compounds are listed in Supplementary Excel File 1, and hub genes are provided in Supplementary Excel File 2.

RESULTS AND DISCUSSION

Screening of Active Compounds and Targets

After searching, filtering, and removal of the duplicates, 6 putative components (Sitosterol, Betulinic acid, crategolic acid, quercitin, kaempferol) with F $\geq$ 30% and DL $\geq$ 0.18 were selected. F30 means that the bioavailability is 30%. Bioavailability is the rate and extent to which the active constituent or active moiety of a drug is absorbed from a drug product and reaches the circulation. The basic drug-likeness properties of the compounds were given in Table 1.Compounds that are formulated to have high bioavailability will be more effective, as they will help the body to absorb more of the appropriate nutrient without having to take higher doses. Drug likeness (DL) measures the likelihood of a chemical becoming an oral drug in terms of bioavailability. DL derived from structures and properties of existing drugs and drug candidates has been widely used to filter out undesirable compounds in the early phases of drug discovery. Further, 500 potential target genes of 6 active constituents were retrieved from the Swiss Target Prediction database. After identifying the promising targets of compounds, a total of 8116 genes affiliated with diabetes mellitus and cardiovascular disease were retrieved from GeneCards and OMIM databases.[14] Later, the common targets of both diabetes mellitus, cardiovascular disease and the compound-related genes were predicted through a Venn diagram. A total of 211 potential diabetes mellitus and cardiovascular disease genes of Syzygium cumini were selected and considered as key targets. The Figure 1 shows theTop ranked genes ranked by degree method For Phytoconstituents.

Compounds-Target Network Construction

A total of 5 satisfactory active compounds were obtained from Syzygium cumini. Further, 5 active compounds, 211 key targets, and their associated pathways with a maximum number of genes were chosen for the construction of an 'active compound–targeted genes–connected pathway' network diagram. Each of these active compounds corresponded to multiple targets. This is a strong indication that many targets may induce a synergistic effect when Syzygium cumini serves as an diabetes mellitus and cardiovascular disease agent.

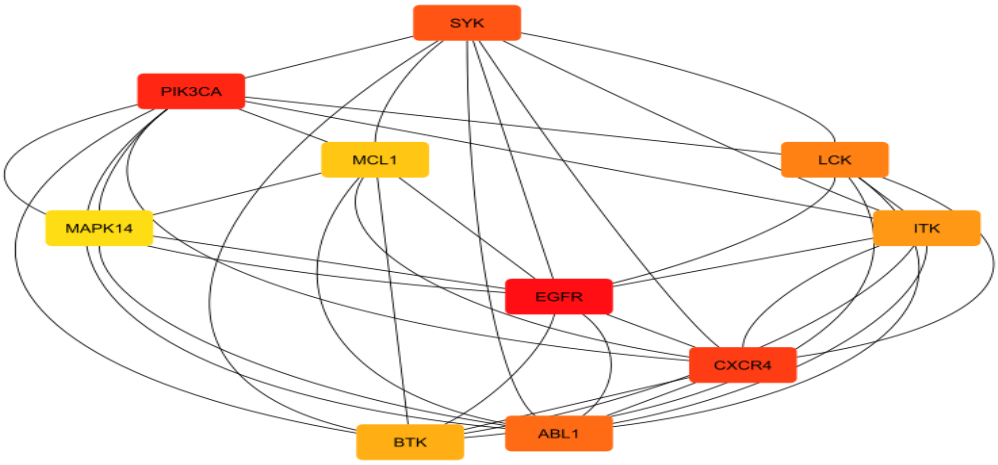

Figure 2. Top ten ranked targets obtained from the PPI network analysis

PPI Network Construction

The 211 overlapped genes were submitted into the STRING database for the construction of the PPI network. In the PPI network, nodes and their associated interactions indicate the interrelationship among multiple targets during disease development. Later, a network analyzer tool was employed for analyzing the PPI network of overlapped genes. The highest degree means that targeted genes are greatly correlated with each other; hence, all these genes might be key targets. After comparing these findings with those supplied by enrichment analysis, four genes, particularly IL6, SRC, ESR, MAPK3, were identified as the main anti-diabetes mellitus and cardiovascular disease targets of Syzygium cumini.[15]The top 10 genes of PPI interaction were given in Table 2.The Top ten ranked targets are depicted in **Figure 2**. The KEGG enrichment pathway of the hub-genes are depicted in **Figure 3**. Followed by Table 3 discuss the Toxicity profile of selected compounds.

