Network Pharmacology insights into the Anti-Diabetic Mechanisms of *Syzygium cumini* Seeds in type 2 Diabetes Mellitus

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Abstract
Type 2 diabetes mellitus (T2DM) is a progressive, multipathological and multifactorial disease. Syzygium cumini seeds (SCS) are used in T2DM management; however, the molecular mechanism is still unexplored. Recently, network pharmacology has emerged as a new approach to study the effects of natural products with the various targets underlying diseases. Therefore, the present study investigated the molecular mechanism of SCS in T2DM via a network pharmacology approach. The bioactive compounds present in SCS were retrieved from the published literature indexed in the PubMed and structure queried from the PubChem database. The protein targets of these genes were predicted via the SwissTargetPrediction web tool. The proteins involved in the pathogenesis of T2DM were retrieved from the TherapeuticTargetDisease database.

Among the 10 bioactive compounds of SCS, the top five, on the basis of their degree of interaction with target proteins, are α-terpineol, ferulic acid, quercetin, 3,3',4'-tri-O-methylellagic acid and caffeic acid. KEGG pathway analysis revealed that twenty target proteins associated with SCS-T2DM were linked to 14 pathways among which important pathways are regulation of lipolysis, diabetic cardiomyopathy and the PPAR signaling pathway. This study demonstrated the multicomponent, multitarget, and multi-pathway properties of SCS, which can be used for further research into its mechanism in the treatment of T2DM.

Keywords: Network pharmacology, *Syzygium cumini*, protein-protein interaction, KEGG pathways

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by high blood glucose levels. T2DM can arise from abnormalities in insulin action, secretion, or both[5]. A lack of insulin action is caused by insufficient insulin secretion or a reduced insulin response in the body[2]. This can occur due to either autoimmune destruction of pancreatic cells or increased insulin resistance[2]. Hyperglycemia is the leading diagnostic indicator for diabetes[5]. The eyes, kidneys, nerves, heart, and blood vessels are particularly affected by chronic hyperglycemia in individuals with diabetes which can result in damage, dysfunction, and failure of these organs[2]. The metabolic disorders associated with carbohydrate, fat, and protein metabolism are caused by the insufficient effectiveness of insulin in target tissues.

The result is chronic hyperglycemia and diabetic microvascular along with macrovascular complications associated with increased morbidity and mortality[2,5]. *Syzygium cumini* (L.) Skeels, or *Eugenia jambolana* Lam., is a plant commonly known as Jamun. It is known for its medicinal properties and is utilized in Ayurveda, Unani, and Chinese practices to treat diabetes[14,23]. The plant has various common names including black plum, jamun, java plum, Indian blackberry, Portuguese plum, Malabar plum, purple plum, Jamaica, and damson plum[3]. It is widely distributed in tropical and subtropical regions of India, the Andaman Islands, Sri Lanka, Bangladesh, and Burma. All parts of the plant including seeds, fruits, leaves, flowers, and bark, are used in traditional medicine[14,23].

Syzygium cumini seeds (SCSs) are used in traditional medicine to treat a wide range of ailments such as diabetes, pimples, mouth blisters and stomach aches. SCS powdered seeds mixed with sugar can be taken orally 2–3 times daily to treat dysentery. Additionally, SCS has astringent and diuretic properties[4,6,24]. SCS has been reported to have several pharmacological activities including chemoprotective, hypoglycemic, hyperglycemic, analgesic, anti-inflammatory, antiallergic, antihyperlipidemic, antiplaque, antimicrobial, antidiarrheal, antioxidant, gastroprotective, ulcer healing and antibacterial properties[23]. The scientific and folk medicine literature reports the antidiabetic effects of SCS extract[5,9,22].

There are preclinical and clinical scientific reports on the anti-T2DM properties of SCS. However, the molecular mechanism of SCS in the treatment of T2DM, which is based on a multicomponent, multitarget and multi-pathway mechanism, is still unexplored[20]. Therefore, the present study aimed to investigate the molecular mechanism of SCS in the treatment of T2DM via a network pharmacology approach. This approach involves the use of bioinformatics, cheminformatics, computational biology, systems biology and network-based mathematical tools[27]. This approach is based on the concept of systems pharmacology, which helps us to understand a holistic and systemic view of traditional medicines and considers the interactions between multiple molecules and targets in the body to elucidate several mechanisms involved with herbal medicine[28-30].

