# Network Pharmacology of Ayurvedic Multicomponent Formulation Diabecon Containing *Gymnema sylvestre*, *Pterocarpus marsupium* and *Asphaltum punjabinum* with Special Reference to Antidiabetic Property

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# ABSTRACT

Network pharmacology, a burgeoning field amalgamating systems biology and computational biology, offers a promising avenue to unravel the intricate mechanisms of multi-component formulations in traditional medicine systems such as Ayurveda. This paper delves into the network pharmacology analysis of Diabecon, an Ayurvedic formulation comprising *Gymnema sylvestre*, *Pterocarpus marsupium*, and *Asphaltum punjabinum*, with a focus on its antidiabetic properties. Leveraging information from diverse databases and employing tools like Cytoscape, we constructed pharmacological networks elucidating the interactions between bioactive and molecular targets, particularly in the context of diabetes. Our analysis unveils novel insights into the synergistic effects of the constituents of Diabecon, shedding light on their mechanisms of action and potential therapeutic applications in managing diabetes mellitus. This research not only enhances our understanding of the pharmacodynamics of Diabecon but also paves the way for the identification of new therapeutic leads and targets for combating diabetes and related metabolic disorders.

Keywords: Network pharmacology, Ayurveda, Diabecon, *Gymnema sylvestre*, *Pterocarpus marsupium*, *Asphaltum punjabinum*, antidiabetic, Multi-component formulation, Molecular targets, Diabetes mellitus.

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# INTRODUCTION

In the realm of modern medicine, there is a growing recognition of the value of traditional and natural remedies as potential sources of novel therapeutic agents.<sup>1-3</sup> Complementing this trend, network pharmacology has appeared as a dominant resource for discovering the multifaceted connections between bioactive botanicals and molecular targets within multicomponent formulations. This integrative approach, which blends systems biology with computational techniques, offers a holistic understanding of the pharmacological effects of such formulations.<sup>4-6</sup>

Diabecon, an Ayurvedic multi-component formulation, encapsulates the essence of this paradigm shift in drug discovery. Comprising *Gymnema sylvestre*, *Pterocarpus marsupium*, and *Asphaltum punjabinum*, Diabecon is renowned for its antidiabetic properties. While traditional drug discovery often hinges on targeting single genes or proteins, the multi-targeted approach of formulations like Diabecon represents a more nuanced strategy in combating complex metabolic disorders such as diabetes mellitus.<sup>7,8</sup> The conventional "one gene-one target-one drug" paradigm is increasingly being challenged by the recognition that many diseases stem from dysregulation across multiple pathways. In this context, formulations like diabecon, which exert their effects through synergistic interactions between multiple bioactive components, hold immense promise. Network pharmacology offers a lens through which to elucidate the intricate interplay between these components and their molecular targets, thereby shedding light on the mechanisms underlying their therapeutic efficacy.<sup>9</sup>

The field of network pharmacology has been widely utilized to investigate the pharmacological mechanisms of intricate formulations in systems such as traditional Chinese medicine; however, its applicability in Ayurvedic medicine has not been as well studied. Thus, this study aims to bridge this gap by employing network pharmacology to unravel the pharmacodynamics of diabecon. By delineating the interactions between its constituents and molecular targets, with a specific focus on its antidiabetic properties, we seek to offer novel insights into the therapeutic potential of this Ayurvedic formulation.

#### MATERIALS AND METHODS

#### **Diabecon**, Bioactives, and Targets

Pharmacology networks were constructed using bioactive chemicals that were identified from the botanicals *G. sylvestre*, *P. marsupium*, and *A. punjabinum* found in diabecon. The names of botanicals and chemical constituents were searched in a Pubmed database accessed on Jan 10 2024 to collect bioactive present in botanicals. Additionally, Indian Medicinal Plants, Phytochemistry and Therapeutics 2.0 were searched for all botanicals in diabecon. Structural information on these bioactive was sourced from the PubChem database in the '.sdf' format along with their PubChem IDs all are reported in Table 1.<sup>10-11</sup>

The dedicated tool 'discover my compounds' target' was utilized to query the structures present in the '.sdf' files within the binding database (Binding DB) in order to determine the targets of these bioactive compounds. This free online database records the interactions that occur between tiny drug-like compounds and proteins. Using this tool, a database search for a compound that is exact or similar is performed, providing information on the targets of the search. The similarity search tool yields structurally related compounds and scores to the query molecule. A score of 0.8 indicates that the chemical is either exactly the same as the one that was requested or that it shares 80% structural similarity with another entry in the database. Targets of bioactives with a score equal to 0.80 were selected to enhance result accuracy.

