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ANTIMICROBIAL AND ANTIDIABETIC PLANT COMPOUNDS DERIVED FROM SPECIFIC MEDICINAL FLORA: POTENTIAL CANDIDATES FOR NOVEL DRUG DEVELOPMENT AND DISCOVERY

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ABSTRACT

Withania Coagulans, *Gymnema Sylvestre*, *Indigofera Tinctoria*, and *Trachyspermum Ammi* are medicinal plants with long histories in traditional and modern medicine for treating indigestion, type 2 diabetes, cancer, asthma, constipation, heart disease, and infections. However, research on their phytochemical and pharmacological properties, particularly their antibacterial and antidiabetic effects, is limited. Analyses using GC-MS and LC-MS identified various bioactive compounds, while IR, UV, and ^{1}H-NMR characterized their functional groups. The bioactive compounds identified through GC-MS and LC-MS included 18, 12, 15, and 15 compounds, corresponding to 17, 26, 17, and 19 peaks, respectively. In vitro studies revealed *Withania Coagulans* showed superior antidiabetic effects, while *Gymnema Sylvestre* and *Indigofera Tinctoria* displayed moderate antibacterial activity against several bacterial strains. *Trachyspermum Ammi* exhibited significant antifungal efficacy against *Aspergillus species*. A docking analysis using Auto Dock Vina investigated the interactions between two proteins, 7myj (linked to diabetes) and 5ESG

(related to fungal activity), and the detected bioactive compounds. The in vitro antidiabetic effects of *Withania Coagulans* extract were assessed using a glucose uptake model in yeast cells, showing greater efficacy compared to standard treatments, as corroborated by docking studies. These findings underscore the potential health benefits of these plants.

KEYWORDS: Ayurveda, Diabetes Management, Molecular Docking, Liquid Chromatography and Mass Spectrometry.

1. INTRODUCTION

Diabetes is a long-term condition that affects how the body processes carbohydrates, fats, and proteins, and it is anticipated that its global prevalence will increase from 4% in 1995 to 5.4% by 2025. In India, approximately 33 million adults are currently diagnosed with diabetes, and this figure is expected to grow to 57.2 million by 2025. Although there are medications available, many patients often turn to alternative methods such as traditional herbal treatments.[1-3] This research aims to investigate the use of herbal medicine and the dynamics of the patient-physician relationship. Antibiotic resistance is becoming an increasingly serious health concern worldwide, contributing to 41% of the global burden of disease. The significant factor in acquired bacterial resistance to antibiotics is largely responsible. The rise of antibiotic-resistant organisms can be attributed to the inappropriate use, overuse, and underuse of antibiotics. If current trends continue, drug-resistant infections may lead to 10 million deaths and result in economic damages of up to $100 trillion by 2050. Efforts on a global scale are being introduced to combat this problem, focusing on preserving life.[4]

Indian Rennet (Paneer Dodi) extracts have shown properties that combat viruses, fungi, and bacteria and have been used in Ayurvedic practices to address numerous health concerns, including issues related to the digestive system, diabetes, and diarrhea. The Sirukurinjan family (Gymnema Sylvestre) and Avuri or Neeli (Indigofera Tinctoria) are used in traditional medicine to manage various ailments, such as inflammation in the mouth and throat, while also functioning as antimicrobial and antifungal agents.[5-8]

Ayurvedic medicine places great importance on plants such as *Trachyspermum Ammi (Thymol), Indigofera Tinctoria (True Indigo), Gymnema Sylvestre (Sirukurinjan),* and *Withania Coagulans (Indian Rennet/ Paneer Dodi)* for their vital nutrients and antioxidant content. These plants tackle a range of health concerns, including diabetes, inflammation, infections, and digestive issues. In South Asia, they are commonly utilized for their

phytochemical properties and are acknowledged as effective supplements to traditional medical practices. Recent research has underscored their antibacterial, antiviral, and antidiabetic capabilities, establishing phytotherapy as a budget-friendly treatment option that carries minimal side effects.[9] These plants are abundant in phytochemicals like alkaloids and flavonoids, which are utilized to manage conditions such as heart disease, asthma, and diabetes. They offer a variety of health advantages, including anti-inflammatory and antimicrobial effects. Medicinal plants play a crucial role in the creation of new pharmaceuticals, with 25% of drugs originating from them.[10-11] They also yield compounds like morphine and quinine, which are effective against different pathogens and offer protection against diseases such as Parkinson's and Alzheimer's. Different extraction methods, including solvent extraction and distillation, are used to isolate biologically active compounds for pharmaceutical applications. Advanced techniques, such as HPLC and GC-MS, are necessary for the separation of effective phytochemicals. Phytochemical methods and spectral analysis are employed to identify and characterize these ayurvedic medicines.[12-13] A review of the literature indicates that there has been insufficient research on the antimicrobial and antidiabetic effects of *Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria,* and *Trachyspermum Ammi*. This study aims to evaluate their spectral data and phytochemical characteristics while conducting in-vitro tests on their health benefits, potentially affirming their traditional medicinal applications.[14]

2. MATERIALS AND METHODS

2.1. Characterization of traditional medicinal plant instruments

^{1}H NMR spectra for the samples were obtained on a 400 MHz Bruker instrument using DMSO-d6 with TMS as the reference standard. The IR spectra were collected with a Bruker FTIR Vector 22 spectrometer in the range of 4400 to 400 cm-1. UV-Vis spectra were gathered using UV light at wavelengths of 254 and 365 nm. All chemicals utilized were sourced from Merck Products.[14] Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of the methanol extracts of the traditional medicinal plants Withania Coagulans, Gymnema Sylvestre, Indigofera tinctoria and Trachyspermum Ammi was performed by using Agilent 7890A GC System. Also, High-Resolution Mass Spectrometry, LC-MS spectrum was carried out by using the Agilent technology instrument Accurate Mass Q-ToF spectrometer to find biologically active substances.[34-39]

2.2. *In-vitro* Antidiabetic Activity by *Glucose Uptake in Yeast Cell Model*

The standard experimental protocol used by Shettar et al.[2] was employed to determine glucose absorption in yeast cells. A 1% (v/v) solution of baker's yeast was prepared using distilled water and maintained at a temperature of 25°C for a duration of 24 hours. The mixture was then subjected to centrifugation at 4200 rpm for 5 minutes repeatedly until a transparent supernatant was achieved. Exactly 10 volumes of the transparent supernatant were mixed with 90 volumes of distilled water to produce a 10% v/v yeast cell suspension. Approximately 1-5 mg w/v of the *Withania Coagulans (Indian Rennet)* extract was diluted in dimethyl sulfoxide (DMSO). This resulting solution was then mixed with different glucose concentrations (5, 10, and 25 mM) in 1 mL of glucose solution, and incubated for 10 minutes at 37°C. Following incubation, the samples underwent centrifugation at 3800 rpm for a duration of 6 minutes, after which the glucose concentrations in the resulting fluid were assessed using a UV-vis spectrophotometer at a wavelength of 520 nm. Control samples and standard (METFORMIN) were included for each test.[31-33]

3. RESULT AND DISCUSSION

3.1. Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis with Gas Chromatography and Mass Spectrometry targets traditional medicinal plants like *Indian Rennet, Gymnema Sylvestre, Indigofera tinctoria,* and *Trachyspermum ammi*

Figure 1a shows the GC-MS analysis of *Withania Coagulans*, revealing 18 peaks corresponding to phytochemicals, detailed in Table 1.[15,16]

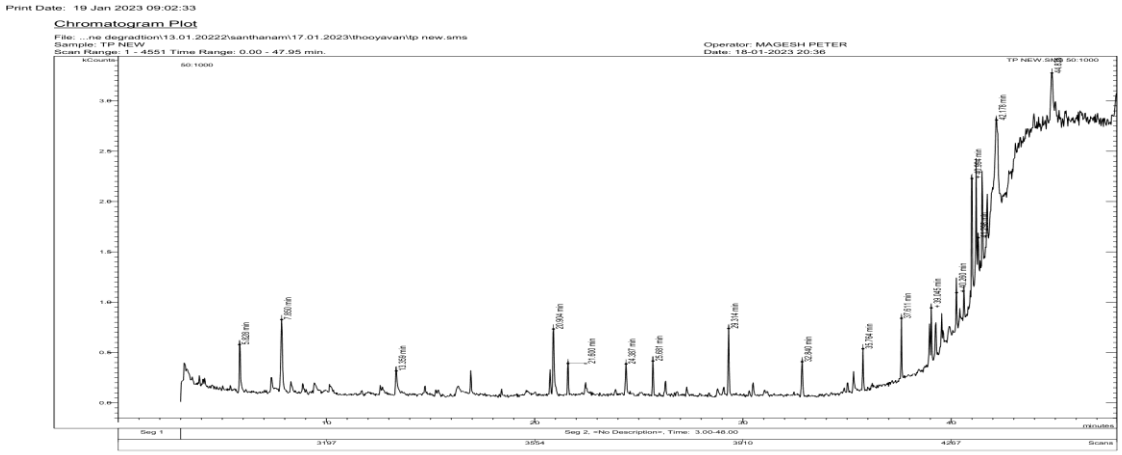


Figure: 1a-GC-MS Chromatogram of *Withania Coagulans (Indian Rennet/Paneer Dodi).*

Table 1: Findings from the GC-MS chromatogram of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

Sl. No.	Compound Name	Molecular Formula	Molecular weight	Area	RT Value	Amount	R. Match
1.	5-((2,4,6-trichlorophenoxy)methyl)furan-2-carbaldehyde	$C_{12}H_7Cl_3O_3$	305.54	2175	5.828	3.558	877
2.	4-ethyl-2-pentadecyl-1,3-dioxolane	$C_{20}H_{40}O_2$	312.54	4332	7.850	7.085	902
3.	butyl phenethyl butylphosphonate	$C_{16}H_{27}O_3P$	298.36	1496	13,359	2.447	858
4.	N-butyl-N-phenylacetamide	$C_{12}H_{17}NO$	191.27	3097	20.904	5.065	664
5.	trimethylsilyl 2,6-bis((trimethylsilyl)oxy)cyclohexane-1-carboxylate	$C_{16}H_{36}O_4Si_3$	376.72	1235	21.600	2.019	664
6.	N-(4-hydroxyphenethyl) acetamide	$C_{10}H_{13}NO_2$	179.22	1320	24.387	2.160	748
7.	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,13-hexadecamethylheptasiloxane	$C_{16}H_{48}O_6Si_7$	533.15	1284	25.681	2.100	602
8.	2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_8H_{24}O_4Si_4$	296.62	2331	29.314	3.812	886
9.	bis(tert-butyldimethylsilyl) ethylphosphonate	$C_{14}H_{35}O_3PSi_2$	338.57	2603	39.045	4.257	833
10.	[1,1'-binaphthalene]-2,2'-diamine	$C_{20}H_{16}N_2$	284.36	1321	39.256	2.160	684
11.	1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione	$C_{16}H_{12}O_5$	284.27	1398	39.562	2.287	609
12.	9-ethyl-3,6-dimethoxy-10-methylphenanthrene	$C_{19}H_{20}O_2$	280.37	805	40.619	1.317	787
13.	4b1-methyl-8b,12b-dihydrodibenzo[2,3:4,5]pentaleno[1,6-ab]inden-4b(4b1H)-ol	$C_{23}H_{18}O$	310.40	4822	40.994	7.886	534
14.	pyrazolo[3,4-b]thiopyrano[4,3-d]pyridin-1-amine	$C_9H_6N_4S$	202.24	1727	41.203	2.825	580
15.	1-(2,4-bis((trimethylsilyl)oxy)phenyl)-2-(4-((trimethylsilyl)oxy)phenyl)propan-1-one	$C_{24}H_{38}O_4Si_3$	474.82	2026	41.298	3.313	606
16.	4,4'-oxybis(tert-butylbenzene)	$C_{20}H_{26}O$	282.43	3127	41.499	5.115	798
17.	2-butoxy-2,4,6,8-tetraethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_{12}H_{32}O_5Si_4$	368.72	15785	42.178	25.817	719
18.	6,6-diphenyl-2,5,7-trioxa-6-silatridecane	$C_{21}H_{30}O_3Si$	358.55	2581	44.835	4.222	825

Similarly, GC-MS analysis of *Gymnema Sylvestre* (Figure 2a) shows twelve peaks corresponding to phytoconstituents, with detailed information provided in Table 2. The chromatogram confirms the presence of these compounds in the methanol extracts.[16,17]

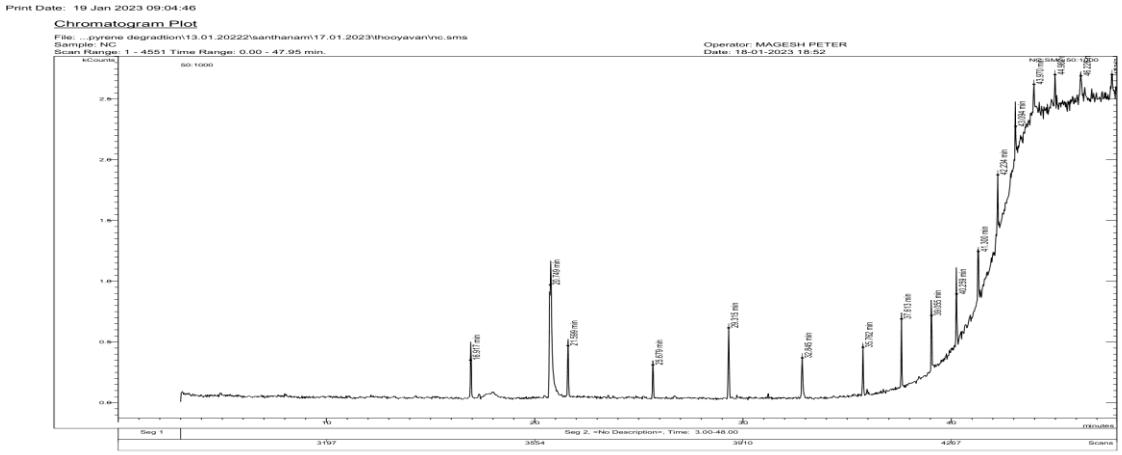


Figure 2a: GC-MS Chromatogram of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