The field of network pharmacology is widely recognized as a contemporary approach for identifying active compounds and potential molecular targets in a diverse range of herbs and herbal formulas[17]. Advanced computer technology is used to study the molecular interactions between drug molecules and cells in the body[7]. This approach has become an essential tool for understanding the complex relationships between botanical formulas and the human body[7]. The utilization of the network pharmacology approach aids in the investigation into the mechanisms of action of bioactive compounds in the treatment of diseases[21]. This integration of network pharmacology into traditional medicine provides distinctive and innovative opportunities for the discovery of active compounds, biomarkers, and the scientific foundation underlying traditional medicine, all of which are based on the intricate biological systems of the human body[21].

Material and Methods

Extraction of bioactive components of SCS: The keywords "*Syzygium cumini*" or "*Eugenia jambolana*" were searched for in PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). A database was prepared on the basis of the phytochemicals obtained from the SCS. Structural and chemical data for the phytochemicals were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Phytochemical information not available in PubChem was obtained from the original literature.

Target screening for bioactive phytochemicals and T2DM: Potential targets for the phytochemicals present in SCS were obtained from SwissTargetPrediction (http://www.swisstargetprediction.ch/)[13]. Canonical SMILES sequences of phytochemicals were uploaded to the SwissTargetPrediction database. The selection criterion for targets in SwissTargetPrediction was a probability of greater than 0.01. gProfiler (https://biit.cs.ut.ee/gprofiler/gost) was used to convert the gene ID to a UniProt ID with *H. sapiens* as the criterion. Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to create a Venn diagram. T2DM-related target data was obtained from the TherapeuticTarget Database (TTD) (http:// db.idrblab.net/ttd) database.

Construction of a PPI Network: Common SCS-T2DM targets were imported into the STRING database (https://string-db.org/), with the species set "*Homo sapiens*" and a confidence score greater than 0.90. The STRING network was imported and analyzed via Cytoscape version 3.10.1. The network was analyzed using the network analyzer plugin in Cytoscape 3.10.1. Nodes were analyzed for degree centrality.

KEGG pathway analysis: The database for Annotation, Visualization, and Integrated Discovery (DAVID 2021, https://david.ncifcrf.gov/)[9] was used for KEGG pathway analysis. The KEGG pathway portrays higher-level functions represented by network relationships, reactions, and molecular interactions[15]. Bubble plots of the top enriched KEGG pathways with a significance level of $p<0.1$ were generated via a free online data analysis and visualization tool https://www.bioinformatics.com.cn/en.

Results

Prediction of Bioactive Compounds and Target Proteins for SCS: A total of 12 compounds from *Syzygium cumuni* seeds were retrieved that complied with all the rules of Lipinski's rule of five with valid PubChem ID. Their structure was retrieved from PubChem. These bioactive compounds belong to different chemical classes, such as tannins, phenolic acids, monoterpenoids, phenylpropanoids, flavonoids, and cyclic polyketides. The SwissTargetPrediction database was used to identify probable targets of the 12 bioactive compounds. Out of 12, target genes/proteins were retrieved for ten phytochemicals. For two phytochemicals, namely 3,5,7,4'-tetrahydroxy flavanone and 5-(hydroxymethyl) furfural, no targets were retrieved from the SwissTargetPrediction database.

A total of 254 target proteins for ten phytochemicals were obtained. Of the T2DM-related targets, 76 were obtained from the TTD database. When intersected with 254 SCS-related targets, 20 were identified as possible T2DM targets from the SCS phytochemicals, as shown in figure 1. Table 1 represents the phytochemical ID, phytochemical name, PubChem ID, phytochemical class, T2DM-associated targets, and number of target proteins.