Binding DB has links to other databases, and these connections were used to obtain further data about the targets. Protein and gene names were retrieved from UniProt using the associated UniProt IDs. Additionally, binding DB made it easier to retrieve data about the kind of target, the bioactive's mode of action (inhibition or activation), the organism that serves as the source, and the chemical that the bioactive is comparable.<sup>12</sup>

#### **Disease Types and Indications**

Furthermore, Uniport IDs were converted into KEEG gene IDs using the KEGG mapper, and further searches were carried out to identify the pathways associated with the functionality of the targets. Using the KEGG pathway finder online, the targets of the bioactive were examined to determine any correlations with particular diseases or indications. In order to accomplish this, the KEGG mapper converted Uniport IDs into KEEG gene IDs and then conducted additional research to find the pathways connected to the targets' functions. This provides a thorough annotation of human genes inside illness networks.<sup>13</sup>

## **Network Construction**

Nodes, which stand for communication or redistribution sites, and edges, which stand for the lines of communication connecting nodes, make up a network pharmacology. The three botanicals that make-up diabecon (GS, PM, and PA) are linked to their bioactive substances, related targets, related disorders, and underlying processes; these are the nodes in the networks. An open-source Java-based program called Cytoscape 3.2.1 was used to build these networks. Several Cytoscape tools were used for network analysis. The diabecon network cohesively incorporates its objectives, bioactive, botanical components, and related disorders. In the process of building the network, duplicate bioactives were removed. To further demonstrate the use of pharmacology networks in diabetes therapies, a subset network centered on the connection between diabecon and diabetes was created.<sup>14</sup>

## RESULTS

#### **Diabecon - Bioactive - Target Network**

The botanical components of diabecon - *G. sylvestre* (GS), *P. marsupium* (PM), and *A. punjabinum* (AP) - were found to contain 120, 72, and 35 bioactives, respectively, based on data retrieved from the Indian Medicinal Plants, Phytochemistry And Therapeutics 2.0 (IMPPAT 2.0) as of February 2024. Among these, several bioactives were identified as common across the three botanicals mentioned in Table 2.

Consequently, the diabecon formulation as a whole comprises 207 bioactives. From this set, 94 bioactives were identified as Score-0.80 based on a Binding Database (Binding DB) search conducted between March and February 2024, specifically after removing duplicates GS, PM, and PA contain 26, 19, and 4 score-0.80 bioactives, respectively (Figure 1).

#### **Diabecon-Disease Network**

An examination of the network interactions between diabecon and various diseases revealed its potential to address a diverse array of health conditions. Diabecon demonstrated efficacy against 60 different disease types, encompassing a total of 13309 distinct disease indications through its modulation of 49 protein targets. Major disease types targeted by diabecon include diabetes mellitus as highlighted in Table 3. Additionally, it can target inflammatory diseases, obesity, retinopathy, vascular diseases and metabolic disorders.

Center node is formulation diabecon, second layer is its three botanicals. The third oval-shaped node is the bioactives of botanicals in sky blue color. Fourth, blue nodes represent the targets associated with bioactives, and the final layer represents diseases associated with the targets (Figure 2).

Several bioactive components of diabecon, such as G. sylvestre, P. marsupium, and A. punjabinum, exhibited connections to multiple disease types. Notably, betaamyrin acetate, deacylgymnemic acid, gymnemagenin, gymnemasaponin II, gymnemic acid I, gymnestrinogenin, gynosaponin TN-2, (-)-epicatechin, (2S)-7-hydroxyflavanone, 3,7,4'-trihydroxyflavone, beta-eudesmol, beta-eudesmol, garbanzol, isoliquiritigenin, naringetol, pseudobaptigenin, pterostilbene, pyrocatechol, and coumarin were identified as key bioactives involved in these interactions. These compounds range from flavonoids like (-)-epicatechin and 3,7,4'-trihydroxyflavone to terpenoids like beta-amyrin acetate and beta-eudesmol, and phenolic compounds like pyrocatechol and coumarin. Some of these compounds have been studied for their antioxidant, anti-inflammatory, and potential therapeutic properties, while others may exhibit