Table 2: Findings from the GC-MS chromatogram of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

Sl. No.	Compound Name	Molecular formula	Molecular weight	Area	RT Value	Amount	R. Match
1.	2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_8H_{24}O_4Si_4$	296.62	1564	16.917	5.079	856
2.	3,3-dimethyl-1-phenylazetidine	$C_{11}H_{15}N$	161.25	8754	20.749	28.436	627
3.	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16-hexadecamethyl-1,3,5,7,9,11,13,15-octaoxa-2,4,6,8,10,12,14,16-octasilacyclohexadecane	$C_{16}H_{48}O_8Si_8$	593.23	1471	21.599	4.779	642
4.	2,2,4,4,6,6,8,8,10,10,12,12,14,14,16,16,18,18-octadecamethyl-1,3,5,7,9,11,13,15,17-nonaoxa-2,4,6,8,10,12,14,16,18-nonasilacyclooctadecane	$C_{18}H_{54}O_9Si_9$	667.39	987	25.679	3.208	607
5.	6-(4-chlorophenyl)-4-phenylpyrimidin-2(1H)-one	$C_{16}H_{11}ClN_2O$	282.73	1912	29.315	6.211	833
6.	bis(tert-butyldimethylsilyl) ethylphosphonate	$C_{14}H_{35}O_3PSi_2$	338.57	1440	32.845	4.678	864
7.	4,6-dimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2H)-one	$C_{18}H_{16}O_5$	312.32	1126	35.762	3.658	797
8.	6-(4-chlorophenyl)-4-phenylpyrimidin-2(1H)-one	$C_{16}H_{11}ClN_2O$	282.73	1747	41.300	5.675	868
9.	(S)-1-(2-(hydroxymethyl)pyrrolidin-	$C_{17}H_{22}N_2O_2$	286.38	1827	42.234	5.935	910

	1-yl)-3-(5-methyl-1H-indol-3-yl)propan-1-one						
10.	4,6-dimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2H)-one	$C_{18}H_{16}O_5$	312.32	1060	43.094	3.444	840
11.	1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane	$C_{10}H_{28}O_6Si_5$	384.75	1064	46.226	3.458	677
12.	1,1,1,3,3,5,5,7,7,9,9,11,11,13,13,13-hexadecamethylheptasiloxane	$C_{16}H_{48}O_6Si_7$	533.15	1077	47.717	3.500	518

Likewise, GC-MS analysis of *Indigofera tinctoria* shows 15 peaks, indicating 15 phytoconstituents, as illustrated in Figure 3a. Details are provided in Table 3.[17,18]

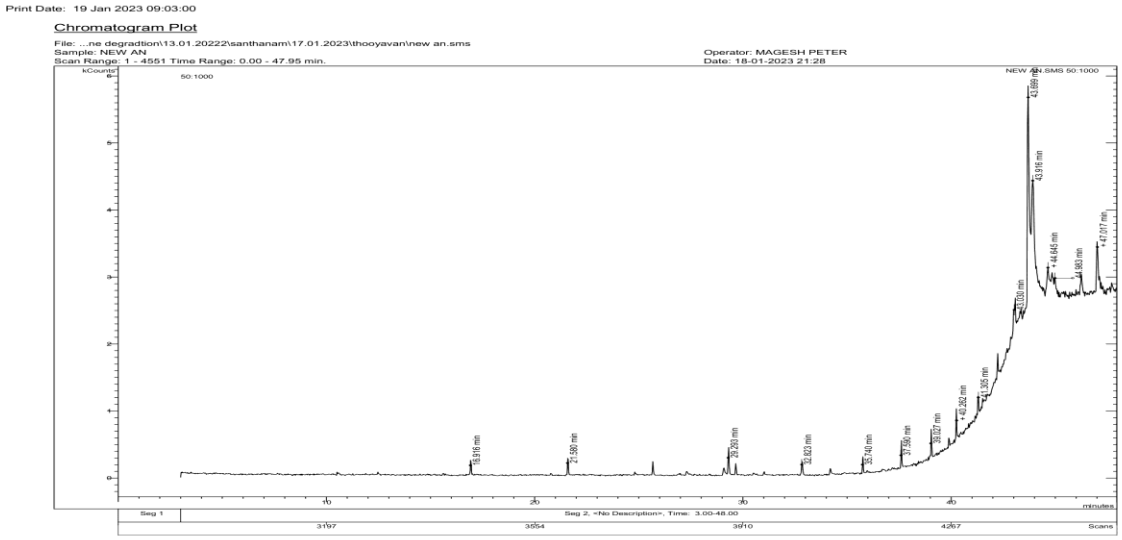


Figure 3a: GC-MS Chromatogram of *Indigofera tinctoria (True Indigo).*

Table 3: Findings from the GC-MS chromatogram of *Indigofera tinctoria (True Indigo).*

Sl. No	Compound Name	Molecular Formula	Molecular weight	Area	RT Value	Amount	R. Match
1	1-(2,5-bis((trimethylsilyl) oxy)phenyl)ethan-1-one	$C_{14}H_{24}O_3Si_2$	296.51	770	16.916	1.154	870
2	trimethylsilyl 2,5-bis ((trimethylsilyl)oxy)benzo ate	$C_{16}H_{30}O_4Si_3$	154.12	370.67	21.580	1.272	683
3.	2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_8H_{24}O_4Si_4$	296.2	1233	29.293	1.848	868
4.	2-butoxy-2,4,6,8-tetraethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_{12}H_{32}O_5Si_4$	368.72	1191	39.027	1.784	656
5.	octyl (2,4,4-trimethyl pentyl) phthalate	$C_{24}H_{38}O_4$	390.56	302	39.905	0.453	714

6.	Octasiloxane, 1,1,3,3,5,5,7,7, 9,9,11,11,13,13,1,5,15 hexadecamethyl	$C_{32}H_{72}O_{12}Si_8$	873.60	1087	40.262	1.629	556
7.	(R,Z)-((1,3-diphenyl hept-1-en-1-yl)oxy)trimethylsilane	$C_{22}H_{30}OSi$	338.57	976	41.305	1.462	852
8.	8-(pyrrolidin-1-yl sulfonyl)dibenzo[b,d]furan-3-amine	$C_{16}H_{16}N_2O_3S$	316.38	2137	43.030	3.201	582
9.	(E)-7-(benzylideneamino)-4-methyl-2H-chromen-2-one	$C_{17}H_{13}NO_2$	263.30	20046	43.699	30.031	332
10.	3-methyl-1,2-dihydrocyclopenta[ij]tetraphen-1-ol	$C_{21}H_{16}O$	284.36	21841	43.916	32.721	646
11.	N,N'-([1,1'-binaphthalene]-2,2'-diyl)diacetamide	$C_{24}H_{20}N_2O_2$	368.44	2913	44.645	4.365	598
12.	2,2,4,4,6,6,8,8-octamethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	$C_8H_{24}O_4Si_4$	296.62	2455	44.853	3.678	834
13.	1-(2,4-bis((trimethylsilyl)oxy)phenyl)ethan-1-one	$C_{14}H_{24}O_3Si_2$	296.51	1237	44.983	1.854	674
14.	7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl propionate	$C_{19}H_{17}ClN_2O_3$	356.81	1534	46.249	2.299	709
15.	(5R,8R,9S,10S,13S,14S)-3-hydroxy-16-(hydroxyimino)-10,13-dimethylhexadecahydro-17H-cyclopenta[a]phenanthren-17-one	$C_{19}H_{29}NO_3$	319.45	5648	47.017	8.462	570

Figure 4a shows the GC-MS analysis of *Trachyspermum ammi. L*, revealing fifteen phytoconstituents identified in methanol extracts. Table 10 details the compound names, molecular formulas, weights, areas, RT values, amounts, and R. Match.[19,20,21]

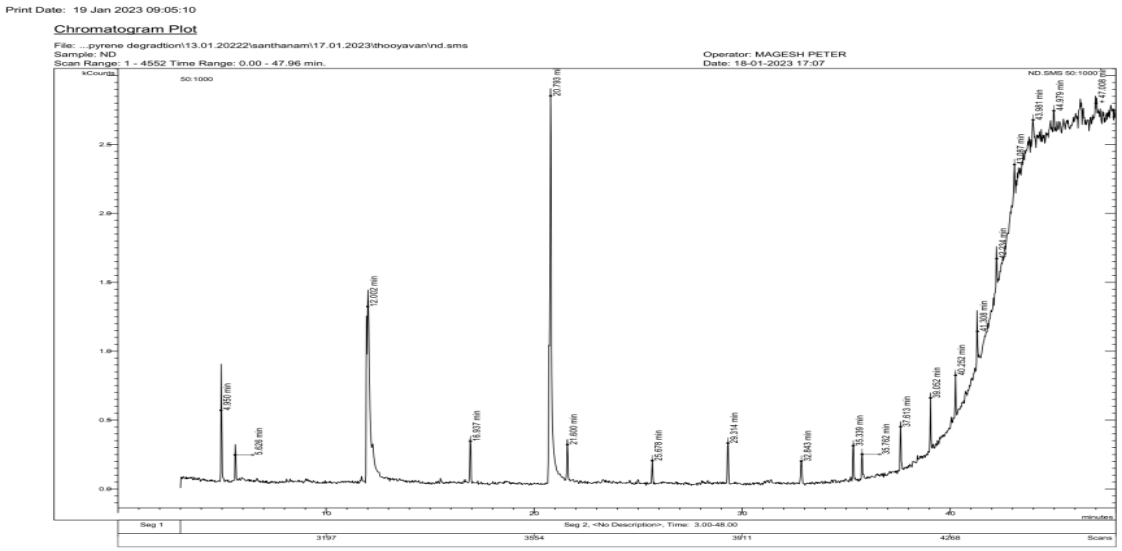


Figure 4a: GC-MS Chromatogram of *Trachyspermum ammi. L (Thymol/ Ajwain).*

Table 4: Findings from the GC-MS chromatogram of *Trachyspermum ammi. L (Thymol/ Ajwain).*

Sl. No.	Compound Name	Molecular Formula	Molecular weight	Area	RT Value	Amount	R. Match
1.	p-cymene	$C_{10}H_{14}$	134.22	2311	4.950	3.915	794
2.	1,2,3,4,5-pentamethyl cyclopenta-1,3-diene	$C_{10}H_{16}$	136.24	769	5.626	1.302	619
3.	((2S,3S,4R,5R)-2,4-dibenzyl-2,3,4-trihydroxy-5-(1-hydroxy-2-phenylethyl)tetrahydro furan-3-yl)(4-nitrophenyl)methanone	$C_{33}H_{31}NO_8$	569.61	12904	12.002	21.857	719
4.	2,2,4,4,6,6,8,8-octamethyl -1,3,5,7,2,4,6,8-tetraoxatetra Silocane	$C_8H_{24}O_4Si_4$	296.62	1150	16.937	1.948	857
5.	3-vinylpyridine	C_7H_7N	105.14	17865	20.793	30.260	567
6.	trimethylsilyl 2,4-bis((trimethylsilyl)oxy) benzoate	$C_{16}H_{30}O_4Si_3$	370.67	1000	21.600	1.693	661
7.	2,2,4,4,6,6,8,8,10,10, 12,12,14, 14,16,16,18,18-octadecamethyl -1,3,5,7,9,11,13,15,17-nonaoxa-2,4,6,8,10,12,14,16,18-nonasila Cyclooctadecane	$C_{18}H_{54}O_9Si_9$	667.39	584	25.678	0.990	595

8.	4-(2-(benzo[d]oxazol-2-yl)vinyl)benzoic acid	$C_{16}H_{11}NO_3$	265.27	873	35.339	1.478	829
9.	6-(4-chlorophenyl)-4-phenylpyrimidin-2(1H)-one	$C_{16}H_{11}ClN_2O$	282.73	767	35.762	1.300	901
10.	(S)-1-(2-(hydroxymethyl) pyrrolidin-1-yl)-3-methyl butan-1-one	$C_{10}H_{19}NO_2$	185.27	825	41.308	1.397	849
11.	(cyclopentyloxy)dimethyl (perfluorophenyl)silane	$C_{13}H_{15}F_5OSi$	310.34	893	42.234	1.513	663
12.	8-chloro-5H-indeno[1,2-b] quinoline-10,11-dione	$C_{16}H_8ClNO_2$	281.70	448	44.979	0.759	857
13.	4H-chromen-4-one	$C_9H_6O_2$	146.04	1171	46.272	1.983	478
14.	2-(6-(2-methylbut-3-en-2-yl)-7-oxo-2,3-dihydro-7H-furo [3,2-g]chromen-2-yl)propan-2-yl acetate	$C_{21}H_{24}O_5$	356.16	342	46.393	0.579	472
15.	Chromium,tetracarbonyl-[.eta.-4-4,5-diethyl-1,2,2	$C_8H_{10}CrO_4$	222.16	1391	47.008	2.357	524

Research suggests that herbal remedies may assist with health conditions such as cancer and diabetes, although scientific support is limited. This study investigates *Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria, and Trachyspermum Ammi*, concentrating on their healing properties and bioactive components. The aim is to bridge traditional usage with scientific proof and examine their potential for contemporary treatments. Gaining insight into the medicinal significance of these herbs could result in innovative therapies and enhanced health outcomes.[22-25]

3.2. LC-MS findings of herbal plants

The LC-MS chromatograms for *Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria* and *Trachyspermum Ammi* showed peaks at 17, 26, 17 and 19. Their spectra are shown in figures 1b, 2b, 3b, and 4b. Details on retention time, area, and % area is in table 5.[25-30]

Table 5: LC-MS findings of herbal plants.