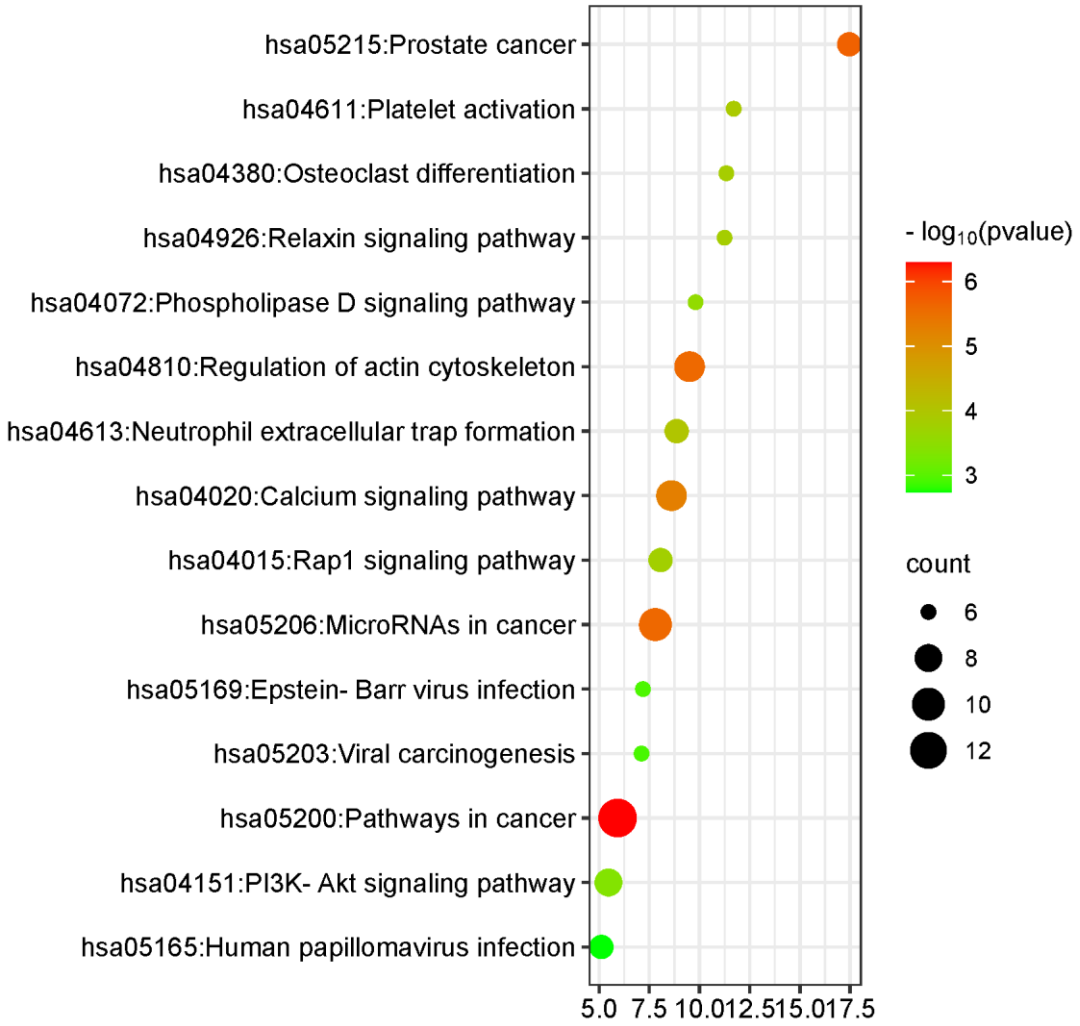

Figure 3.KEGG enrichment pathway

Molecualr docking analysis

Moleculr docking analysis was perfomed through CB-Dock online tool. The compound structures were drawn and optimised. Followed by the Protein was obtained from Protein data bank. (https://www.rcsb.org/). In this current stuyd, PDB:1N26[16] target was chosen to estimate the binding potential. PDB:1N26 is a Crystal Structure of the extra-cellular domains of Human Interleukin-6 Receptor alpha chain. The Pharmacokientic profile of the selcetd compounds were givne in Tabale S4. The molecualr docking reports was given in Table 4. Before the discovery of insulin, numerous plant-based medicinal substances were utilized to treat diabetes mellitus (DM), and several hundred plants have shown antidiabetic efficacy. Traditional healers worldwide mainly rely on medicinal plants and herbs to treat diabetes, even though several different forms of oral hypoglycaemic medicines and insulin are available for treating diabetes. Since ancient times, plant compounds having hypoglycaemics properties have been employed in folk medicine worldwide. Since natural substances have been used for years without showing any hazardous side effects, they have the advantage of not causing side effects when treating diabetes. Additionally, herbal medications prevent glucose swings and safeguard -cells.Syzygium cumini (L.) that have been utilized for millennia by traditional healers to cure diabetes. The clinical evidence shows that the part of Syzygium cumin (L.), mainly fruits, seeds, and stem bark, has significant therapeutic potential on diabetes-related cardiometabolic conditions.The ethanolic extract of seeds of Syzygium cumini shows significant falls in the Fasting blood sugar (FBS) concentration in Alloxan induced mild and severely diabetic rabbits. Followed by there was aimprovement in the histopathology of islets in Alloxan albino rats when treated with ethanolic extract of Syzygium cumini seeds. Not only ethanolic extract, Aqueous extract of seed powder shows a reduction in FBS on STZ induced albino Wistar diabetic rats study. In