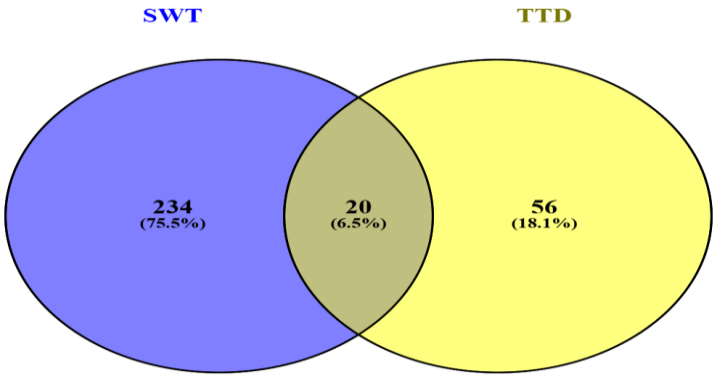

Figure 1: Potential targets of *Syzygium cumini* seeds in T2DM

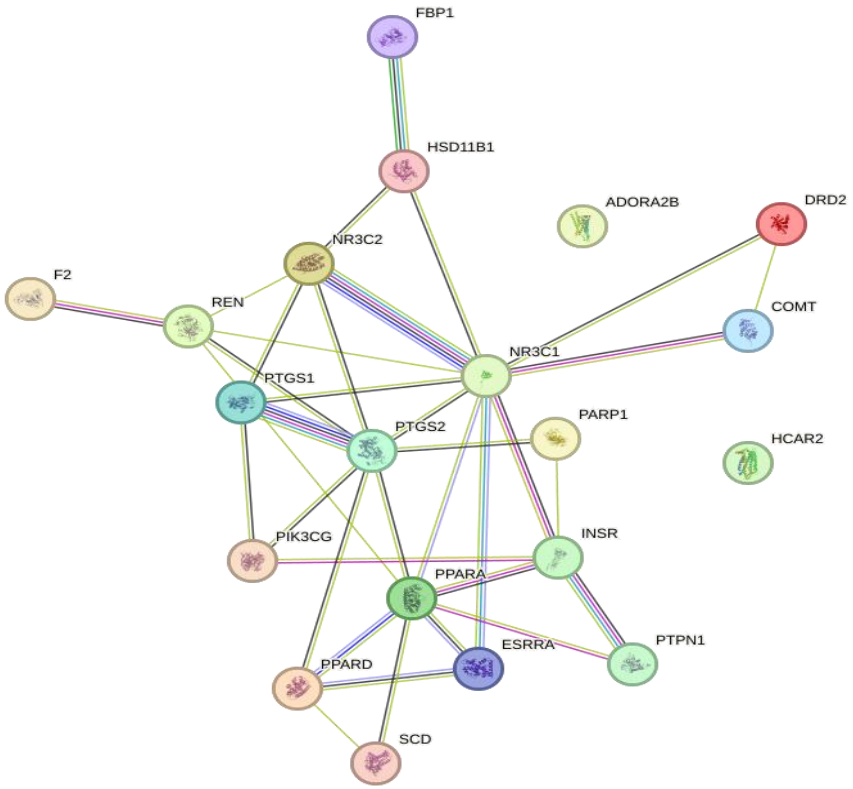

Figure 2: Protein-protein interaction network of the potential targets of *Syzygium cumini* seeds in T2DM using STRING database

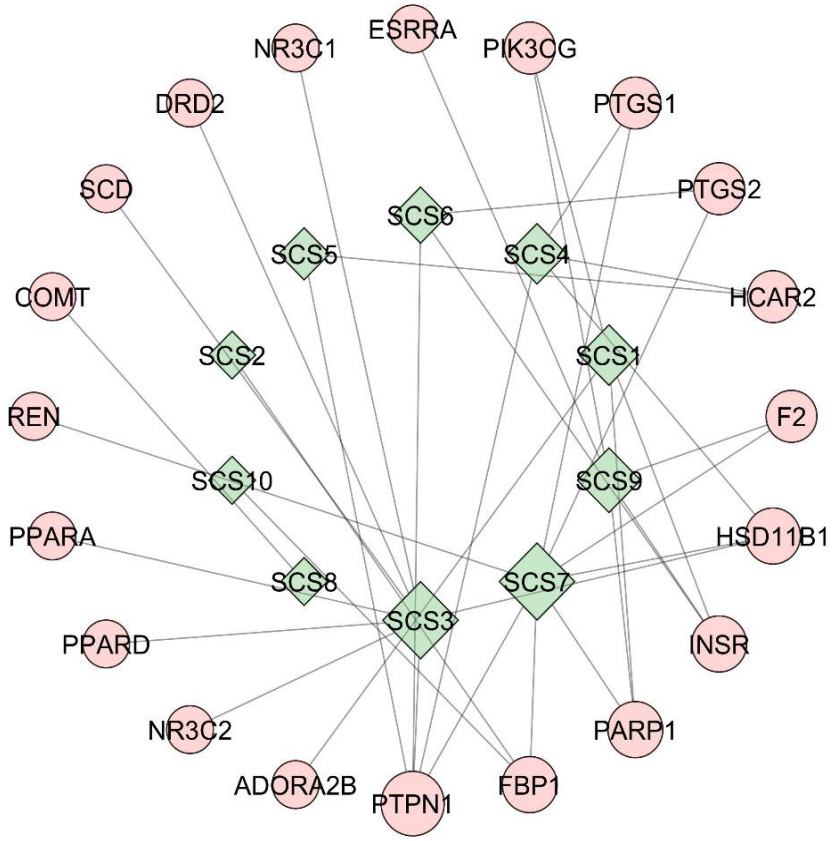

Figure 3: A network comprising bioactive SCS bioactive compounds' interaction with SCS-T2DM intersecting target proteins. The network was constructed using Cytoscape 3.10.1. Green diamonds represent SCS bioactive compounds, and red circles represent SCS-T2DM intersecting target proteins