Gymnema Sylvestre			*Withania Coagulans*			*Indigofera Tinctoria*			*Trachyspermum Ammi*		
Ret. time	**Area**	**Area**	**Ret. time**	**Area**	**Area**	**Ret. time**	**Area**	**Area**	**Ret. time**	**Area**	**Area**
0.581	17495	0.371	0.727	13543813	53.090	1.616	811016	24.536	0.738	1281242	43.134
0.758	796328	16.877	0.933	1547417	6.066	1.7582	1799184	54.431	0.901	319920	10.770
1.176	12338	0.261	1.083	482898	1.893	2.081	8718	0.264	1.104	36534	1.230
1.680	88007	1.865	1.799	3397519	13.318	2.225	6066	0.184	1.477	39975	1.346
1.776	163490	3.465	1.885	1276467	5.004	2.305	20214	0.612	1.541	53870	1.814
1.883	365035	7.737	2.064	1065027	4.175	2.341	190077	5.750	1.724	49523	1.667

1.993	1730554	36.677	2.124	1066097	4.179	2.384	68191	2.063	1.840	93979	3.164
2.214	166685	3.533	2.211	472434	1.852	2.618	8242	0.249	1.909	246735	8.306
2,282	294960	6.251	2.289	795999	3.120	2.709	169691	5.134	1.985	65386	2.201
2.341	132632	2.811	2.431	578438	2.267	2.955	7758	0.235	2.039	192500	6.481
2.405	250801	5.315	2.657	635020	2.489	3.098	2408	0.073	2.149	39097	1.316
2.584	206546	4.378	2.778	132726	0.520	3.183	17653	0.534	2.292	21150	0.712
2.703	170680	3.617	2.861	97538	0.382	3.280	36072	1.091	2,428	8131	0.274
2.857	26801	0.568	2.953	256513	1.006	3.586	98960	2.994	2.539	8260	0.278
2.934	20580	0.436	3.353	95241	0.373	3.816	19850	0.601	2.630	19097	0.643
3.098	11940	0.253	3.462	4883	0.019	4.086	16609	0.502	2.705	70573	2.376
3.379	31914	0.676	3.879	62835	0.246	4.220	24712	0.748	3.159	8453	0.285
3.532	28672	0.608							3.287	406270	13.677
3.568	11306	0.240							3.655	9704	0.327
3.706	5343	0.113	***Gymnema Sylvestre***			***Withania Coagulans***			***Trachyspermum Ammi***		
3.947	46473	0.985	Ret. Time	Area	Base Peak m/z	Ret. Time	Area	Base Peak m/z	Ret. Time	Area	Base Peak m/z
4.192	33158	0.703	0.740	933138	266.15	0.748	2921858	273.05	0.682	745896	266.15
4.335	62346	1.321	1.794	193588	340.25	0.983	58641	282.05	1.001	110660	143.05
4.582	7076	0.150	2.040	2137381	679.65	1.802	49109	340.25	3.372	75061	159.25
4.696	15823	0.335	2.345	1449302	183.05				3.505	57438	318.25
4.893	21348	0.452									

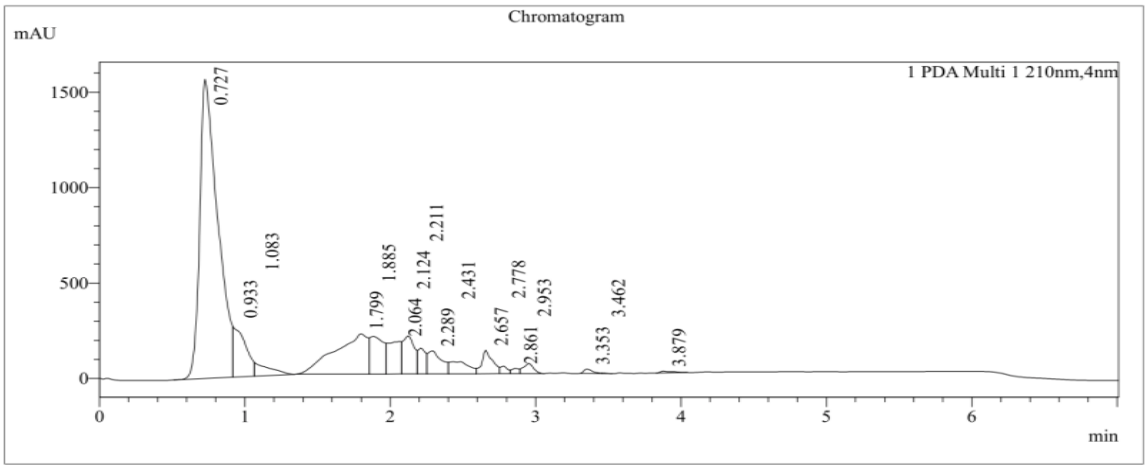


Figure 1b: LC-MS Chromatogram of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

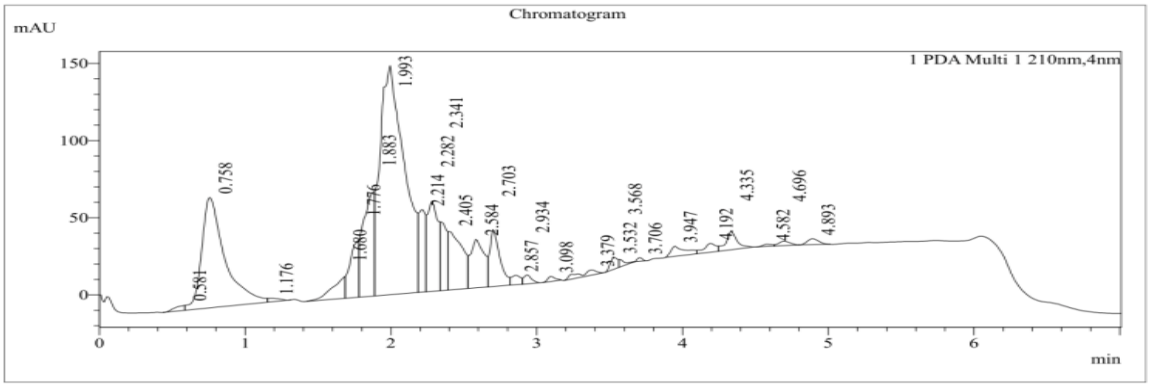


Figure 2b: LC-MS Chromatogram of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

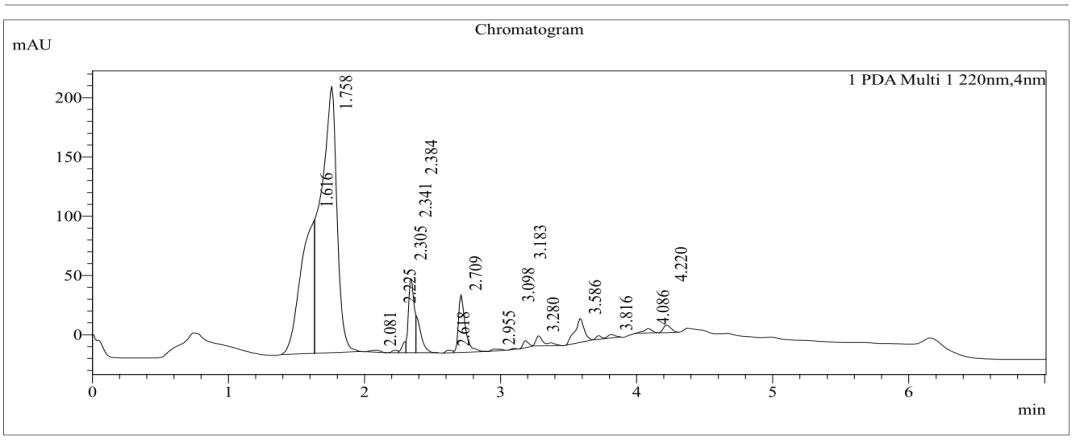


Figure 3b: LC-MS Chromatogram of *Withania Coagulans Indigofera Tinctoria (True Indigo)*.

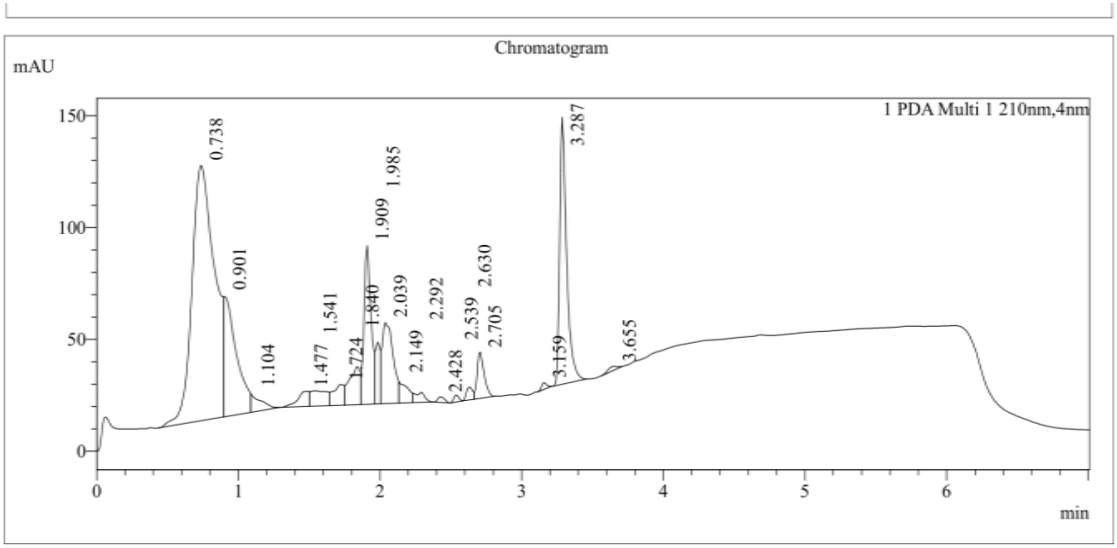


Figure 4b: LC-MS Chromatogram of *Trachyspermum Ammi (Thymol/Ajwain)*.

4. Spectroscopic Studies

Plant medicines are integral to modern life and effective in treating diseases, but quality research on the safety of herbal products is essential. People increasingly favour complementary and alternative medicine (CAM) for its low cost and fewer side effects. Therefore, authentication of herbal medicine through analytical techniques is necessary. Modern spectroscopic methods like IR, UV, ^{1}H-NMR, and LC-MS are used for identifying and quantifying compounds from plant matrices, aiding in the study of biologically active compounds.[31-39]

4.1. *Medicinal Plant Withania coagulans (Indian Rennet)*

IR (KBr, cm^{-1}): 3334 (O-H stretch, strong, broad), 1586 (C=C stretch), 1369 (O-H bending), 1230 (C-O stretch, strong) and 1044 (S=O stretch, strong) cm^{-1} (Figure 1c). UV/Vis (CH_3OH): λmax (ε) = 330 and 390 nm (Figure -1d). ^1H NMR (400 MHz, DMSO): δ= 8.05 (s, -NH, secondary amine), 3.87-4.24 (m, OH, alcohol), 2.48-2.49 (t, $-CH_2-$, methylene), 1.63 (s, 2H, $-NH_2$, amine) (Figure 1e). m/z: 984, 945 and 950 (Figure 1f).[31-39]

4.2. *Medicinal Plant Gymnema sylvestre (Sirukurinjan/ Gurmar)*

IR (KBr, cm^{-1}): 3287 (O-H stretch, weak, broad), 2917 (C-H stretch), 1623 (C=C stretch), 1316 (O-H bending) and 1026 (S=O stretch) cm^{-1} (Figure 2c). UV/Vis (CH_3OH): λmax (ε) = 260, 320 and 390 nm (Figure 2d). ^1H NMR (400 MHz, DMSO): δ= 5.49 (s, OH, alcohol), 4.52 (s, H, ethylene proton), 4.32 (s, 2H, $-CH_2-$), 3.80-3.01 (m, 2H, $-CH_2-$, methylene), 2.49 (s, 2H, methylene protons), 2.16 (d, 1H, -NH), 1.80 (d, 1H, -NH), 1.28 (s, 1H, $-CH_2-$, methylene) (Figure 2e). m/z: 979, 984, 940 and 950 (Figure 2f).[31-39]

4.3. *Medicinal Plant Indigofera tinctoria (True Indigo)*

IR (KBr, cm^{-1}): 3270 (C-H stretching, strong, broad), 1622 (C=C stretch) and 1032 (S=O stretch, strong) cm^{-1} (Figure 3c). UV/Vis (CH_3OH): λmax (ε) = 320, 390, 550 and 660 nm (Figure 3d). ^1H NMR (400 MHz, DMSO): δ= 7.59-7.57 (d, -CH, aromatic proton), 7.27-7.25 (d, -CH, aromatic hydrogen atoms), 7.06-7.02 (q, -CH, aromatic hydrogen atoms), 6.95-6.91(t, -CH, aromatic proton), 5.31 (s, 2H, ethylene protons), 4.56-4.55 (d, 1H, -OH), 3.30-3.01 (q, 2H, methylene protons), 2.51-2.49 (m, 2H, $-CH_2-$), 2.46 (s, 1H, -CH) and 1.24 (m, $-CH_2$, methylene) (Figure 3e). m/z: 982, 977 and 922 (Figure 3f).[31-39]

4.4. *Medicinal Plant Trachyspermum ammi (Thymol/Ajwain)*

IR (KBr, cm^{-1}): 3287 (O-H stretch, weak, broad), 2923 (N-H stretch, strong), 2853 (C-H stretch), 1744 (C=O stretch, strong), 1620 (C=C stretch), 1456 (C-H bend), 1378 (O-H bend, medium), 1227 (C-N stretch, medium), 1150 (C-O stretch, strong), 1028 (S=O stretch, strong) and 805 (C=C bend) cm^{-1} (Figure 4c). UV/Vis (CH_3OH): λmax (ε) = 250, 280 and 330 nm (Figure 4d). ^1H NMR (400 MHz, DMSO): δ= 9.04 (s, -CH, aromatic hydrogen protons), 7.11-7.09 (q, -CH, aromatic hydrogen protons), 6.95-6.93 (d, -CH, ethylene proton,), 6.55-6.31 (t, -CH, 1-ethylene), 5.49 (m, -CH-, ethylene), 4.38 (s,-OH, alcohol), 4.24 (s,-OH, alcohol), 3.46 (s, -CH, methine), 2.94 (s, -CH, methine), 2.49-2.48 (t, 2H,

methylene), 2.07-1.96 (t, 2H, methyl), 1.89 (s, 2H, methyl) and 1.60-1.11 (m, 3H, -CH_3) (Figure 4b). m/z: 979, 984, 940 and 950 (Figure 4f).[31-39]