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	Table 1: Botanical - Bioactives	
Botanical	PubChem name	PubChem CID
G. sylvestre	Gymnemic acid II	91617872
G. sylvestre	Gymnemic acid III	14264066
G. sylvestre	Gymnemic acid IV	14264063
G. sylvestre	Gymnemic acid IX	91617808
G. sylvestre	Gymnemic acid V	14683206
G. sylvestre	Gymnemic acid VI	91617894
G. sylvestre	Gymnemic acid VII	91617592
G. sylvestre	Gymnemic acid VIII	91617623
G. sylvestre	Gymnemic acid X	15674686
G. sylvestre	Gymnemic acid xi	15674687
G. sylvestre	Gymnemic acid XII	91826975
G. sylvestre	Gymnestrogenin	15560302
G. sylvestre	Gynosaponin TN-2	319538106
G. sylvestre	Gypenoside II	53232197
G. sylvestre	Gypenoside LV	53581228
G. sylvestre	Gypenoside LXIII	52669293
G. sylvestre	Gypenoside LXXIV	50155152
G. sylvestre	Gypenoside V	53730223
G. sylvestre	Gypenoside XVII	44584555
G. sylvestre	Gypenoside XXVIII	274140052
G. sylvestre	Gypenoside XXXVII	274140053
G. sylvestre	Lupeol	259846
G. sylvestre	Methyl alpha-D- galactopyranoside	76935
G. sylvestre	Methyleugenol	7127
G. sylvestre	Resiniferonol	162824
G. sylvestre	Stigmasterol	5280794
P. marsupium	(-)-Epicatechin	72276
P. marsupium	(2S)-7-hydroxyflavanone	688857
P. marsupium	Abscisic acid	5280896
P. marsupium	Flavone 1	133775
P. marsupium	3,7,4'-Trihydroxyflavone	5281611
P. marsupium	Phenolic Flavone	185124
P. marsupium	7,4'-Dihydroxyflavone	5282073
P. marsupium	Isoflavone	44257328
P. marsupium	beta-Eudesmol	91457
P. marsupium	Ebanol	6504499
P. marsupium	Garbanzol	442410
P. marsupium	Isoliquiritigenin	638278
P. marsupium	Liquiritigenin	114829
P. marsupium	Marsupsin	134369
P. marsupium	Naringetol	439246
P. marsupium	Propterol-b	185124
P. marsupium	Pseudobaptigenin	5281805
P. marsupium	Pterostilbene	5281727
P. marsupium	pterosupin	23498438

A. punjabinumCoumarin323A. punjabinumhumic acid90472028A. punjabinumPyrocatechol289	A. punjabinum	fulvic acid	5359407
$T \sim f^{-1}$	A. punjabinum	Coumarin	323
A. punjabinum Pyrocatechol 289	A. punjabinum	humic acid	90472028
	A. punjabinum	Pyrocatechol	289

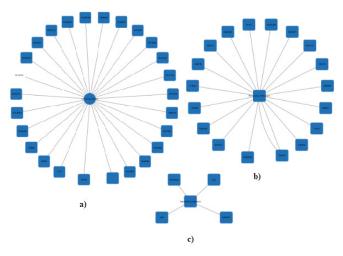


Figure 1: Diabecon: bioactives network. a) G. sylvestre b) P. marsupium c) A. punjabinum

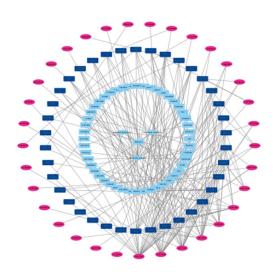


Figure 2: Diabecon-disease network

antidiabetic or cardioprotective effects. The diversity of these compounds reflects the rich biochemical profile of natural sources and highlights their potential for pharmaceutical and nutraceutical applications.