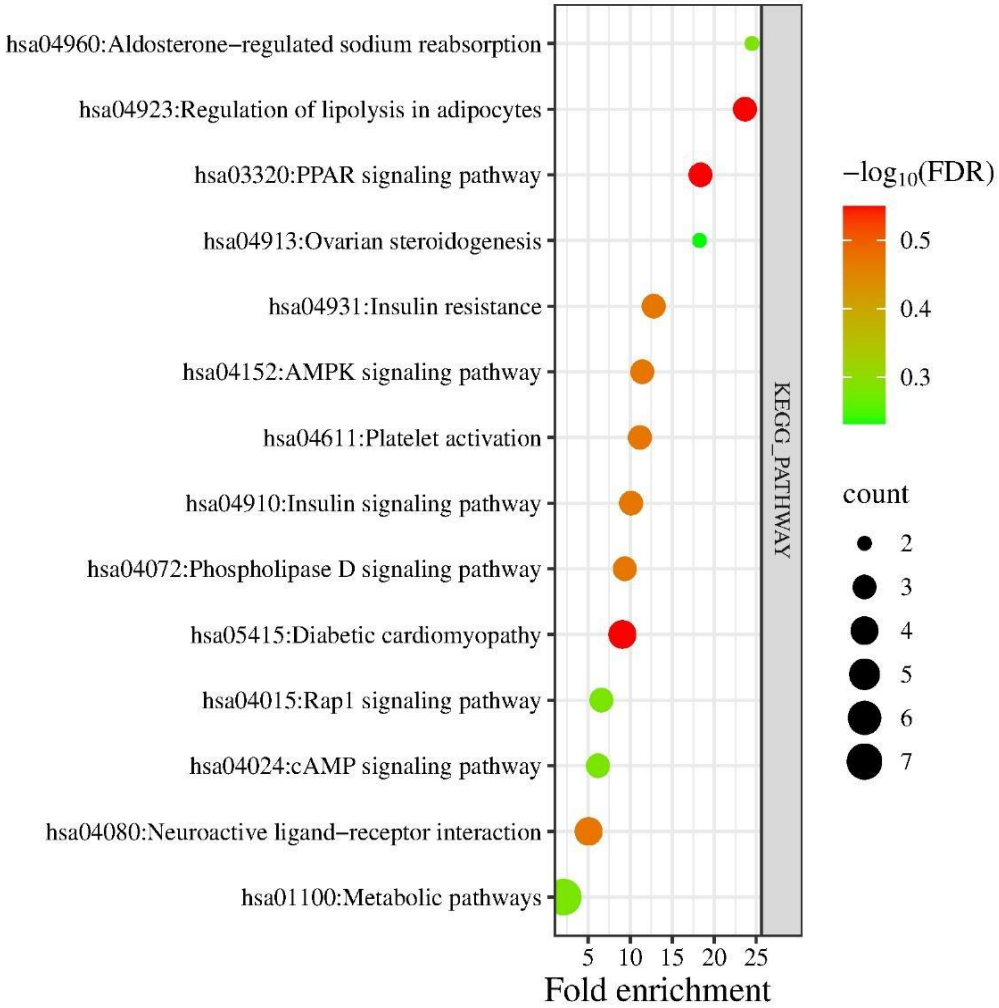

Figure 4: KEGG pathway enrichment analysis. The red color shows most pathways with least false discovery rate (FDR). Size represents the number of gene counts.

Analysis of the protein-protein interaction (PPI) network and SCS bio actives-T2DM Target network: PPI interactions of the 20 common targets were analyzed via STRING and Cytoscape v3.10.1. Common targets were uploaded to STRING with parameters set to "*Homo sapiens*" and a confidence score greater than 0.4. The resulting PPI network was subsequently imported into Cytoscape v3.10.1. The topological properties of the nodes in the PPI network were calculated via a Cytoscape plugin called a network analyzer. The network showed 20 nodes and 35 edges (Table 2). The PPI network topology revealed that NR3C1, PPARA, PTGS2, INSR, NR3C2, REN, PPARD, PTGS1, ESRRA, PIK3CG and HSD11B1 target proteins showed a betweenness degree ≥ 0.1 and degree centrality 3.

These are important target proteins for ten SCS bioactive compounds that could play an important role in the management of diabetes. To further delve into the interactions of SCS bioactive components with the target proteins, an SCS bioactive interaction with the SCS-T2DM intersecting target network was created as shown in figure 3, with topological parameters shown in table 3. SCS3 (α-terpineol) and SCS7 (ferulic acid) showed the highest interaction with eight targets each. SCS9 (quercetin), SCS1 (3,3',4'-tri-O-methylellagic acid), and SCS4 (caffeic acid) showed interaction with five, four and four target proteins.