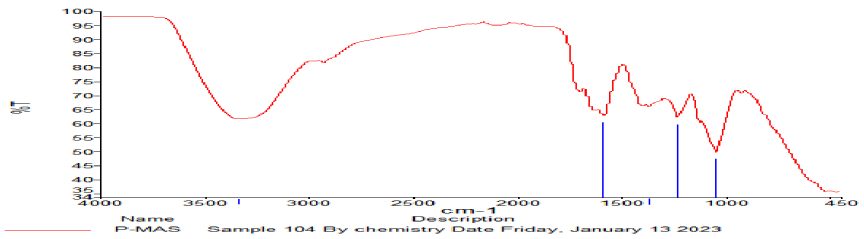


Figure 1c: IR Spectrum of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

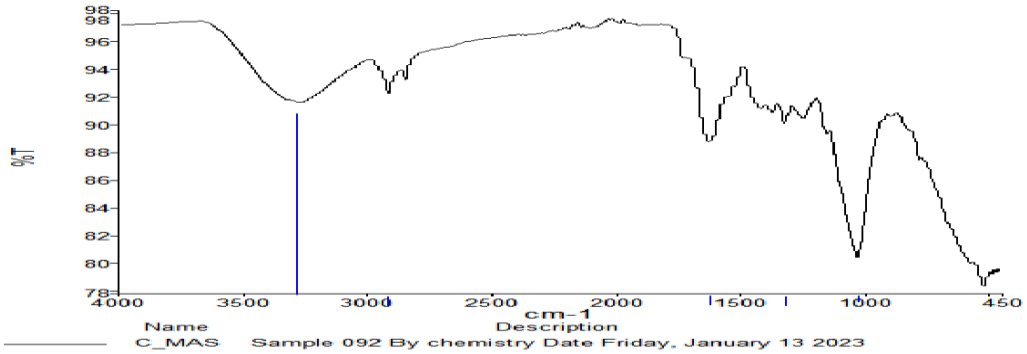


Figure 2c: IR Spectrum of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

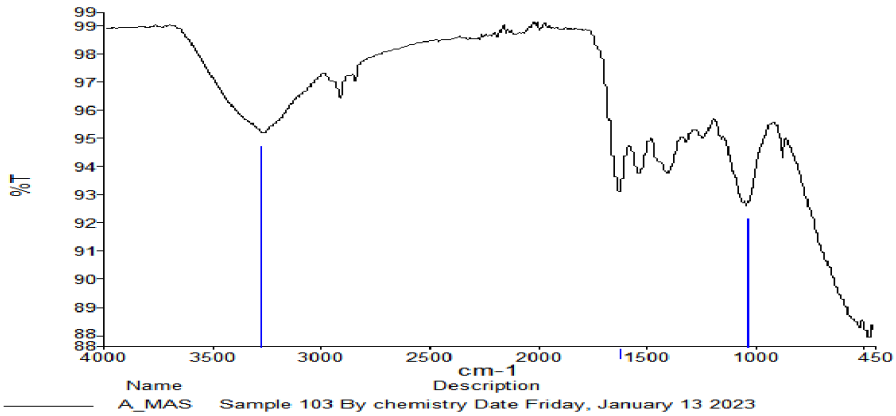


Figure 3c: IR Spectrum of *Withania Coagulans Indigofera Tinctoria (True Indigo)*.

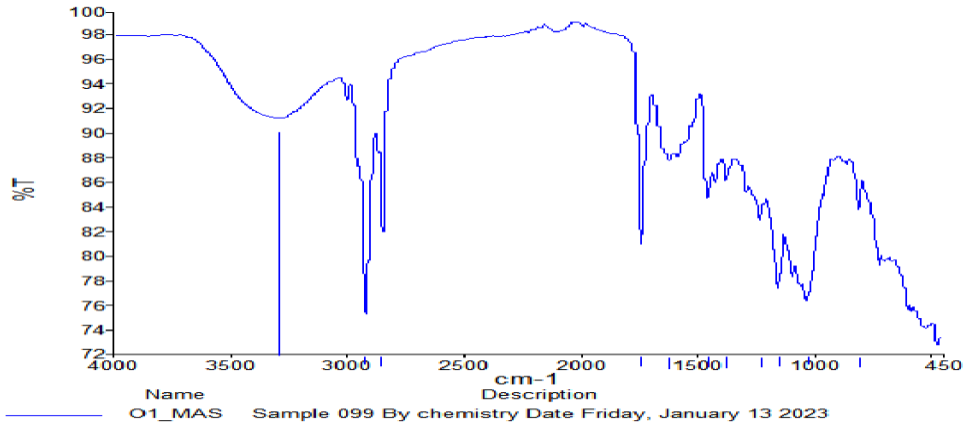


Figure 4c: IR Spectrum of *Trachyspermum Ammi (Thymol/Ajwain)*.

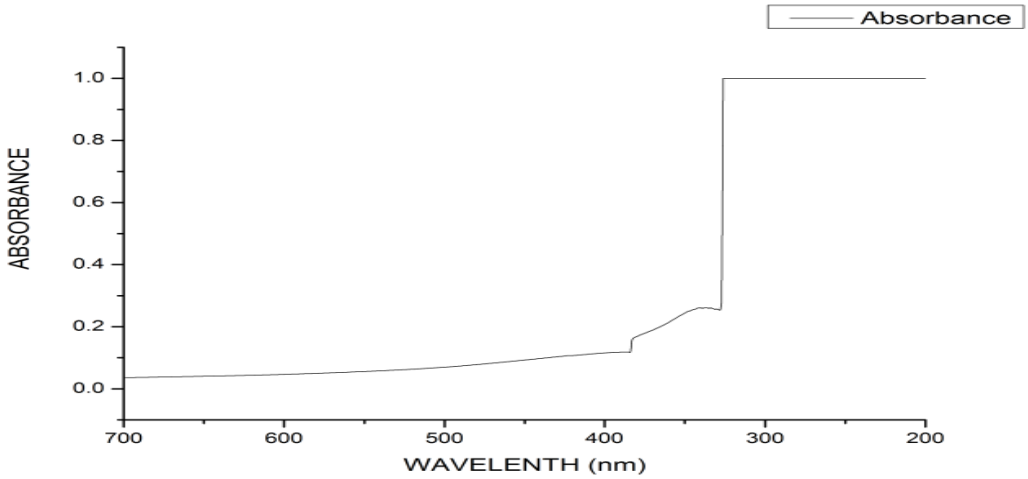


Figure 1d: UV Spectrum of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

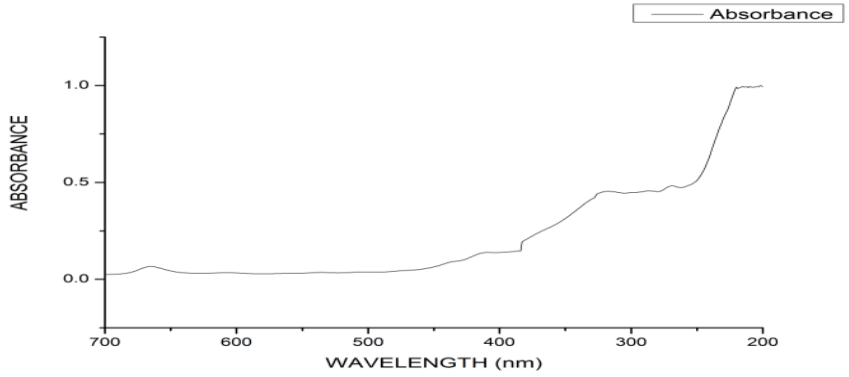


Figure 2d: UV Spectrum of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

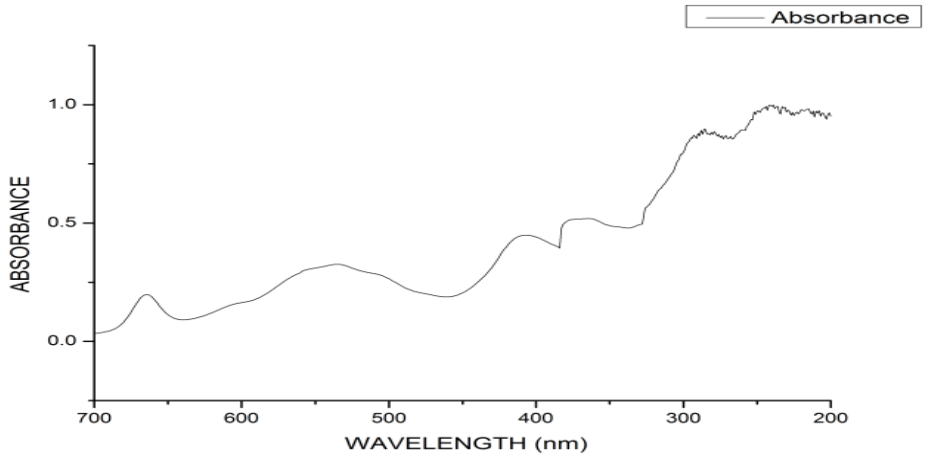


Figure 3d: UV Spectrum of *Withania Coagulans Indigofera Tinctoria (True Indigo)*.

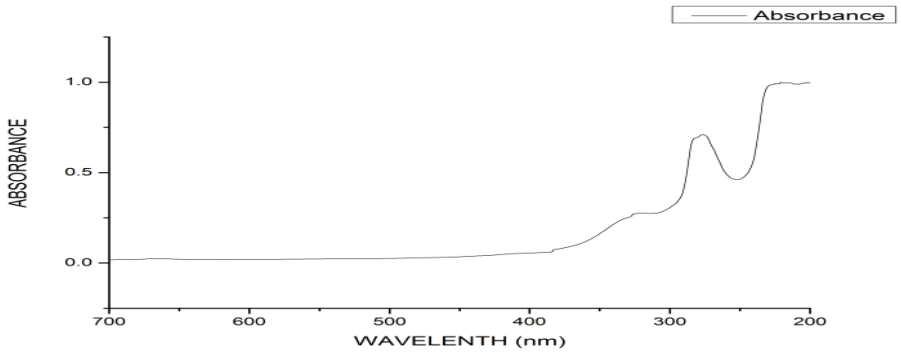


Figure 4d: UV Spectrum of *Trachyspermum Ammi (Thymol/Ajwain)*.

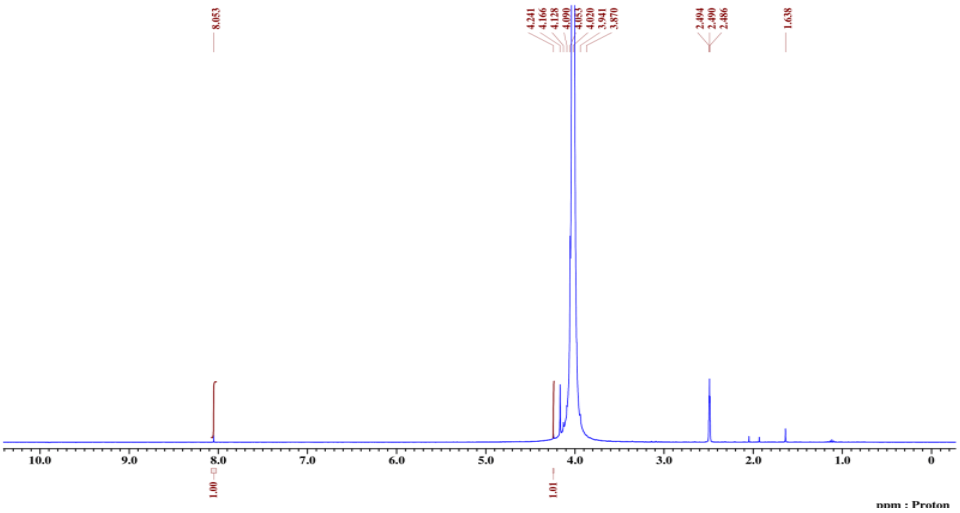


Figure 1e: ^{1}H-NMR Spectrum of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

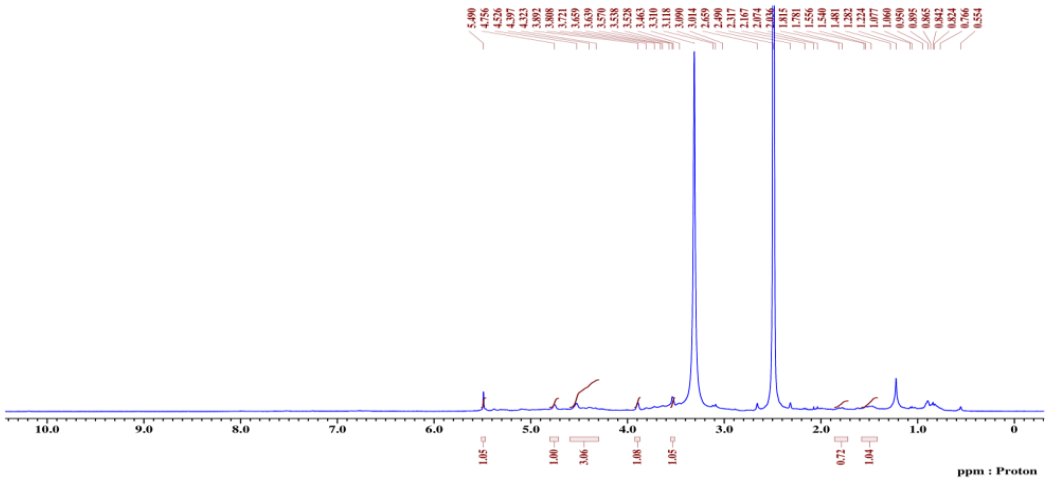


Figure 2e: ^{1}H-NMR Spectrum of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

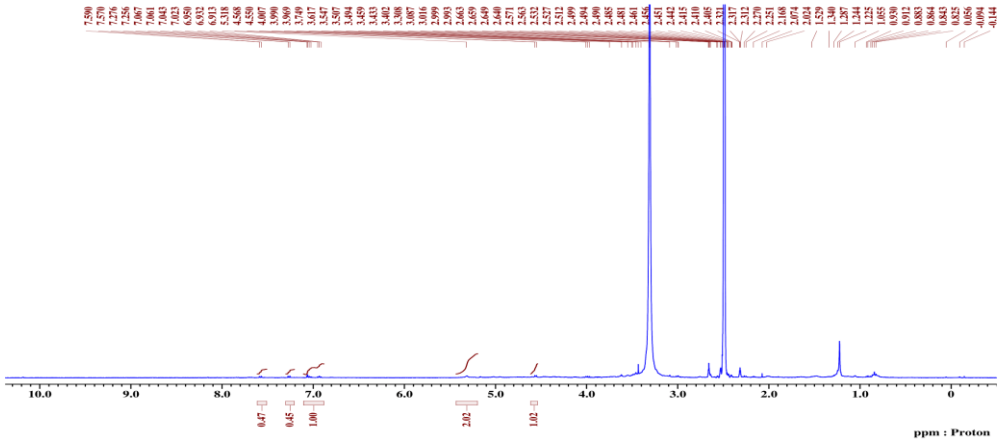


Figure 3e: ^{1}H-NMR Spectrum of *Withania Coagulans Indigofera Tinctoria (True Indigo)*.