Our analysis revealed that 50 diabecon bioactive can interact with 72 targets associated with 158 diseases, indicating a potential link to diabetes. Specific diabetes indications targeted by diabecon include coagulation factor X, delta(24)sterol reductase, prostaglandin E synthase, bifunctionalepoxide hydrolase 2, tissue factor, signal transducer and activator of transcription 3, alpha-synuclein, apoptosis regulator Bcl-2, carbonic anhydrase 7, alkaline phosphatase, tissue-nonspecific isozyme, dipeptidyl peptidase 4, estrogen receptor, estrogen

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	Table 2: Re	sults of bindi	ng DB database	e with target	s associat	ed with bi	oactive		
Target name	Uploaded compound general hits	Max Similarity	Hits (All compound)	Ki Data	IC50 Data	Kd Data	EC50 Data	UniProt ID	Gene
Coagulation factor X	1	0.81	1	0	1	0	0	P00742	F10
Prostaglandin E synthase	1	0.81	2	0	2	0	0	O14684	PTGES
11-beta-hydroxysteroid dehydrogenase 1	1	0.85	2	0	7	0	2	P50172	Hsd11b1
11-beta-hydroxysteroid dehydrogenase type 2	1	0.85	2	0	3	0	1	P80365	HSD11B2
Coagulation factor X	1	0.84	2	0	2	0	0	P00742	F10
Bifunctional epoxide hydrolase 2	1	0.83	1	0	1	0	0	P34913	EPHX2
Tissue factor	1	0.8	8	0	8	0	0	P13726	F3
Coagulation factor X	1	0.86	2	0	2	0	0	P00742	F10
Alpha-synuclein	1	1	1	0	1	0	0	P37840	SNCA
Apoptosis regulator Bcl-2	1	0.8	6	6	0	0	0	P10415	BCL2
Carbonic anhydrase 7	1	1	5	5	0	0	0	P43166	CA7
Alkaline phosphatase, tissue- nonspecific isozyme	<u>1</u>	1	<u>1</u>	0	1	0	0	P05186	ALPL
Androgen receptor	1	0.86	1	0	1	0	0	P10275	AR
Carbonic anhydrase 1	1	0.97	1	1	0	0	0	P00915	CA1
Carbonic anhydrase 12	1	0.88	3	3	0	0	0	O43570	CA12
Carbonic anhydrase 2	1	0.97	1	1	0	0	0	P00918	CA2
Carbonic anhydrase 4	1	0.88	3	3	0	0	0	P22748	CA4
Carbonic anhydrase 7	1	0.88	3	3	0	0	0	P43166	CA7
Dipeptidyl peptidase 4	1	0.88	2	0	2	0	0	P27487	DPP4
Estrogen receptor	1	0.8	23	1	21	0	4	P03372	ESR1
Estrogen receptor beta	1	0.98	17	1	15	0	2	Q92731	ESR2
17-beta-hydroxysteroid dehydrogenase type 2	1	0.96	1	0	2	0	0	P37059	HSD17B2
Carbonic anhydrase 12	1	0.86	5	6	0	0	0	O43570	CA12
Carbonic anhydrase 2	1	0.86	2	2	0	0	0	P00918	CA2
Carbonic anhydrase 4	1	0.83	4	4	0	0	0	P22748	CA4
Carbonic anhydrase 7	1	0.86	5	5	0	0	0	P43166	CA7
Glycogen synthase kinase-3 beta	1	0.83	2	0	2	0	0	P49841	GSK3B
Inositol hexakisphosphate kinase 2	1	0.91	4	0	4	0	0	Q96PC2	IP6K3
Dipeptidyl peptidase 4	1	0.88	2	0	2	0	0	P27487	DPP4
Liver carboxylesterase 1	1	0.82	1	0	1	0	0	P23141	CES1
Aromatase	1	0.87	9	1	14	0	0	P11511	CYP19A1
Carbonic anhydrase 1	1	0.87	1	1	0	0	0	P00915	CA1
Carbonic anhydrase 12	1	0.8	3	3	0	0	0	O43570	CA12
Carbonic anhydrase 2	1	0.87	1	1	0	0	0	P00918	CA2
Carbonic anhydrase 4	1	0.8	3	3	0	0	0	P22748	CA4
Dipeptidyl peptidase 4	1	0.8	2	0	2	0	0	P27487	DPP4
Estrogen receptor beta	1	0.88	16	0	15	0	2	Q92731	ESR2
72 kDa type IV collagenase	1	1	1	0	1	0	0	P08253	MMP2
Estrogen receptor beta	1	1	1	0	1	0	0	Q92731	ESR2