KEGG pathway analysis: KEGG pathway analysis was performed by submitting 20 common targets to the DAVID database to study the putative pathways associated with the targets. Among the 20 targets, 19 were significantly enriched in 14 KEGG pathways. The details of the 14 KEGG pathways are listed in table 4 and enrichment analysis is shown in figure 4. Among the 14 signaling pathways, the regulation of lipolysis in adipocytes, diabetic cardiomyopathy, the PPAR signaling pathway, insulin resistance, AMPK signaling pathway, platelet activation, the insulin signaling pathway, the phospholipase D signaling pathway, and neuroactive ligand-receptor interactions were significant. This shows that SCS bioactive compounds can influence the several signaling pathways associated with diabetes.

Table 1
Data mining for Phytoconstituents/bioactive

Phytochemical ID	Phytochemical Name	PubChem ID	Phytochemical Class	SCS-T2DM intersecting Targets	Count
SCS1	3,3',4'-tri-O-methylellagic acid	11674590	Tannin	PIK3CG, INSR, ADORA2B, PARP1	4
SCS2	3-hydroxy-4,5-dimethoxybenzoic acid	74709	Phenolic acids	FBP1	1
SCS3	α-terpineol	17100	Monoterpenoids	PTPN1, HSD11B1, PPARA, PPARD, NR3C1, DRD2, SCD, NR3C2	9
SCS4	Caffeic acid	689043	Phenylpropanoids	PTPN1, HSD11B1, PTGS1, HCAR2	4
SCS5	Cinnamic acid	444539	Phenylpropanoids	PTPN1, HCAR2	2
SCS6	Ellagic acid	5281855	Phenolic acids	PTPN1, PTGS2, INSR	3
SCS7	Ferulic acid	445858	Phenylpropanoids	PTPN1, FBP1, PARP1, HSD11B1, PTGS2, PTGS1, F2, REN	8
SCS8	Gallic acid	370	Phenolic acids	COMT	1
SCS9	Quercetin	5280343	Flavonoids	INSR, PARP1, PIK3CG, F2, ESRRA	5
SCS10	Syringic acid	10742	Phenolic acids	FBP1	1
SCS11	3,5,7,4'-tetrahydroxy flavanone	74709	Phenolic acids	-	0
SCS12	5-(hydroxymethyl) furfural	237332	Cyclic polyketides	-	0

Discussion

Diabetes is not a single disease; rather, it is a group of metabolic disorders affecting a large population worldwide. It is characterized mainly as chronic hyperglycemia resulting from defects in insulin secretion or insulin action. The number of diabetic people in the world is predicted to reach 366 million by the year 2030[2]. Over the last three decades, the use of herbal medicinal and pyto-products has increased[12]. Phytotherapy is considered as an alternative therapy for the management of diseases. The therapeutic efficacy of phytotherapy is based on the combined action of a number of bioactive components that act by specifically targeting and disrupting the cell membrane, by binding to and inhibiting certain proteins, or by adhering to or intercalating into RNA or DNA[11].

In recent years, network pharmacology techniques have been successfully employed to decipher the mechanism of action of conventional medicine in the management of complex disorders. The complexity of T2DM pathophysiology is due to several genetic, metabolic, and environmental variables[16]. Such diseases require treatment at multiple targets in which numerous bioactive components of medicinal plants may exert pharmacological effects through synergistic or

antagonistic interactions[11]. In this study, a network pharmacology-based methodology was applied to understand the mechanisms of action of SCS phytochemicals. It aimed to identify the mechanism of phytochemicals that have a potential drug-like nature by complying with Lipinski's rule of five (RO5).

The drug-like nature of phytochemicals influences their solubility and permeability. In these approaches, RO5 indicates that poor permeation and absorption are more likely when there are more than 5 H-bond donors and 10 H-bond acceptors, the molecular weight is greater than 500 and calculated log P (CLogP) is greater than 5 (or MlogP) > 4.15)[18]. Lipinski's rule of drug-likeness is a useful guideline for orally bioavailable small molecules[26]. Moreover, the chemical class was defined for each phytochemical from the ClassyFire database[10].

The targets of these ten phytochemicals, obtained from the SwissTargetPrediction database, consisted of a total of 254 target genes. SwissTargetPrediction is a web server for accurately predicting the targets of bioactive molecules on the basis of a combination of 2D and 3D similarity measurements with known ligands. Predictions can be performed in five different organisms, and mapping predictions on the basis of homology within and between different species is possible for close paralogs and orthologs[8]. A total of 76 target genes associated with T2DM were subsequently identified from the TTD database, which provides knowledge of existing drug targets (both approved and in clinical trials) used for target discovery, selection, research and development[19]. It provides information on 1118 drugs from clinical trials, 1511 approved drugs, and 2331 experimental drugs linked to their primary targets (3257 drugs with available structural data)[19].