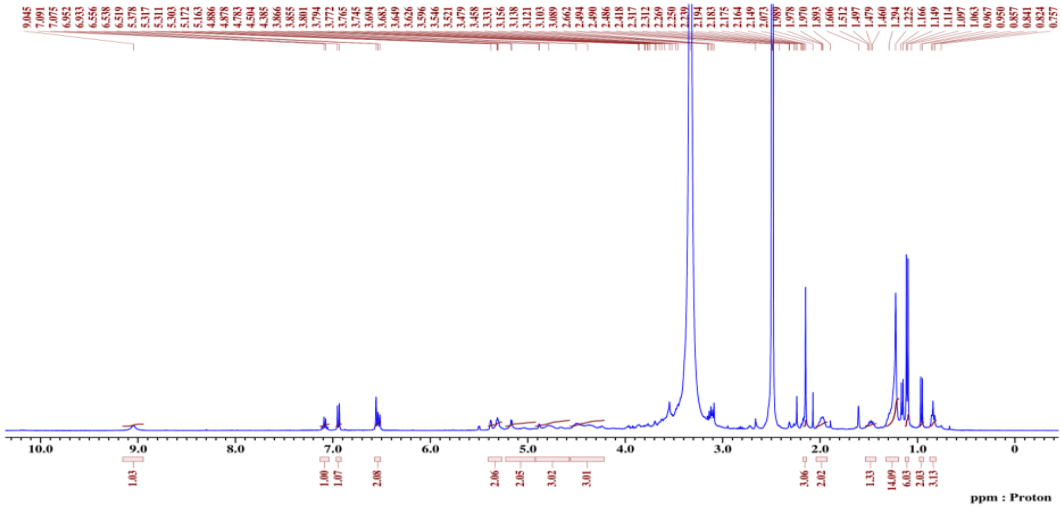


Figure 4e: ^{1}H-NMR Spectrum of *Trachyspermum Ammi (Thymol/Ajwain)*.

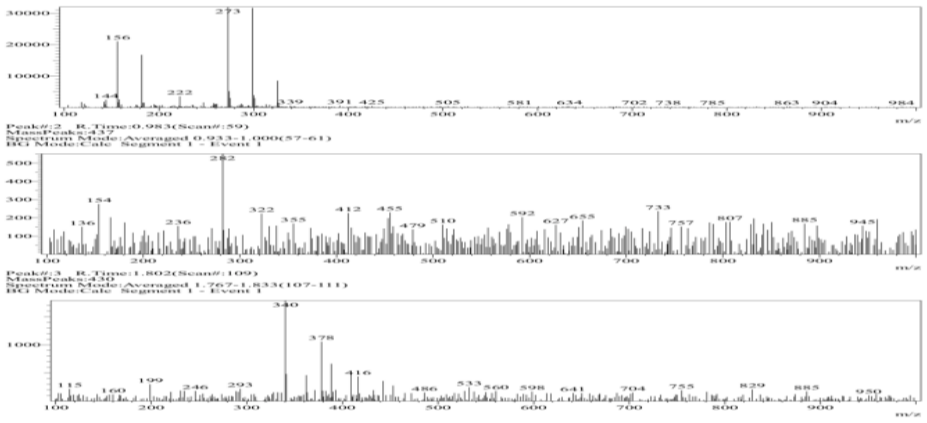


Figure 1f: Mass Spectrum of *Withania Coagulans (Indian Rennet/Paneer Dodi)*.

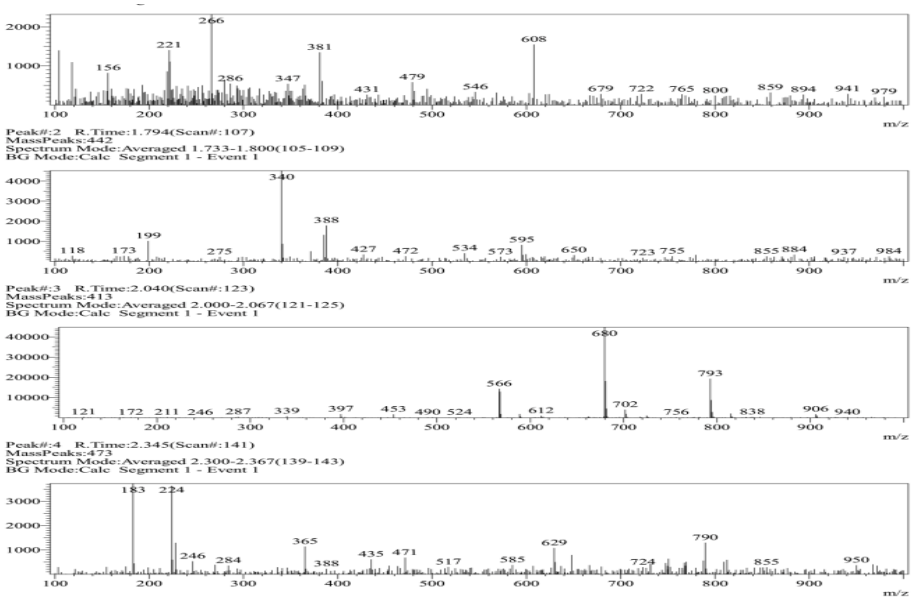


Figure 2f: Mass Spectrum of *Gymnema Sylvestre (Sirukurinjan/ Gurmar)*.

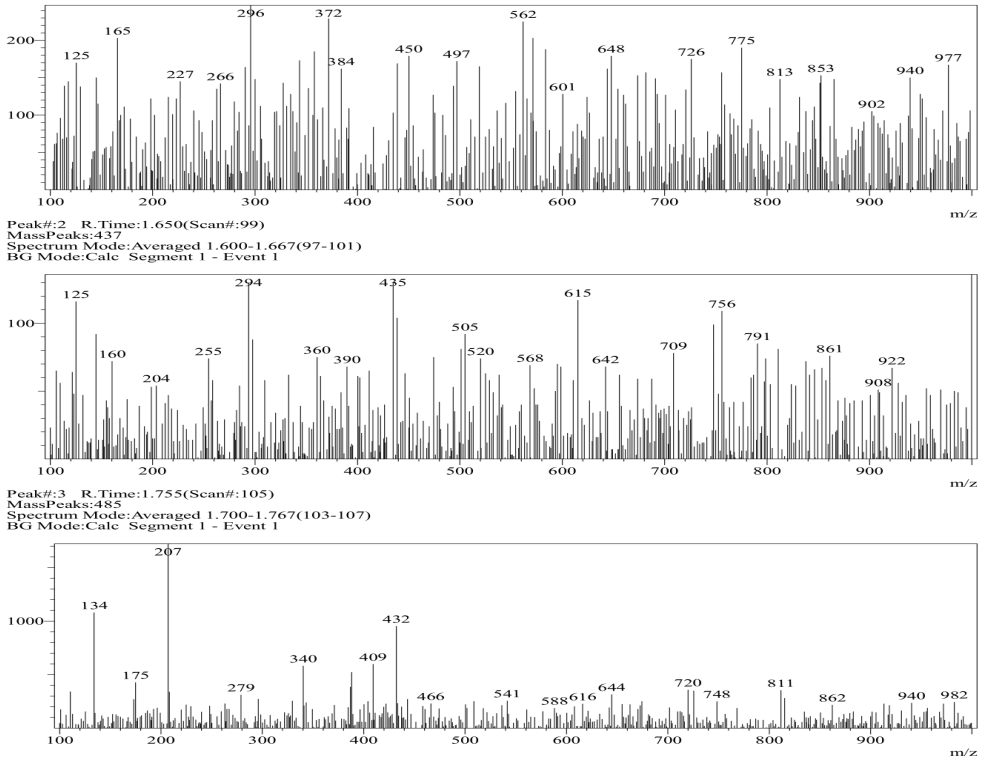


Figure 3f: Mass Spectrum of *Withania Coagulans Indigofera Tinctoria (True Indigo)*.

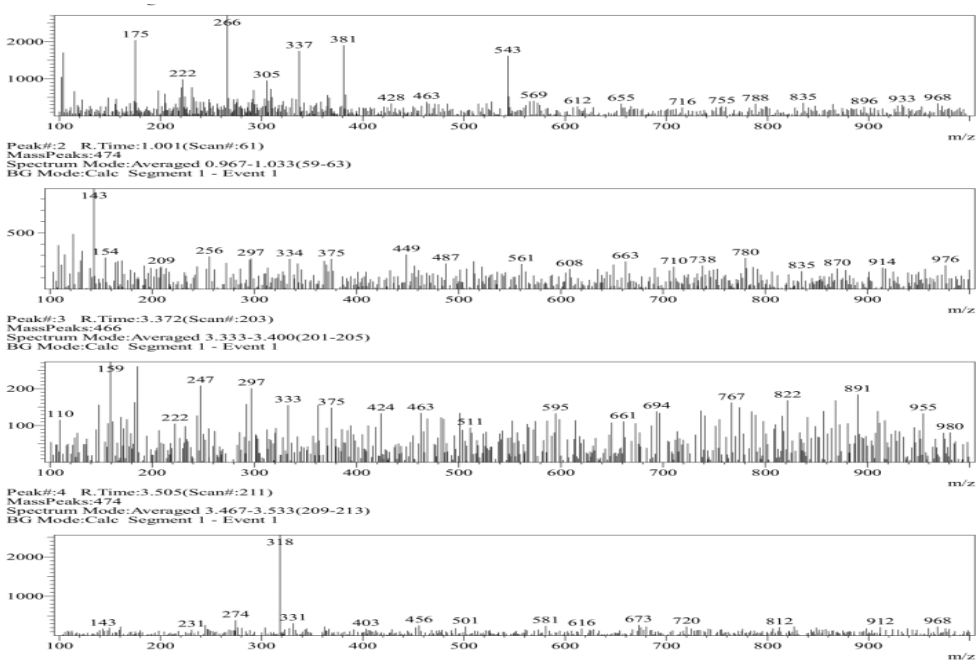


Figure 4f: Mass Spectrum of *Trachyspermum Ammi (Thymol/Ajwain)*.

4.5. In vitro Antidiabetic Activity of *Indian Rennet (Paneer Dodi)*

Glucose Uptake by Yeast Cell Assay

The present study investigated the antidiabetic effects of *Indian Rennet* extract using both Alpha-amylase and glucose uptake assays in a yeast model.[40-41] Antidiabetic activity was assessed for *Indian Rennet* extracts at five different concentrations (1, 2, 3, 4, and 5 mg) through a glucose uptake assay utilizing various glucose concentrations (5, 10, and 20 mM). Control optical density (OD) values were recorded at 0.918 (5Mm), 1.019 (10Mm) and 1.325 (20 Mm). *Indian Rennet* extracts showed OD values are 0.520 [5Mm (1 mg)], 0.710 [10Mm (1 mg)], 0.820 [20Mm (1 mg)], 0.571 [5Mm (2 mg)], 0.730 [10Mm (2 mg)], 0.843 [20Mm (2 mg)], 0.601 [5Mm (3 mg)], 0.750 [10Mm (3 mg)], 0.857 [20Mm (3 mg)], 0.612 [5Mm (4 mg)], 0.792 [10Mm (4 mg)], 0.932 [20Mm (4 mg)], 0.692 [5Mm (5 mg)], 0.800 [10Mm (5 mg)], 0.957 [20Mm (5 mg)] for glucose uptake capacity by yeast cells. The standard drug metformin exhibited OD values of 0.723 at 5 mM (5 mg), 0.821 at 10 mM (5 mg), and 0.998 at 20 mM (5 mg) in terms of glucose uptake capacity by yeast cells. The *Indian Rennet* extracts showed optical density (OD) values of 0.520, 0.571, 0.601, 0.612, and 0.692 at concentrations of 1, 2, 3, 4, and 5 mg/ml, respectively, in comparison to the standard *Metformin*, which had an OD of 0.723 at 5 mg/ml, for assessing glucose uptake capacity by yeast cells.[42,43] For the standard *Metformin* with an optical density (OD) of 0.821 at a concentration of 5 mg/ml, the *Indian Rennet* extracts exhibited OD values of 1.019 (control), 0.710, 0.730, 0.750, 0.792, and 0.800 corresponding to concentrations of 1, 2, 3, 4, and 5 mg/ml, respectively, in relation to glucose uptake by yeast cells. In contrast to the conventional *Metformin* which has an optical density (OD) of 0.998 (5 mg/ml), the *Indian Rennet* extracts demonstrated OD values of 1.325 (control µml), 0.820, 0.843, 0.857, 0.932, and 0.957 (for concentrations of 1, 2, 3, 4, and 5 mg/ml respectively) in terms of glucose uptake efficacy by yeast cells. Extracts from Indian Rennet showed a calculated rise in glucose uptake, using the formula [(Increase in glucose uptake) = [(Abs control – Abs sample) × 100/Abs control]].[44-45] The values recorded were 55.95, 62.22, 63.35, 66.02, 75.05, and 78.52 for 5 mM, 57.25, 60.33, 61.56, 64.95, 66.23, and 66.99 for 10 mM, and 61.12, 62.94, 64.00, 69.72, 71.63, and 74.75 for 20 mM, respectively. The glucose-bound G1 values displayed by *Indian Rennet* extracts were 0.921 [5 mM (1 mg)], 1.021 [10 mM (1 mg)], and 1.312 [20 mM (1 mg)], while the G6 values were 0.723 [5 mM (1 mg)], 0.821 [10 mM (1 mg)], and 0.998 [20 mM (1 mg)] in a sample volume of 100 ml, respectively. A dose-dependent increase in the percentage of glucose uptake with rising concentrations (5 to 20 mM) of *Indian Rennet* extracts was noted (see Figures 5a, 5b and 5c). The sequence of

glucose uptake activity for the extracts was 5 mg > 4 mg > 3 mg > 2 mg > 1 mg and 20 mM > 5 mM > 10 mM. The amount of bound glucose was calculated using the formula G1-G6/ Mass of the sample multiplied by the volume of the sample. The outcomes of the glucose absorption assay indicated 71.4% at a 5 mM concentration (1 mg in 100 ml sample volume), 82% at a 10 mM concentration (1 mg in 100 ml sample volume), and 98.5% at a 20 mM concentration (1 mg in 100 ml sample volume), respectively. The herbal medicinal plant *Indian Rennet* aids the pancreas in producing and releasing more insulin and helps maintain normal blood (glucose) sugar levels. Additionally, it contributes to the prevention of influenza, pneumonia, and hepatitis B, as well as protecting damaged eyes and nerves.[46-47] This effect is attributed to biologically active compounds such as Methyl-2-furaldehyde, 4-hydroxyphenethyl, Hexadecamethyl heptasiloxane, Tert-butyldimethylsilyl, ethyl phosphate, Pyrazoles, thiopyrano, pyridine, butoxy, tetraethyl, tetrasilocane, trimethylsilyloxy, and phenyl silatridecane found in *Indian Rennet*. The in vitro antidiabetic effects of *Indian Rennet* are illustrated in Figures 5a, 5b, and 5c.