human clinical trial the Aqueous and ethanolic extract of seeds in diabetic patients are reported with Signiant therapeutic end points at different concentration. [17]The fruits, seeds, and stem bark of the Syzygium cuminitree have promising antidiabetic properties.[18-21] Through this NP approach, we suggest the major targeted active sites, such as IL6, AKT1, TNF, SRC, EGFR, PPARG, PTGS2, ESR1, HIF1A, and MAPK3 for diabetes.The identification of a network of bioactive compounds through an integrated NP approach helps in the development of novel successful chemical entity.

CONCLUSION

Traditional systems of medicine reveal a strong history of use to support the antidiabetic action of plants. However, the target sites of the bioactive compounds remain questionable. Hence, we performed a network-based estimation of high-degree active sites of the selected bioactive compounds from Syzygium cumini (L.) towards the diabetes target. Through this integrated Network pharmacological approach, identification of active sites for the new chemical entities can be achieved at the earlier stages of Drug discovery.

Declaration

Funding

The present review did not receive any funding.

Availability of data and materials Information

Data collected from open sources.

Declarations Competing interests

The author(s) declare that they have no conflict of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

All the authors have approved the manuscript for publication

Acknowledgment

We would like to thank our institute for providing technical support throughout the study.

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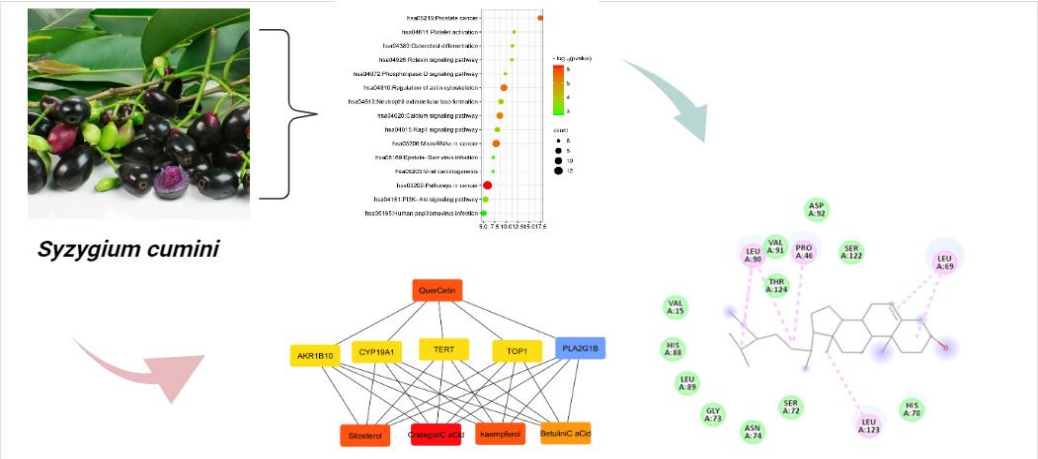


Graphical Abstract

Table 1. Selected Active compounds, their properties

Phyto chemical name	Drug-likeness model score	Bioavailability Score	Molecular weight
Sitosterol	0.29	0.55	414.71 g/mol
Betulinic acid	0.25	0.85	456.70 g/mol
Crategolic acid-	0.55	0.56	472.70 g/mol
quercetin	0.52	0.55	302.24 g/mol
Kaempferol	0.5	0.55	286.24 g/mol

Table 2. Top 10 genes ranked by degree

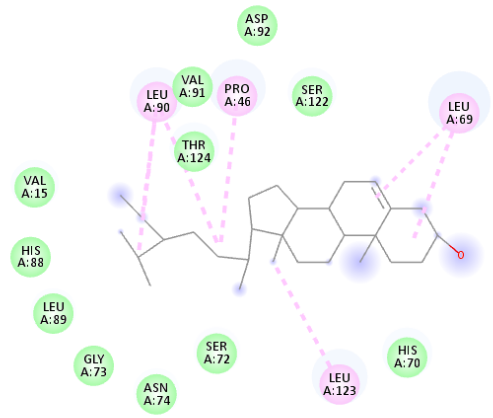
Rank	Name	Score
1	IL6	108
2	AKT1	106
3	TNF	102
4	SRC	91
5	EGFR	85
6	PPARG	83
7	PTGS2	81
8	ESR1	76
9	HIF1A	73
10	MAPK3	68