A total of 20 target genes were identified by intersecting the acquired SCS target proteins and target proteins related to T2DM. The interactions between putative T2DM targets of 10 SCS phytochemicals were subjected to network analysis. Among the SCS bioactive compounds, α-terpineol, ferulic acid, quercetin, 3,3',4'-tri-O-methylellagic acid, caffeic acid, ellagic acid and cinnamic acid were the SCS bioactive compounds that interact with more than one target protein. In earlier studies, caffeic acid and its derivatives have shown protective effects against diabetes complications such as diabetic liver damage, endothelial dysfunction, cardiomyopathy, retinopathy, cognitive deficits, neuropathy and nephropathy[1].

KEGG pathway enrichment analysis further revealed that the pathways related to the regulation of lipolysis in adipocytes, diabetic cardiomyopathy, the PPAR signaling pathway, insulin resistance, platelet activation, the insulin signaling pathway, the phospholipase D signaling pathway and neuroactive ligand–receptor interactions were significant. These pathways presented a lower false discovery rate (FDR) value, suggesting that they may play a prominent role as antidiabetic agents in the SCS mechanism.

Table 2
Topological parameters of the PPI network of SCS-T2DM-intersecting targets

Name	Degree	Betweenness centrality	Closeness centrality	Target developmental level	Target family
NR3C1	10	0.430882353	0.708333333	Tclin	Nuclear Receptor
PPARA	8	0.211887255	0.62962963	Tclin	Nuclear Receptor
PTGS2	8	0.185539216	0.62962963	Tclin	Enzyme
INSR	5	0.086151961	0.548387097	Tclin	Kinase
NR3C2	5	0.04877451	0.548387097	Tclin	Nuclear Receptor
REN	5	0.12377451	0.566666667	Tclin	Enzyme
PPARD	4	0.021691176	0.472222222	Tchem	Nuclear Receptor
PTGS1	4	0.018137255	0.5	Tclin	Enzyme
ESRRA	3	0.011029412	0.485714286	Tchem	Nuclear Receptor
PIK3CG	3	0.007352941	0.435897436	Tclin	Kinase
HSD11B1	3	0.117647059	0.459459459	Tclin	Enzyme
PTPN1	2	0	0.425	Tchem	Enzyme
SCD	2	0	0.404761905	Tchem	Enzyme
DRD2	2	0	0.435897436	Tclin	GPCR
PARP1	2	0.001838235	0.425	Tclin	Enzyme
COMT	2	0	0.435897436	Tclin	Enzyme
F2	1	0	0.369565217	Tclin	Enzyme
FBP1	1	0	0.320754717	Tchem	Enzyme
HCAR2	0	0	0	Tclin	GPCR
ADORA2B	0	0	0	Tclin	GPCR

Table 3
Topological parameters of SCS bio-actives and Target proteins

Name	Degree	Betweenness centrality	Closeness centrality
SCS3	8	0.404490571	0.38028169
SCS7	8	0.554644121	0.473684211
SCS9	5	0.134773251	0.310344828
SCS1	4	0.103864654	0.303370787
SCS4	4	0.06950719	0.341772152
SCS6	3	0.104526955	0.38028169
SCS5	2	0.021355651	0.325301205
SCS10	1	0	0.257142857
SCS2	1	0	0.257142857
SCS8	1	0	1
PTPN1	5	0.352109002	0.457627119
INSR	3	0.072272172	0.325301205
FBP1	3	0.145299145	0.341772152
PARP1	3	0.173186073	0.38028169
HSD11B1	3	0.159040805	0.415384615
PIK3CG	2	0.005223172	0.247706422
HCAR2	2	0.00308642	0.262135922
PTGS1	2	0.019273473	0.341772152
PTGS2	2	0.014177181	0.341772152
F2	2	0.064879565	0.36
ADORA2B	1	0	0.234782609
ESRRA	1	0	0.238938053
NR3C2	1	0	0.278350515
NR3C1	1	0	0.278350515
DRD2	1	0	0.278350515
PPARA	1	0	0.278350515
PPARD	1	0	0.278350515
SCD	1	0	0.278350515
REN	1	0	0.325301205
COMT	1	0	1

Table 4
KEGG pathway analysis of 20 targets of SCS-T2DM intersecting target proteins

