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Pharmacoinformatic based screening of phytochemicals from *Ashwagandha (Withania somnifera)* against serine/arginine splicing factor 1 protein in treatment of pancreatic cancer

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Pancreatic cancer sometimes referred to as pancreatic ductal adenocarcinoma, a disease in which the tissues of the pancreas develop malignant (cancerous) cells. The main objective of this work was to use *in silico* molecular modeling tools to predict highly efficacious therapeutic molecules from *Withania somnifera*, a medicinal plant also known as winter cherry or *Ashwagandha,* to bind promising targets against pancreatic cancer. Thirty four phytocompounds produced from *Withania somnifera* were identified using the IMPPAT database, and their structures were found in the PubChem database. A putative target protein called Serine/arginine-rich splicing factor 1 (SRSF1) was matched to every phytocompound. Somniferine (–10.4 kcal × mol^{-1}), Physagulin-D (–10.2 kcal × mol^{-1}), and 27-Deoxywithaferin -A (–10.1 kcal × mol^{-1}), the phytocompounds from *Withania somnifera* with the highest scores, were selected for further examination and compared with the reference drug CID60750 (–6.7 kcal × mol^{-1}). To verify their druggability, a few top-scoring phytocompounds drug-likeness, pharmacokinetic, and toxicological properties were evaluated. These expected results suggest that in the treatment of pancreatic cancer, phytocompounds inhibit the SRSF1 protein. Additional *in vitro* and *in vivo* investigations are required to validate the anticipated characteristics of these substances.

Keywords: *Ashwagandha,* Molecular docking, Pancreatic cancer, SRSF1 receptor, *Withania somnifera*

Pancreatic cancer is among the most-deadly diseases in the world. Pancreatic cancer ranks on global incidence at 14th and on fatality rate at 7th. It is ranked 18th in mortality and 24th in new cases in India[1]. The worst aspect is that, in comparison to all other cancers, it metastasizes more quickly[2]. The pancreas, which is located behind the lower portion of the stomach[3], is the source of pancreatic cancer. It produces hormones that aid in blood sugar regulation and enzymes that aid in food digestion[4]. Pancreatic Ductal Adenocarcinoma (PADC) is the most frequent kind of pancreatic cancer in humans[5].Although the exact cause of pancreatic cancer is unknown, there are a number of variables that can raise one's risk, including age, smoking, obesity, chronic pancreatitis, and a family history of the disease[6]. The primary challenge in identifying pancreatic cancer is that symptoms do not appear until the cancer has progressed or spread to other areas of the body[7]. However, jaundice, weight loss, exhaustion, gastrointestinal (GI) issues[8], and abdominal discomfort are some of the primary symptoms[9]. Positron emission tomography, ultrasound, CT, MRI, and biopsies are some of the initial diagnostic techniques[10]. The doctors would determine whether or not the patient is affected by these tests. 10% of the population, or 10 out of every 100, survive cancer for a year or more following diagnosis[11]. Just 1 person in 100(1%) has cancer and lives for three years or longer following diagnosis[12].Pancreatic cancer can be cured by a variety of treatment options, including as immunotherapy, chemotherapy, radiation therapy, and surgery[13]. Surgery is the most appropriate and effective treatment option out of all of these because it has a higher potential to cure pancreatic cancer[14], whereas chemotherapy and other immunotherapies are rarely advised. The greatest likelihood of both treating pancreatic cancer and guaranteeing that all tumour cells are eliminated is by surgical means of removing the entire tumour[15]. Surgery is only performed when the malignancy can be completely eliminated, as partial tumour cell removal does not improve the patient's prognosis[16].Despite the fact that surgery is a successful treatment, doctors will not always recommend it because chemotherapy can also

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cure some people[17]. Chemotherapy is therefore the first line of treatment for pancreatic cancer[18]. Surgery is scheduled if the condition is too serious and in accordance with the advice and concerns of the doctor[19]. Chemotherapy for pancreatic cancer is usually administered systemically; it can be injected directly into veins or taken orally[20]. The primary goal of chemotherapy is to deliver the medications to the tumour cells at their precise site of invasion by getting them into the bloodstream[21]. The American Cancer Society states that irinotecan (Camptosar), 5-fluorouracil (5-FU), and gemcitabine (Gemzar)are the medications used to treat pancreatic cancer[22]. The remaining drugs that are prescribed include leucovorin, capecitabine, and oxaliplatin[23]. Chemotherapy drugs are usually given in cycles, with treatment and recovery periods occurring in between[24]. A cycle typically lasts two or three weeks, though it might last longer depending on the drugs taken[25]. Some drugs are given once a week or for a few days in a row, while others are only given on the first day of the cycle[26].