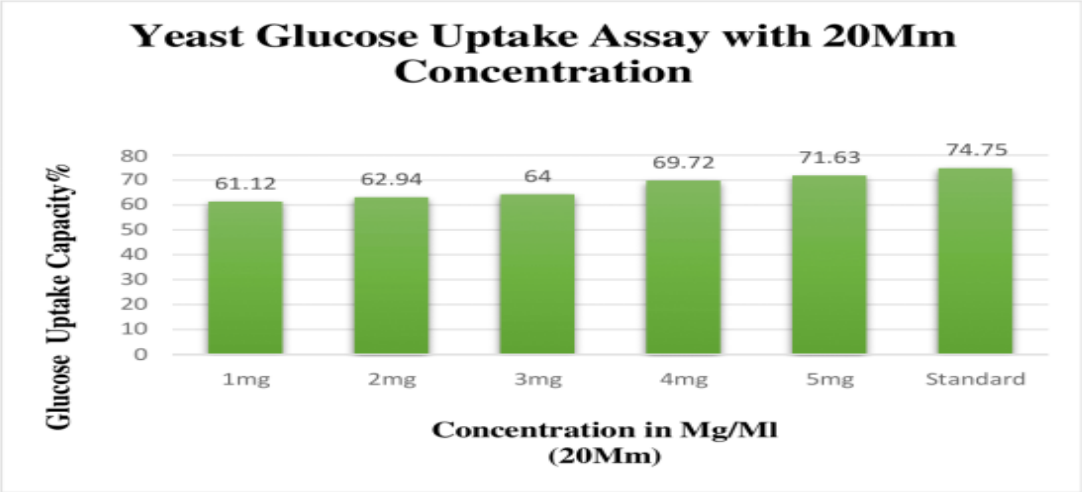


Figure 5a: Yeast Glucose Uptake Assay with 5 and 10 mM Concentration.

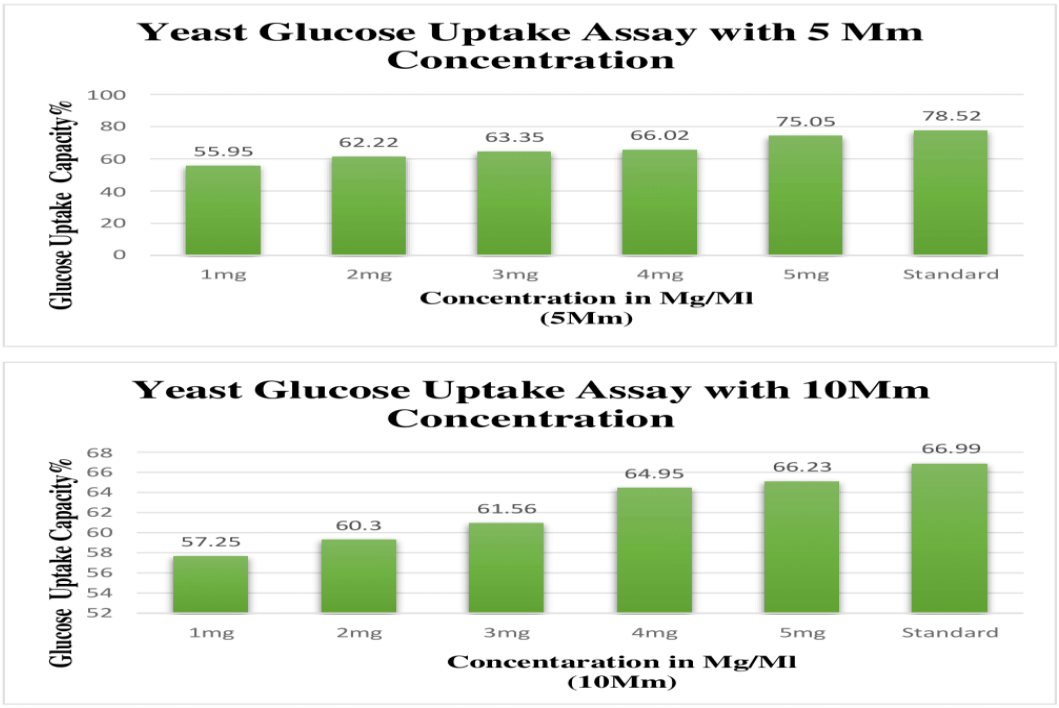


Figure 5b: Yeast Glucose Uptake Assay with 20 mM Concentration.

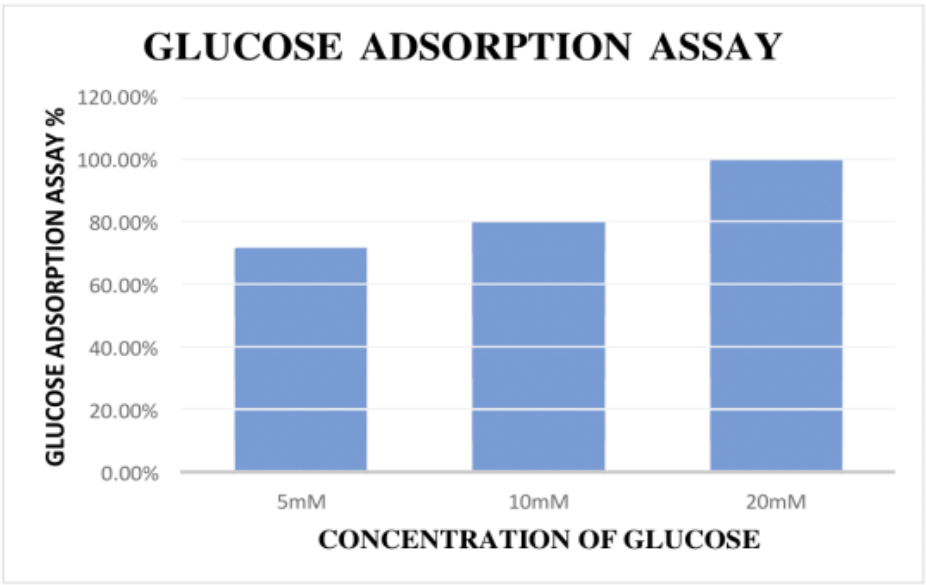


Figure 5c: Glucose Adsorption Assay with 5 mM,10 mM and 20mM Concentration.

4.6.Antibacterial properties of the traditional medicinal plant *Sirukurinjan (Gymnema Sylvestre)* extract against bacteria

In densely populated urban areas, human health is at risk from ailments and infections caused by viruses, bacteria, and fungi. While many medications available in the market are of questionable quality and often fail to combat emerging microbes, they may also have adverse side effects. In response to this challenge, chemists, in collaboration with life science researchers, have sought to identify new herbal medicines. The creation of novel drugs derived from these herbal remedies could help address the current situation. Consequently, various new pharmaceutical agents have been reported and developed from medicinal plants rich in phytoconstituents.[48-49]

The traditional medicinal plant *Gymnema Sylvestre* serves as a treatment for various bacterial, fungal, viral, and diabetic conditions. This research article examines the antibacterial effects of extracts from *Gymnema Sylvestre*, which were tested on the Gram-positive bacterium *Enterococcus faecalis* and the Gram-negative bacteria *Klebsiella pneumoniae* and *Pseudomonas fluorescens*.[50,51] Antimicrobial testing was conducted using the Mueller Hinton agar method. The bacterial strains used in this study were sourced from Hetro Gene Biotech Pvt Ltd, located in Egmore, Chennai, Tamil Nadu, India.

Bacteria such as *Enterococcus faecalis, Pseudomonas fluorescens,* and *Klebsiella pneumoniae* were evaluated in comparison to the standard medications *Ofloxacin* and *Amoxicillin/clavulanic acid*. Antibacterial effectiveness was assessed using the Mueller-Hinton agar method. The antimicrobials found in the designated sample were allowed to spread into the medium and interact within a plate that had just been inoculated with the test organisms.[52] Results indicated that a zone of inhibition formed uniformly where growth was present. The diameter of this restricted area was recorded in millimeters. *Ofloxacin* and *Amoxicillin/clavulanic acid solutions* were used as standard positive and negative controls to evaluate antibacterial effectiveness. Activities were analysed at varying concentrations of 100, 50, and 25 mg/mL in water solvent using *Enterococcus faecalis* and *Klebsiella pneumoniae*, along with *Pseudomonas fluorescens*. The standard medications *Ofloxacin* and *Klebsiella pneumoniae* were utilized for the bacteria. The medicinal plant Gymnema Sylvestre was observed to show effectiveness against Enterococcus faecalis, displaying zones of inhibition of 9, 10, and 12 mm at concentrations of 25, 50, and 100 mg/ml respectively. A zone of inhibition due to the medicinal plant *Gymnema Sylvestre* was recorded at 9 mm, 10

mm, and 12 mm at concentrations of 25, 50, and 100 mg/ml, respectively. Additionally, a zone of inhibition was found for the standard *Ofloxacin* at 12 mm in a concentration of 30 µg/ml against *Enterococcus faecalis.* It was observed that standard *Ofloxacin* (30 µg/ml) exhibited a higher activity level of 12 mm against *Enterococcus faecalis* compared to the 50 and 25 mg/mL concentrations of traditional medicinal plant *Gymnema Sylvestre*, which showed lesser activity. Likewise, when comparing the standard *Ofloxacin* (30 µg/ml) to *Gymnema Sylvestre* at 100 mg/mL, it demonstrated equivalent activity. Based on these results, the order of activity from highest to lowest was found to be 12 mm (30 µg/ml) > 12 mm (100 mg/ml) > 10 mm (50 mg/ml) > 9 mm (25 mg/ml), with the medicinal plant *Gymnema Sylvestre* showing comparable effectiveness at 12 mm (100 mg/ml) to the standard Ofloxacin, which also displayed 12 mm (30 µg/ml). The concentrations of (50 mg/ml) and (25 mg/ml) of the traditional medicinal plant *Gymnema Sylvestre* displayed lower activity in comparison to the standard Ofloxacin. In conclusion, the traditional medicinal plant *Gymnema Sylvestre* demonstrated moderate to high activity overall. In addition, a zone of inhibition measuring 12 mm was observed for the medicinal plant *Gymnema Sylvestre* at a concentration of 30 µg/ml when tested against the standard Ofloxacin.[53]

The zone of inhibition for the medicinal plant Gymnema Sylvestre was recorded as 9 mm, 10 mm, and 12 mm for the concentrations of 25, 50, and 100 mg/ml, respectively. The herb *Gymnema Sylvestre* displayed effectiveness against *Klebsiella pneumoniae*, showing inhibition zones measuring 11, 13, and 15 mm at concentrations of 25, 50, and 100 mg/ml, respectively. In comparison, the standard *Amoxicillin/clavulanic acid* (30µg/ml) demonstrated greater activity with a zone of 16 mm against *Klebsiella pneumoniae*. When compared to the standard *Amoxicillin/clavulanic acid* (30 µg/ml), the 25 mg/ml and 50 mg/ml concentrations of *Gymnema Sylvestre* showed reduced activity. Likewise, the 100 mg/ml concentration of *Gymnema Sylvestre* also exhibited lower activity compared to the standard. Based on these findings, the activity level can be ranked as 16 mm (30 µg/ml) > 15 mm (100 mg/ml) > 13 mm (50 mg/ml) > 11 mm (25 mg/ml), indicating that the medicinal plant *Gymnema Sylvestre* demonstrated slightly lower activity at 15 mm (100 mg/ml) compared to the standard *Amoxicillin/clavulanic acid* at 16 mm (30 µg/ml). The concentrations of 50 mg/ml and 25 mg/ml from *Gymnema Sylvestre* exhibited diminished activity in contrast to the standard *Amoxicillin/clavulanic acid*. In conclusion, the traditional medicinal plant *Gymnema Sylvestre* showed moderate to high antibacterial activity overall. The antibacterial activity results are depicted in figures 6a and 6b. The report on the zone of inhibition against

the three organisms, *Enterococcus faecalis* and *Pseudomonas fluorescens*, is presented in figures 6a and 6b. This figure illustrates the antagonistic effect of the control and the extract of *Gymnema Sylvestre* across three concentrations 25, 50, and 100 mg/ml against the three microorganisms. The extract of *Gymnema Sylvestre* inhibits *Enterococcus faecalis* with a zone of 11 mm at a concentration of 100 mg/ml, which is comparable to the control ofloxacin (12 mm). Similarly, *Gymnema Sylvestre* extracts inhibit *Pseudomonas fluorescens*, showing an inhibition zone of 20 mm when compared with the control Amoxicillin/Clavulanic acid.[51-53] The in vitro antibacterial activity of *Gymnema Sylvestre* is displayed in figures 6a and 6b. The antimicrobial activity results from medicinal plants are presented in table 6.