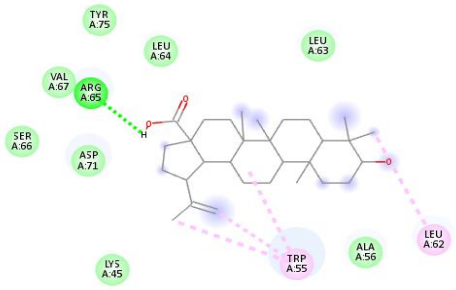
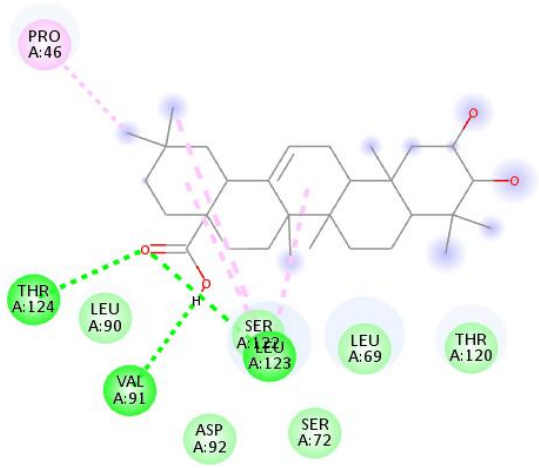
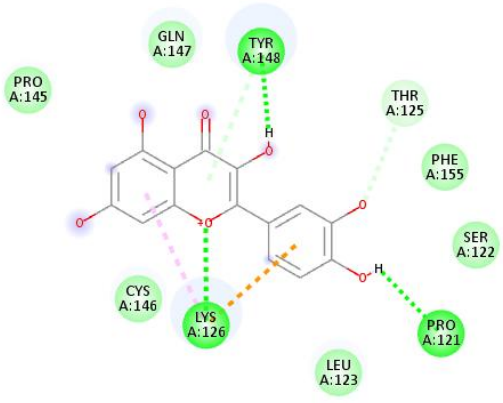
Table 3. Toxicity profile of selected compounds

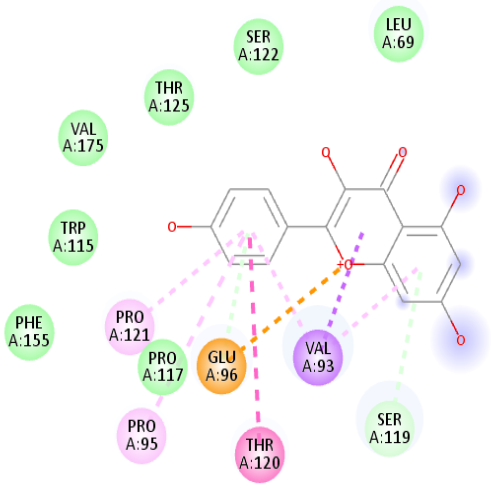
Compound	Predicted LD50	TOXICITY CALSS
Sitosterol	**890mg/kg**	4
Betulinic acid	**2610mg/kg**	5
Crategolic acid	**2000mg/kg**	4
Quercetin	**159mg/kg**	3
Kaempferol	**3919mg/kg**	5

-toxicity class justification.

Table 4. Docking score of selected compounds

Compound	Molecular docking Score towards PDB:1N26 (kcal/mol)	Amino acid residues
Sitosterol	-7.3 	VAL15 PRO46 LEU69 HIS70 SER72 GLY73 ASN74 HIS88 LEU89 LEU90 VAL91 SER122 LEU123 THR124
Betulinic acid	-7.2	LEU43 LYS45 TRP55 ALA56 GLY57 LEU62 LEU63 LEU64 ARG65 SER66 VAL67 ASP71 TYR75

		
Crategolic acid-	-7.5 	LEU69 HIS70 SER72 LEU90 VAL91 ASP92 THR120 PRO121 SER122 LEU123 THR124
quercetin	-6.9 	THR120 PRO121 SER122 LEU123 THR125 LYS126 ALA127 PRO145 CYS146 GLN147 TYR148 SER149 PHE155
Kaempferol	-6.9	LEU69 VAL93 PRO94 PRO95 GLU96 TRP115 PRO117 SER119 THR120 PRO121 SER122 THR125 PHE155 VAL175

	 SER A:122 LEU A:69 THR A:125 VAL A:175 TRP A:115 PHE A:155 PRO A:121 PRO A:117 GLU A:96 VAL A:93 PRO A:95 THR A:120 SER A:119	