Ayurvedic medicine, which incorporates herbal remedies, massage treatment, yoga, acupuncture, and breathing techniques, is an alternative to chemotherapy[27]. Of them, using chemotherapy in conjunction with Ayurveda is the most effective strategy to treat pancreatic cancer[28]. In addition to treating cancer, they also aid in the treatment of obesity, stress, anxiety, and asthma[29]. A few of the herbal remedies that are quite effective in treating pancreatic cancer are pippali, *Ashwagandha,* amalaka[30], turmeric, and kalmegh[31].Studies reveal that the active ingredients in *Ashwagandha,* such as withanolides and withaferin A, may stop tumour cells from proliferating and cause apoptosis, or programmed cell death, which in turn may stop the growth ofcancer cells[32]. To bolster its anticancer effect, *Ashwagandha* has also been demonstrated to improve the immune system's capacity to identify and eliminate malignant cells[33]. Moreover, *Ashwagandha* has antioxidant and anti-inflammatory properties that help lessen chronic inflammation and oxidative stress, two factors that are connected to the development of pancreatic cancer[34]. On addition, it has been noted that *Ashwagandha* increases the potency of chemotherapy medications against pancreatic cancer cells while lowering their toxicity to healthy cells[35]. To sum up, *Ashwagandha* 's complex mechanisms make it a useful natural medicine in the search for potent anticancer medications[36]. However, more research and clinical trials are necessary to completely understand *Ashwagandha*'s therapeutic potential in the management of pancreatic cancer[37].

Materials and Methods

Phytochemical retrieval and preparation

In the context of pancreatic cancer, ligand identification and retrieval from *Withania somnifera (Ashwagandha)* play a pivotal role as a therapeutic agent[38]. *Withania somnifera* is renowned for its rich repertoire of bioactive compounds that exhibit diverse pharmacological activities, including potential anti-cancer properties. This rigorous process of ligand identification and retrieval ensures that the selected ligands from *Withania somnifera* exhibit high potential for inhibiting pancreatic cancer progression, paving the way for subsequent experimental validation. The phytochemicals (Ligands) from the plant *Withania somnifera* were identified form the IMPPAT database (Indian Medicinal Plants, Phytochemicals and Therapeutics)[39] (https://cb.imsc.res.in/imppat/)[40]. Initially the identified phytochemicals were tabulated, then the phytochemicals are retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/)[41] in the 3D SDF format and were clustered using BIOVIA discovery studio software[42] and were saved as Mol/SDF files.

Protein preparation

Target identification and retrieval for pancreatic cancer involves a systematic approach to pinpoint specific molecular targets or pathways that play crucial roles in the progression and development of the disease[43]. The first step in this process is to gather comprehensive information from scientific literature, databases, and experimental studies to identify potential targets associated with pancreatic cancer, such as specific proteins, enzymes, or signalling pathways. The retrieved targets are often represented as three-dimensional structures, which are essential for subsequent molecular docking and virtual screening processes. These structures serve as the templates for predicting the binding interactions between potential drug candidates and the identified targets. This target-centric approach ensures that drug discovery efforts are focused on molecules that have a high likelihood of effectively modulating the disease's progression, ultimately leading to the development of novel and more targeted therapeutic agents for pancreatic cancer. From the literature review, it is

found that the protein SRSF1 (serine/arginine splicing factor 1)[44] as a potential target for pancreatic cancer. The identified target's 3D structure is retrieved from the Publicly available database PDB (Protein Data Bank) (https://www.rcsb.org/)[45]. After the protein structure was obtained from the TTD database, the water molecules, protein cofactors, metal ions, and non-amino acid residues were separated. These alteration procedures are all carried out with the Swiss PDB Viewer[46]. This facilitates the effective removal of the non-amino acid residue. The non-polar hydrogen atoms merged with the polar hydrogen atoms after they were joined.