Term	Count	%	P Value	Genes
hsa04923:Regulation of lipolysis in adipocytes	3	15	0.006255729	INSR, PTGS2, PTGS1
hsa05415:Diabetic cardiomyopathy	4	20	0.007754983	PARP1, INSR, REN, PPARA
hsa03320:PPAR signaling pathway	3	15	0.010209319	SCD, PPARA, PPARD
hsa04931:Insulin resistance	3	15	0.020266264	PTPN1, INSR, PPARA
hsa04152:AMPK signaling pathway	3	15	0.02502103	SCD, INSR, FBP1
hsa04611:Platelet activation	3	15	0.026177721	F2, PIK3CG, PTGS1
hsa04910:Insulin signaling pathway	3	15	0.031437259	PTPN1, INSR, FBP1
hsa04072:Phospholipase D signaling pathway	3	15	0.036189494	INSR, F2, PIK3CG
hsa04080:Neuroactive ligand-receptor interaction	4	20	0.036690827	ADORA2B, F2, DRD2, NR3C1
hsa04015:Rap1 signaling pathway	3	15	0.068099693	ADORA2B, INSR, DRD2
hsa04960:Aldosterone-regulated sodium reabsorption	2	10	0.0746814	INSR, NR3C2
hsa04024:cAMP signaling pathway	3	15	0.076136865	HCAR2, DRD2, PPARA
hsa01100:Metabolic pathways	7	35	0.082229281	HSD11B1, SCD, COMT, PTGS2, FBP1, PIK3CG, PTGS1
hsa04913:Ovarian steroidogenesis	2	10	0.098997667	INSR, PTGS2

Conclusion

This study demonstrated the multicomponent, multitarget, and multi-pathway characteristics of SCSs. This study highlighted 10 SCS bioactive compounds, 20 target proteins and 14 signaling pathways that may play important roles in the anti-T2DM mechanism of SCS. α-terpineol, ferulic acid, quercetin, 3,3',4'-tri-O-methylellagic acid, caffeic acid, ellagic acid and cinnamic acid are SCS bioactive compounds that interact with more than one target protein and could be marker compounds. These compounds can affect nine signaling pathways significantly, which can explain SCS antidiabetic effects.

References

1. Akhlaghipour I., Shad A.N., Askari V.R., Maharati A. and Rahimi V.B., How caffeic acid and its derivatives combat diabetes and its complications: A systematic review, *Journal of Functional Foods*, **110**, 105862 **(2023)**

2. American Diabetes Association, Diagnosis and classification of diabetes mellitus, *Diabetes Care*, **27(1)**, S5–S10 **(2004)**

3. Ayyanar M. and Subash-Babu P., Syzygium cumini (L.) Skeels: a review of its phytochemical constituents and traditional uses, *Asian Pacific Journal of Tropical Biomedicine*, **2(3)**, 240–246 **(2012)**

4. Baliga M.S., Fernandes S., Thilakchand K.R., D'souza P. and Rao S., Scientific validation of the antidiabetic effects of Syzygium jambolanum DC (black plum), a traditional medicinal plant of India, *Journal of Alternative and Complementary Medicine*, **19(3)**, 191–197 **(2013)**

5. Banday M.Z., Sameer A.S. and Nissar S., Pathophysiology of diabetes: An overview, *Avicenna Journal of Medicine*, **10(4)**, 174–188 **(2020)**

6. Chagas V.T., França L.M., Malik S. and Paes A.M. de A., Syzygium cumini (L.) skeels: a prominent source of bioactive molecules against cardiometabolic diseases, *Frontiers in Pharmacology*, **6**, 259 **(2015)**

7. Chandran U., Mehendale N., Patil S., Chaguturu R. and Patwardhan B., Network Pharmacology, In Patwardhan B., ed., Innovative Approaches in Drug Discovery, Elsevier, **1**, 127–164 **(2016)**

8. Daina A., Michielin O. and Zoete V., SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules, *Nucleic Acids Research*, **47(W1)**, W357–W364 **(2019)**

9. Dennis G., Sherman B.T., Hosack D.A., Yang J., Gao W., Lane H.C. and Lempicki R.A., DAVID: Database for Annotation, Visualization, and Integrated Discovery, *Genome Biology*, **4**, 1-11 **(2003)**

10. Djoumbou Feunang Y., Eisner R., Knox C., Chepelev L., Hastings J., Owen G., Fahy E., Steinbeck C., Subramanian S., Bolton E. and Greiner R., ClassyFire: automated chemical classification with a comprehensive, computable taxonomy, *Journal of Cheminformatics*, **8**, 61 **(2016)**

11. Efferth T. and Koch E., Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy, *Current Drug Targets*, **12(1)**, 122–132 **(2011)**

12. Ekor M., The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, *Frontiers in Pharmacology*, **4**, 177 **(2014)**