Table 2: Results of binding DB database with targets associated with bioactive

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Androgen receptor	1	0.84	1	0	1	0	0	P10275	AR
Aromatase	1	1	9	1	14	0	0	P11511	CYP19A1
Carbonic anhydrase 1	1	1	1	1	0	0	0	P00915	CA1
Carbonic anhydrase 12	1	0.91	3	3	0	0	0	O43570	CA12
Carbonic anhydrase 2	1	1	1	1	0	0	0	P00918	CA2
Carbonic anhydrase 4	1	0.91	3	3	0	0	0	P22748	CA4
Dipeptidyl peptidase 4	1	0.91	2	0	2	0	0	P27487	DPP4
Estrogen receptor	1	0.83	23	1	21	0	4	P03372	ESR1
Estrogen receptor beta	1	0.99	17	1	15	0	2	Q92731	ESR2
ATP-dependent translocase ABCB1	1	0.9	1	0	2	0	0	P08183	ABCB1
Carbonic anhydrase 12	1	0.83	1	1	0	0	0	O43570	CA12
Dipeptidyl peptidase 4	1	0.83	1	0	1	0	0	P27487	DPP4
Estrogen receptor	1	0.88	5	5	0	0	0	P03372	ESR1
Acetylcholinesterase	1	0.8	1	1	0	0	0	P22303	ACHE
Carbonic anhydrase 2	1	0.8	3	7	0	0	0	P00918	CA2
Carbonic anhydrase 4	1	0.85	1	1	0	0	0	P22748	CA4
Carbonic anhydrase 12	1	0.8	3	6	0	0	0	O43570	CA12

# Table 3: Summary of major disease types targeted by diabecon

	Samuely of I	hajor alsease types largeled by alabeeon			
Gene	Disease id	Disease	SLCO1B3	C0011849	Diabetes mellitus
F10	C0011847	Diabetes	SNCA	C0011847	Diabetes
F10	C0011849	Diabetes mellitus	BCL2	C0085207	Gestational diabetes
F10	C0011881	Diabetic nephropathy	BCL2	C0342257	Complications of diabetes mellitus
DHCR24	C1257958	Glucose metabolism disorders	ALPL	C0011860	Diabetes mellitus, non-insulin-dependent
HSD11B1	C0002152	Alloxan diabetes	AKR1C3	C0495706	elevated blood glucose level
HSD11B1	C0021655	Insulin resistance	AR	C0011860	Diabetes mellitus, non-insulin-dependent
HSD11B1	C0920563	Insulin sensitivity	AR	C0021655	Insulin resistance
HSD11B1	C0085207	Gestational diabetes	AR	C0085207	Gestational diabetes
HSD11B1	C0271650	Impaired glucose tolerance	AR	C0920563	Insulin sensitivity
HSD11B2	C0011881	Diabetic nephropathy	AR	C0011849	Diabetes mellitus
HSD11B2	C0085207	Gestational diabetes	AR	C0011847	Diabetes
HSD11B2	C0271650	Impaired glucose tolerance	AR	C0011854	Diabetes mellitus, insulin-dependent
EPHX2	C0011860	Diabetes mellitus, non-insulin-dependent	AR	C0271650	Impaired glucose tolerance
F3	C0085207	Gestational diabetes	CA1	C0011849	Diabetes mellitus
F3	C0362046	Prediabetes syndrome	CA2	C0011849	Diabetes mellitus
STAT3	C0158981	Neonatal diabetes mellitus	CA4	C0011860	Diabetes mellitus, non-insulin-dependent
STAT3	C0205734	Diabetes, autoimmune	DPP4	C0011847	Diabetes
STAT3	C0271650	Impaired glucose tolerance	DPP4	C0011849	Diabetes mellitus
STAT3	C0342257	Complications of diabetes mellitus	DPP4	C0011854	Diabetes mellitus, insulin-dependent
STAT3	C1257958	Glucose metabolism disorders	DPP4	C0011860	Diabetes mellitus, non-insulin-dependent
STAT3	C1849157	Resistance to insulin-like growth factor I	DPP4	C0011881	Diabetic nephropathy
		Deficiency of glucose-6-phosphate	DPP4	C0271650	Impaired glucose tolerance
SLCO1B1	C2939465	dehydrogenase	DPP4	C1739108	Latent autoimmune diabetes in adults
SLCO1B1	C0011847	Diabetes			Latent autoimmune diabetes mellitus in
SLCO1B1	C0011849	Diabetes mellitus	DPP4	C1960272	adult
SLCO1B1	C0011854	Diabetes mellitus, insulin-dependent	DPP4	C0085207	Gestational diabetes
SLCO1B3	C0011847	Diabetes Cont	DPP4	C0205734	Diabetes, autoimmune