4.7.The antibacterial properties of the traditional medicinal plant *Avuri or Neeli (Indigofera Tinctoria))* extract against bacteria

The antibacterial properties of the traditional medicinal plant *Avuri or Neeli (Indigofera Tinctoria)* are demonstrated in figures 6c and 6d, which depicts the inhibition effects against two pathogens, *Streptococcus pyogenes* and *Klebsiella pneumoniae*. The dimension of the inhibition zone suggests that Indigofera Tinctoria shows more effective inhibition than the control. The extract is effective against both *Streptococcus pyogenes* and *Klebsiella pneumoniae*, showing inhibition zones of 12 mm and 11 mm respectively at a concentration of 100 µg/mL. Additionally, the medicinal plant *Indigofera Tinctoria* displayed activity against *Streptococcus pyogenes* with inhibition zones measuring 9 mm (25 µg/ml), 10 mm (50 µg/ml), and 11 mm (100 µg/ml). The standard antibiotic *Amikacin* exhibited an activity with a 14 mm zone of inhibition at 30 µg/ml against *Streptococcus pyogenes*. In the case of Klebsiella pneumoniae, inhibition zones measuring 10 mm, 11 mm, and 12 mm were noted at concentrations of 25 µg/ml, 50 µg/ml, and 100 µg/ml, respectively; this was in comparison to the standard antibiotic Gentamycin, which displayed a 14 mm zone at 30 µg/ml. Similarly, when assessed against the standard Amikacin, the traditional medicinal plant Indigofera Tinctoria exhibited notable antibacterial activity. These findings reveal that the increase in activity follows the pattern of 9 mm (25 µg/ml) < 10 mm (50 µg/ml) < 11 mm (100 µg/ml), while the extract exhibited equivalent activity with 10 mm (25 µg/ml) < 11 mm (50 µg/ml) < 12 mm (100 µg/ml). At concentrations of 50µg/ml and 25 µg/ml, the traditional medicinal plant *Indigofera Tinctoria* displayed moderate activity when compared with the standard antibiotics *Amikacin* and *Gentamycin*. In conclusion, the traditional medicinal plant Indigofera Tinctoria demonstrated a moderate to high level of overall activity. The antibacterial properties are illustrated in figures 6c and 6d. The in vitro antibacterial effect of

Indigofera Tinctoria is displayed in figures 6c and 6d. The antimicrobial activity results from medicinal plants are presented in table 6.

Table 6: Results of antimicrobial activity from medicinal plants.

Traditional medicinal plant	Organisms	Control (STD) (mm)	Zone of inhibition (mm)		
			25 mg/ml	50 mg/ml	100 mg/ml
Sirukurinjan (Gymnema Sylvestre)	**Enterococcus faecalis**	12 (Ofloxacin 30µg/ml)	9	10	12
	Klebsiella pneumoniae	16(Amoxicillin/clavulanic acid 30 µg/ml)	11	13	15
Avuri or Neeli (Indigofera Tinctoria)	**Streptococcus pyogenes**	14 mm(Amikacin 30 µg/ml)	9	10	11
	Klebsiella pneumoniae	14 mm (Gentamycin 30 µg/ml)	10	11	12
Ajwain (Trachyspermum ammi)	**Aspergillus niger**	SD067-1PK	11	12	12
	Aspergillus fumigate	SD067-1PK	10	11	11

4.8.Antifungal activity of Traditional medicinal plant *Ajwain or Ajowan (Trachyspermum ammi))* extract against fungi

The traditional medicinal plant *Trachyspermum ammi* exhibits numerous pharmacological properties, including anticancer, asthma relief, treatment for cough and digestive issues, constipation alleviation, antibacterial, antifungal, anticonvulsant, and anti-inflammatory effects. Antifungal assessments of the herbal plant *Trachyspermum ammi* were conducted using the Mueller-Hinton agar method.[54] The fungi selected for this research were *Aspergillus niger* and *Aspergillus fumigatus*, which were sourced from Hetro Gene Biotech Pvt Ltd, located in Egmore, Chennai, Tamil Nadu, India. The fungi chosen for examination are *Aspergillus niger* and *Aspergillus fumigatus*, and these were compared to the standard Original McFarland standards, which consist of sterile distilled water (SD067-1PK). The fungi *Aspergillus niger* and *Aspergillus fumigatus* were evaluated against the standard Original McFarland standards (Barium chloride and Sulfuric acid combined with sterile distilled water).[54]

The traditional medicinal plant *Trachyspermum ammi* was evaluated for its antifungal properties in vitro using the Mueller-Hinton agar method. The antifungal compounds in the sample were allowed to diffuse into the medium and interacted with a plate newly inoculated with the test organisms. The findings indicated that the zone of inhibition was consistently present in the growth areas. The diameter of this restricted area was measured in

micromillimeters. Sterile distilled water (SD067-1PK) served as the standard negative control for antifungal activity. The antifungal effects were examined at various concentrations of 100, 50, and 25 µg/mL in a water solvent against *Aspergillus niger* and *Aspergillus fumigatus*. The standard drug used was based on the Original McFarland standards sterile distilled water (SD067-1PK) for fungi. The extract from the medicinal plant Trachyspermum ammi demonstrated activity against Aspergillus niger, with inhibition zones measuring 11, 12, and 12 mm at concentrations of 25, 50, and 100 µg/ml, respectively. Likewise, inhibition zones of 10, 11, and 11 mm were observed against Aspergillus fumigatus at the same concentrations. The standard Sterile distilled water (SD067-1PK) showed no activity against *Aspergillus niger* and *Aspergillus fumigatus*. When comparing the standard Sterile distilled water (SD067-1PK) with the 50 and 25 µg/mL concentrations of the traditional medicinal plant *Trachyspermum ammi*, a higher activity was noted. Likewise, when compared with the standard Sterile distilled water (SD067-1PK), the traditional medicinal plant *Trachyspermum ammi* at 100 µg/mL exhibited significantly higher activity. Based on these findings, the recorded activity levels were arranged in the sequence of 11 < 12 < 12 mm (20 < 50 < 100 µg/ml), suggesting that the medicinal plant Trachyspermum ammi exhibited significantly greater activity at 12 mm (100 µg/ml), whereas the standard Sterile distilled water (SD067-1PK) displayed no activity.[54] The concentrations of 50 µg/ml and 25 µg/ml of the traditional medicinal plant Trachyspermum ammi exhibited greater effectiveness when compared to the standard Sterile distilled water (SD067-1PK). In conclusion, the traditional medicinal plant *Trachyspermum ammi* demonstrated moderate to high antifungal activity overall. The in vitro antifungal activity of *Trachyspermum ammi* is illustrated in figures 6e and 6f. The antimicrobial activity results from medicinal plants are presented in table 6.

ANTIMICROBIAL IMAGES OF MEDICINAL HERBAL PLANTS

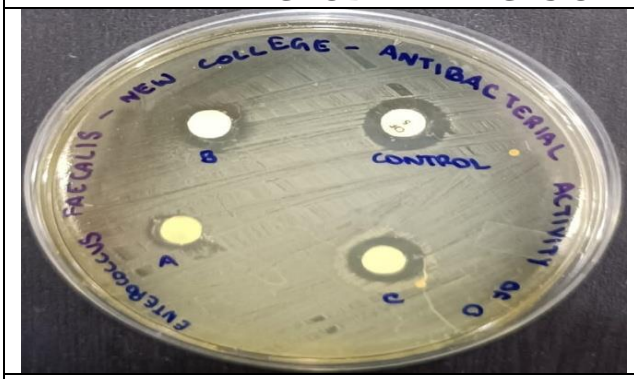


Figure 6a: Zone of Inhibition by the *Gymnema Sylvestre* and standard (control). a. *Enterococcus faecalis*.

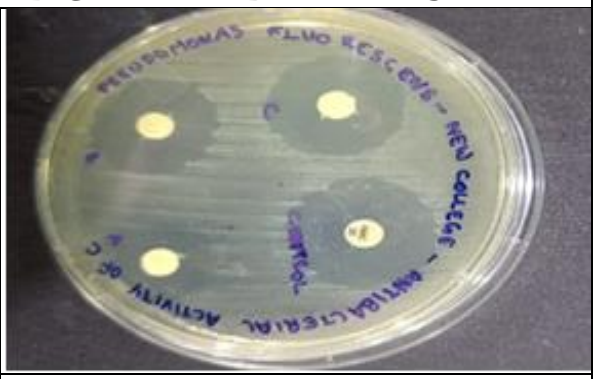


Figure 6b: Zone of Inhibition by the *Gymnema Sylvestre* and standard (control). b. *Pseudomonas fluorescens.*

Figure 6c: Zone of Inhibition by the *Indigofera Tinctoria* and standard (control). a. *Streptococcus Pyogenes.*

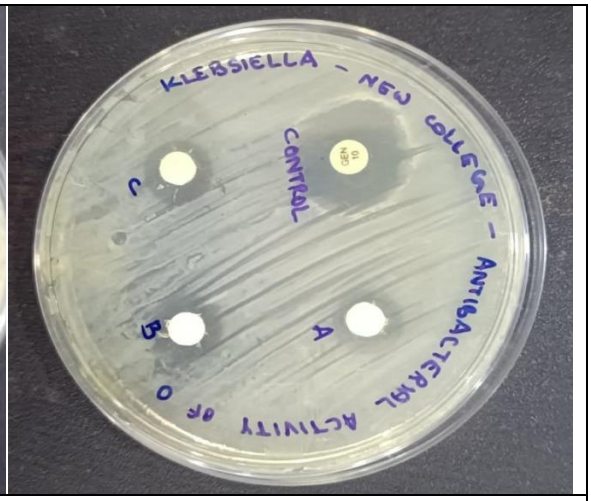


Figure 6d: Zone of Inhibition by the *Indigofera Tinctoria* and standard (control). b. *Klebsiella Pneumonia*

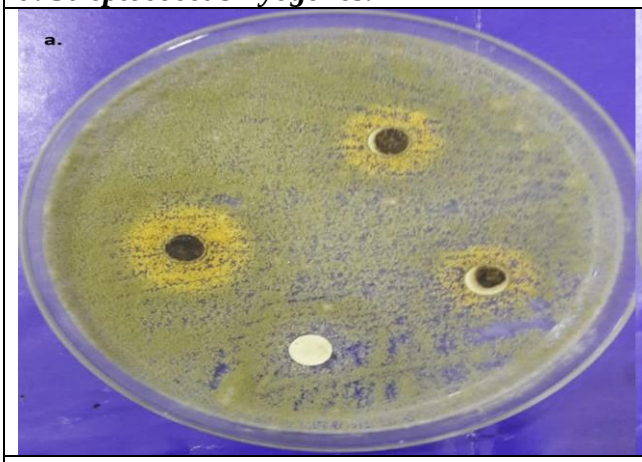


Figure 6e: Zone of Inhibition by the *Trachyspermum Ammi* and standard (control). a. *Aspergillus niger*

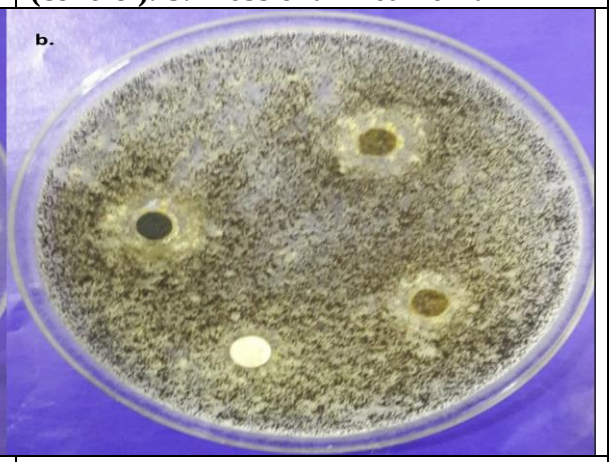


Figure 6f: Zone of Inhibition by the *Trachyspermum Ammi* and standard (control b. *Aspergillus fumigate*

4.9. Molecular docking experiments

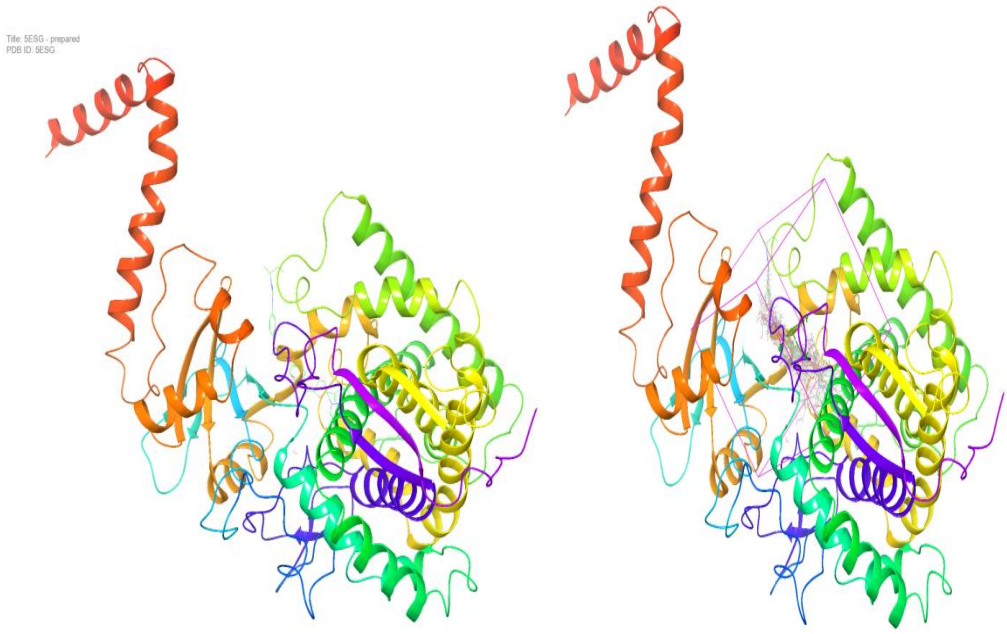
Molecular docking analyses were conducted on phytoconstituents whose 2D structures were created using Chem Draw Ultra Version 8.0.3 and saved in SDF format via the Open Babel GUI version 2.4.1.[55] The autodock software facilitated this molecular docking study, resulting in the identification of bioactive compounds and their receptors, followed by interactions classified based on lipophilicity, hydrogen bond donor capacity, molecular weight, and hydrogen bond acceptor, generating molecular docking scores.[56] From these docking scores and interactions, the best bioactive compounds were selected from all phytoconstituents, which could potentially serve as future drugs. The molecular docking studies were performed on herbal medicinal plants, specifically *Withania Coagulans* and *Gymnema Sylvestre*, with each phytoconstituent analysed against the 7myj diabetic protein using Autodock Vina 4.2 software.[57] Likewise, molecular docking studies were carried out

on herbal plants such as *Indigofera Tinctoria* and *Trachyspermum Ammi*, testing each phytoconstituent against a specified fungal protein using Autodock Vina 4.2 software. All observed docking scores were negative, indicating a stronger binding, as well as stable complex formation between the phytoconstituents of herbal medicinal plants and the protein during the docking studies.[58-62] When comparing standard drugs to the herbal medicinal plants *Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria,* and *Trachyspermum Ammi*, the latter were found to be more effective based on docking scores, which is clear. The docking images are presented in the following figures. The docking reports can be found in Table 7.