13. Gfeller D., Grosdidier A., Wirth M., Daina A., Michielin O. and Zoete V., SwissTargetPrediction: a web server for target prediction of bioactive small molecules, *Nucleic Acids Research*, **42**, W32-W38 **(2014)**

14. Jasmine R., Selvakumar B.N., Daisy P. and Ignacimuthu S., Activity of Eugenia jambolana, an ethnomedical plant, against drug-resistant bacteria, *Pharmaceutical Biology*, **48(4)**, 405–410 **(2010)**

15. Kanehisa M., Furumichi M., Tanabe M., Sato Y. and Morishima K., KEGG: new perspectives on genomes, pathways, diseases and drugs, *Nucleic Acids Research*, **45(D1)**, D353–D361 **(2017)**

16. Kaur P., Reis M.D., Couchman G.R., Forjuoh S.N., Greene Jr. J.F. and Asea A., SERPINE 1 links obesity and diabetes: A pilot study, *Journal of Proteomics & Bioinformatics*, **3(6)**, 191–199 **(2010)**

17. Klionsky D.J., Abdel-Aziz A.K., Abdelfatah S., Abdellatif M., Abdoli A., Abel S., Abeliovich H., Abildgaard M.H., Abudu Y.P., Acevedo-Arozena A. and Adamopoulos I.E., Guidelines for the use and interpretation of assays for monitoring autophagy, 3rd edition, *Autophagy*, **12(1)**, 1–222 **(2016)**

18. Lipinski C.A., Lombardo F., Dominy B.W. and Feeney P.J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Advanced Drug Delivery Reviews*, **46(1–3)**, 3–26 **(2001)**

19. Liu X., Zhu F., Ma X., Tao L., Zhang J., Yang S., Wei Y. and Chen Y.Z., The Therapeutic Target Database: an internet resource for the primary targets of approved, clinical trial and experimental drugs, *Expert Opinion on Therapeutic Targets*, **15(8)**, 903–912 **(2011)**

20. Makhoba X.H., Viegas C., Mosa R.A., Viegas F.P.D. and Pooe O.J., Potential Impact of the Multi-Target Drug Approach in the Treatment of Some Complex Diseases, *Drug Design, Development and Therapy*, **14**, 3235–3249 **(2020)**

21. Noor F., Tahir ul Qamar M., Ashfaq U.A., Albutti A., Alwashmi A.S. and Aljasir M.A., Network pharmacology approach for medicinal plants: review and assessment, *Pharmaceuticals*, **15(5)**, 572 **(2022)**

22. Ravi K., Sekar D.S. and Subramanian S., Hypoglycemic activity of inorganic constituents in *Eugenia jambolana* seed on streptozotocin-induced diabetes in rats, *Biological Trace Element Research*, **99(1–3)**, 145–155 **(2004)**

23. Sawant L., Singh V.K., Dethe S., Bhaskar A., Balachandran J., Mundkinajeddu D. and Agarwal A., Aldose reductase and protein tyrosine phosphatase 1B inhibitory active compounds from *Syzygium cumini* seeds, *Pharmaceutical Biology*, **53(8)**, 1176–1182 **(2015)**

24. Sharma H.K., Chhangte L. and Dolui A.K., Traditional medicinal plants in Mizoram, India, *Fitoterapia*, **72(2)**, 146–161 **(2001)**

25. Sharma S.B., Nasir A., Prabhu K.M., Murthy P.S. and Dev G., Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits, *Journal of Ethnopharmacology*, **85(2–3)**, 201–206 **(2003)**

26. Zhang M.Q. and Wilkinson B., Drug discovery beyond the 'rule-of-five', *Current Opinion in Biotechnology*, **18(6)**, 478–488 **(2007)**

27. Zhang R., Li X., Zhang X., Qin H. and Xiao W., Machine learning approaches for elucidating the biological effects of natural products, *Natural Product Reports*, **38(2)**, 346–361 **(2021)**

28. Zhang R., Zhu X., Bai H. and Ning K., Network pharmacology databases for traditional chinese medicine: review and assessment, *Frontiers in Pharmacology*, **10**, 123 **(2019)**

29. Zhang W., Huai Y., Miao Z., Qian A. and Wang Y., Systems pharmacology for investigation of the mechanisms of action of traditional chinese medicine in drug discovery, *Frontiers in Pharmacology*, **10**, 743 **(2019)**

30. Zhou W., Wang Y., Lu A. and Zhang G., Systems pharmacology in small molecular drug discovery, *International Journal of Molecular Sciences*, **17(2)**, 246 **(2016)**.

(Received 10th September 2024, accepted 16th November 2024)