DPP4C0362046Prediabetes syndromeDPP4C0948379Impaired insulin secretionDPP4C1864903Hyperinsulinemic hypoglycemiaESR1C0271650Impaired glucose toleranceESR1C1849157Resistance to insulin-like growth factor IGSK3BC0011847DiabetesIGF1RC0085207Gestational diabetesIGF1RC0085207Gestational diabetesIGF1RC0011847Diabetes encreased serum insulin-like growthIGF1RC0011847DiabetesIGF1RC0011854Diabetes mellitus, insulin-dependentIGF1RC0011854Diabetes mellitus, non-insulin-dependentIGF1RC0021655Insulin resistancePTPN1C0021655Insulin sensitivityPTPN1C1257963Endogenous hyperinsulinismPTPN1C0011847DiabetesPTPN1C0011847DiabetesPTPN1C0011847DiabetesPTPN1C0011847DiabetesPTPN1C0011847DiabetesPTPN1C0011849Diabetes mellitusPTPN1C0011849Diabetes mellitusPTPN1C0011849Diabetes mellitus, insulin-dependentPTPN1C0011851Diabetes mellitus, esperimentalCYP19A1C0011851Diabetes mellitus, esperimentalCYP19A1C0011853Diabetes mellitus, esperimentalCYP19A1C0011849DiabetesCYP19A1C0011849DiabetesCYP19A1C0011849Diabetes <tr< th=""></tr<>
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receptor beta, 17-beta-hydroxysteroid dehydrogenase type 2, inositol hexakisphosphate kinase 3, liver carboxylesterase 1 acetylcholinesterase. These are a variety of enzymes, receptors, and transporters involved in different physiological processes, ranging from coagulation and steroid metabolism to signal transduction and drug transport. Their diverse functions underscore their significance in maintaining homeostasis and regulating various cellular pathways.

The provided obtained in KEGG pathway reveals several pathways and genes implicated in diabetic disease processes. The insulin signaling pathway (hsa:04910) emerges prominently, involving critical genes like IRS1 and PIK3R1, essential for insulin signaling and glucose uptake. Dysregulation here often leads to insulin resistance, a hallmark of type 2 diabetes. Similarly, the insulin resistance pathway (hsa:04931) involves genes like IRS1, PIK3R1, and AKT2, crucial in cellular insulin responsiveness. Furthermore, the AMPK signaling pathway (hsa:04152) is implicated, with genes such as PRKAA1, PRKAA2, PIK3R1, and AKT2 playing significant roles in cellular metabolism and survival. These pathways collectively underscore the intricate molecular mechanisms underlying diabetes, encompassing insulin sensitivity, glucose metabolism, and cellular responses to metabolic stress. Dysregulation in these pathways contributes to insulin resistance, impaired glucose tolerance, and the development of type 2 diabetes and its associated complications.

# CONCLUSION

Network pharmacology is a powerful computational technique that systematically mines data, synthesizes information, and correlates bioactives, targets, pathways, and related indications to reveal the underlying intelligence of conventional medicine. This strategy bridges the gap between intricate etiopathology, molecular targets, and the complex phytochemistry of natural compounds. It is a practical application of systems biology. Diabecon's network pharmacology research has uncovered novel connections between bioactives, targets, and possible uses in a range of illnesses, with a focus on diabetes and its consequences. This comprehensive method provides insightful information on the complex processes that underlie the Ayurvedic multi-component formulation's medicinal efficacy.

By leveraging network pharmacology, we have uncovered new avenues for understanding the pharmacological properties of diabecon and its constituents, *G. sylvestre*, *P. marsupium*, and *A. punjabinum*. These insights extend beyond traditional pharmacological approaches, providing a comprehensive systems-level understanding of the pharmacology of natural products. In summary, network pharmacology provides a holistic and integrative approach to unraveling the pharmacological complexities of Ayurvedic multi-component formulations like diabecon. By elucidating the interconnectedness of bioactives, targets, and pathways, this approach paves the way for accelerated drug discovery, advanced clinical research, and the development of effective therapeutics for complex diseases.

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