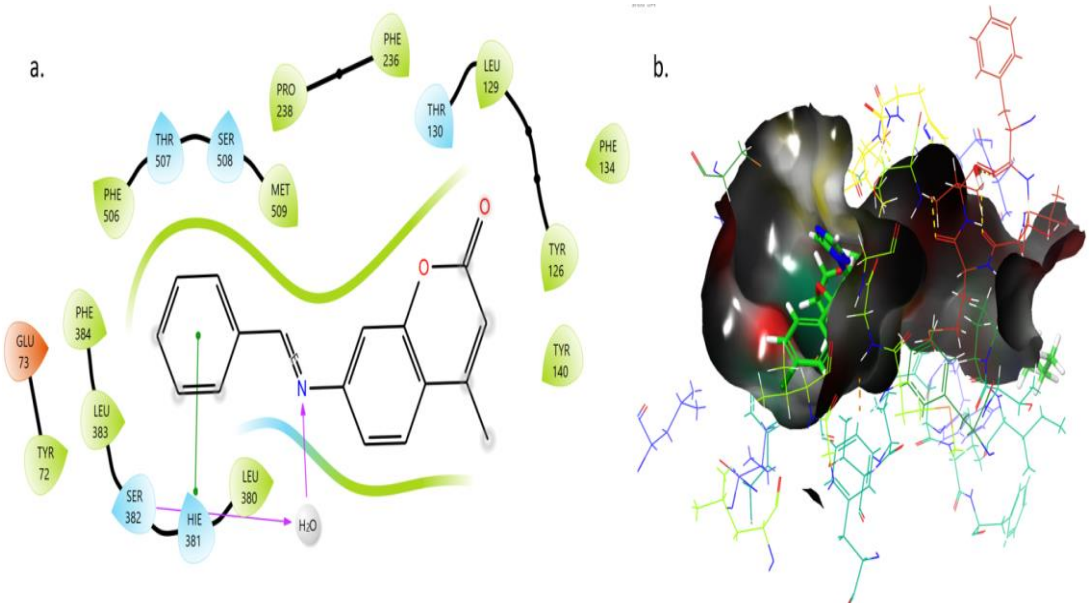
Table 7: Docking Score of the molecule against the 7myj -diabetic protein.

Sl. No.	Herbal medicinal plant *Withania Coagulans* bioactive compound name for *7myj -diabetic protein*	Docking Score	Herbal medicinal plant *Gymnema Sylvestre* bioactive compound name for *7myj -diabetic protein*	Docking Score
1	5-((2,4,6-trichlorophenoxy)methyl)furan-2-carbaldehyde	-5.227	3,3-dimethyl-1-phenylazetidine	-4.642
2	4-ethyl-2-pentadecyl-1,3-dioxolane	-4.856	4,6-dimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2H)-one	-5.344
3	Butyl phenethyl butylphosphonate	-3.976	6-(4-chlorophenyl)-4-phenylpyrimidin-2(1H)-one	-5.302
4	N-butyl-N-phenylacetamide	-5.075	(S)-1-(2-(hydroxymethyl)pyrrolidin-1-yl)-3-(5-methyl-1H-indol-3-yl)propan-1-one	-5.886
5	N-(4-hydroxyphenethyl) acetamide	-4.942		
6	1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione	-6.647		
7	9-ethyl-3,6-dimethoxy-10-methylphenanthrene	-3.976		
8	Pyrazolo[3,4-b]thiopyrano[4,3-d]pyridin-1-amine	-4.790		
9	1-(2,4-bis((trimethylsilyl)oxy)phenyl)-2-(4-((trimethylsilyl)oxy)phenyl)propan-1-one	-3.909		
10	4,4'-oxybis(tert-butylbenzene)	-5.263		
11	2-butoxy-2,4,6,8-tetraethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	-3.852		
12	METFORMIN (STANDARD DRUG)	-2.742	METFORMIN (STANDARD DRUG)	-2.742
Docking Score of the molecule against the 5ESG -fungal protein				
Sl. No	**Herbal medicinal *plant Indigofera tinctoria* bioactive compound name**	**Docking Score**	**Herbal medicinal plant *Trachyspermum ammi.L* bioactive**	**Docking Score**

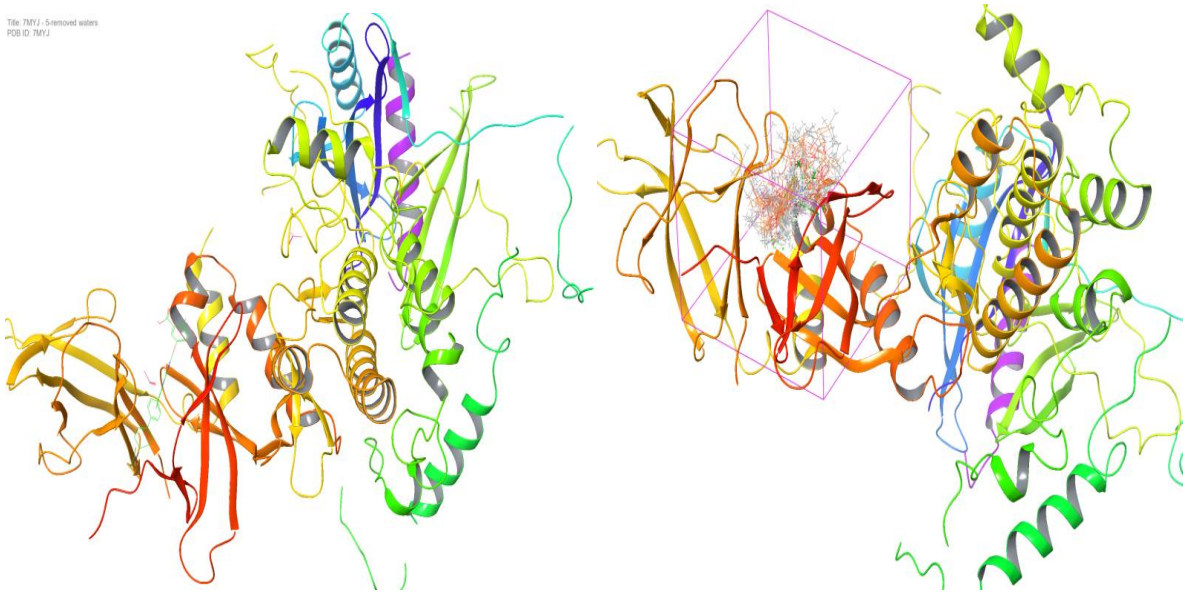
	for *5ESG -fungal protein*		**compound name for *5ESG -fungal protein***	
1	1-(2,5-bis((trimethylsilyl) oxy)phenyl)ethan-1-one	-5.848	p-cymene	-5.522
2	trimethylsilyl 2,5-bis ((trimethylsilyl)oxy)benzoate	-5.926	1,2,3,4,5-pentamethyl cyclopenta-1,3-diene	-5.774
3	2-butoxy-2,4,6,8-tetraethyl-1,3,5,7,2,4,6,8-tetraoxatetrasilocane	-4.261	3-vinylpyridine	-5.089
4	octyl (2,4,4-trimethyl pentyl) phthalate	-6.944	trimethylsilyl 2,4-bis((trimethylsilyl)oxy) benzoate	-5.306
5	Octasiloxane, 1,1,3,3,5,5,7,7, 9,9,11,11,13,13,1,5,15 hexadecamethyl	-6.625	2,2,4,4,6,6,8,8,10,10, 12,12,14, 14,16,16,18,18-octadecamethyl -1,3,5,7,9,11,13,15,17-nonaoxa-2,4,6,8,10,12,14,16,18-nonasila Cyclooctadecane	-6.500
6	(R,Z)-((1,3-diphenyl hept-1-en-1-yl)oxy)trimethylsilane	-4.843	4-(2-(benzo[d]oxazol-2-yl)vinyl)benzoic acid	-8.806
7	8-(pyrrolidin-1-yl sulfonyl)dibenzo[b,d]furan-3-amine	-5.374	6-(4-chlorophenyl)-4-phenylpyrimidin-2(1H)-one	-7.855
8	(E)-7-(benzylideneamino) -4-methyl-2H-chromen-2-one	-9.386	(S)-1-(2-(hydroxymethyl) pyrrolidin-1-yl)-3-methyl butan-1-one	-6.717
9	3-methyl-1,2-dihydrocyclopenta[ij]tetraphen-1-ol	-7.477	(cyclopentyloxy)dimethyl (perfluorophenyl)silane	-6.007
10	N,N'-([1,1'-binaphthalene]-2,2'-diyl)diacetamide	-6.007	8-chloro-5H-indeno[1,2-b] quinoline-10,11-dione	-5.608
11	1-(2,4-bis((trimethylsilyl)oxy)phenyl)ethan-1-one	-5.556	4H-chromen-4-one	-4.851
12	7-chloro-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepin-3-yl propionate	-4.050	2-(6-(2-methylbut-3-en-2-yl)-7-oxo-2,3-dihydro-7H-furo [3,2-g]chromen-2-yl)propan-2-yl acetate	-9.307



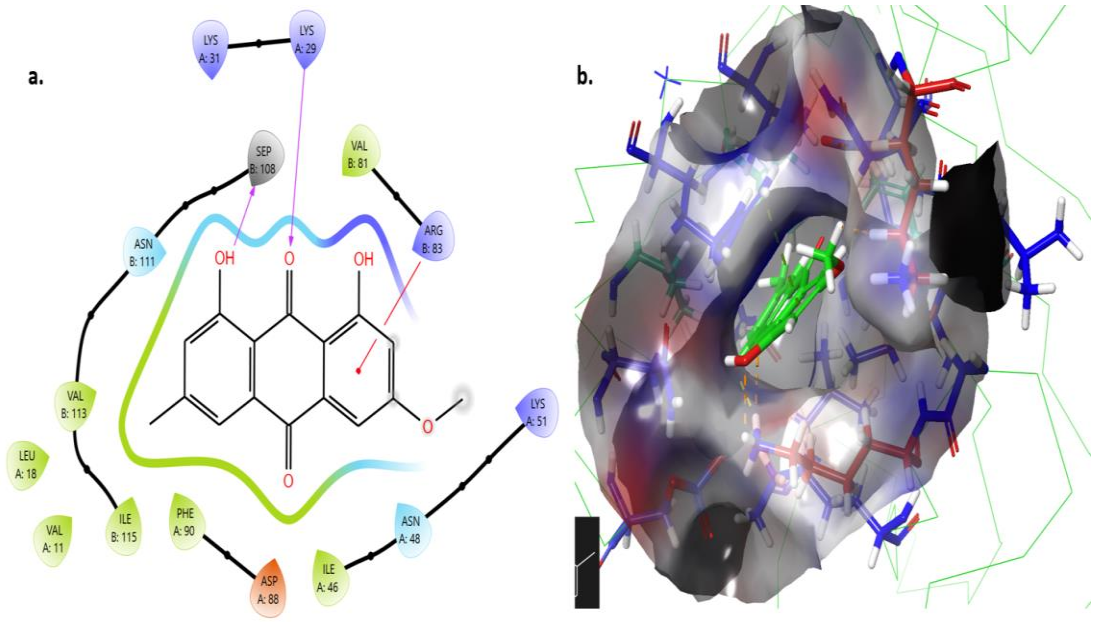
Secondary structure of fungal target protein 5ESG. Docked compounds inside the fungal protein 5ESG binding pocket



Interaction of highest dock scored molecule (E)-7-(benzylideneamino)-4-methyl-2H-chromen-2-one with 5ESG protein.



Docked compounds inside the protein 7myj binding pocket Secondary structure of diabetic target protein 7MYJ.



Interaction of highest dock scored molecule 1,8-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione with 7MYJ protein.

5. CONCLUSION

This study highlights the existence of phytochemical compounds in the medicinal plants Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria, and Trachyspermum Ammi. These plants are abundant in various substances, including heterocyclic compounds, resins, essential oils, phosphonic esters, alkaloids, organosiloxanes, heptasiloxanes, flavonoids, organosilicon compounds, organophosphorus compounds, chiral coordination compounds,

flavonones, steroids, terpenoids, and flavonoids. These compounds are utilized in the treatment of diabetes, as well as bacterial and fungal infections, particularly in the early to moderate stages. The results indicate that herbs like Withania Coagulans, Gymnema Sylvestre, Indigofera Tinctoria, and Trachyspermum Ammi are effective for managing oral infections caused by both bacteria and fungi. These natural herbs can be ingested in multiple forms without posing any toxicity or adverse reactions. Diabetes mellitus is a major health concern that impacts both economic factors and society at large. Ongoing research is focused on traditional remedies and extracts from Indian Rennet (Paneer Dodi) for potential use in combination therapies. Additional studies are required to explore their biological functions, drug metabolism, safety, toxicity, and the feasibility of clinical trials.

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CONFLICTS OF INTEREST

We declare that we have no conflict of interest.

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