Active site prediction

In the context of pancreatic cancer, identifying the active sites on key proteins involved in cancer progression, metastasis, or resistance to therapy is essential. These active sites serve as the docking points for potential drug molecules, allowing them to exert their therapeutic effects by modulating the protein's activity or inhibiting its function. Active site prediction is a critical aspect of drug discovery, especially for targeting specific molecular pathways involved in pancreatic cancer. Using computational tools like Prankweb (https://prankweb.cz/)[47] can streamline this process by predicting the potential active sites on target proteins relevant to pancreatic cancer. Target locations are displayed as pockets, and the rank and probability score of each pocket can be used to identify which pocket is best. Using the PyRx program, the pocket coordinates are noted to create the docking grid.

Molecular docking

In the context of lead molecule research, molecular docking has been increasingly useful in recent times. It is a computational technique used in the field of structural biology and drug design. It predicts the preferred orientation of one molecule (ligand) when bound to another molecule (receptor/ target) to form a stable complex. This method plays a pivotal role in understanding molecular interactions, especially in drug discovery and development processes. The process involves searching for the best-fitting position and orientation of a ligand within a receptor's binding site. Various scoring functions evaluate the binding affinity and stability of the docked complex, guiding researchers in selecting potential drug candidates with high efficacy and low side effects. Molecular docking can simulate thousands to millions of potential interactions rapidly, making it a powerful tool in virtual screening of large chemical libraries. The molecular docking is usually carried out by the open-source software tool PyRx (https://pyrx.sourceforge.io/)[48], virtual screening software that helps users in the computational drug discovery process. The PyRx 0.8 AutoDockVina wizard module was used for docking[48]. Its user-friendly layout makes docking wizard and vina wizard easy to operate. This is a useful tool for the CADD method. Following the identification of the target protein's active binding site, a receptor grid box was created using the PyRx built-in tool. It can be used to screen compound libraries against potential drug targets, and helps users with every step of the process, from data preparation to analysis of the results. Using the Protein-Ligand Interaction Profiler (PLIP)[49], the Target-Ligand Interaction is examined. The distance between the interaction and the bonds between them, as well as the interactions with ligands and targets at the amino acid level, may all be analyzed using this open-source platform.

Pharmacokinetics and physiochemical properties prediction

ADME properties refer to Absorption, Distribution, Metabolism, and Excretion, which are fundamental pharmacokinetic parameters that determine the fate of a drug molecule in the body is referred to as the Pharmacokinetics (PK) in CADD[50]. Understanding these properties is crucial in drug development to ensure efficacy, safety, and optimal dosing of potential therapeutic agents. The Absorption describes how a drug is taken up from its administration site into the bloodstream. Factors influencing absorption include the drug's solubility, size, and formulation. A drug must be sufficiently absorbed to reach therapeutic concentrations in the bloodstream. Distribution is defined as a drug that is distributed to various tissues and organs. Distribution depends on factors like the drug's binding to plasma proteins, tissue permeability, and blood flow to different organs. Metabolism involves the biochemical transformation of the drug into metabolites, primarily by enzymes in the liver. This process can either activate or deactivate the drug and often leads to the formation of water-soluble metabolites that are easier to excrete. Excretion refers to the removal of the drug and its metabolites from the body, mainly through the kidneys in urine or via the liver in bile[51]. SwissADME (http://www.swissadme.ch/)[50] is a valuable tool in CADD that offers insights into Absorption, Distribution, Metabolism, and Excretion (ADME) properties of small

molecules. This online platform simplifies the prediction of these crucial pharmacokinetic parameters, aiding in early-stage drug discovery.

Toxicity analysis

Toxicity prediction plays a pivotal role in various industries, from pharmaceuticals to environmental science. In drug discovery, toxicity prediction aids in identifying candidate compounds with minimal side effects, thereby streamlining the drug development process and reducing costs. In Computer-Aided Drug Design (CADD), one of the widely used methods for toxicity prediction is pkCSM (pharmacokinetics and toxicity prediction) (https://biosig.lab.uq.edu.au/pkcsm/)[52]. pkCSM employs machine learning algorithms trained on large datasets of known compounds to predict various pharmacokinetic and toxicity properties of new molecules. The pkCSM model utilizes molecular descriptors and fingerprints to estimate toxicity parameters such as mutagenicity, hepatotoxicity, and cardiotoxicity. These predictions are based on the chemical structure of the compounds and their similarity to known toxic or non-toxic molecules in the training set. Therefore, the toxicity profile for the phytochemical Somniferine is obtained from pkCSM.

Results

Phytochemical retrieval and preparation

Using the IMPPAT database, thirty-four phyto-chemicals from the *Ashwagandha* (*Withania somnifera*) plant were extracted, and each phytochemical's 3D structure was recorded in SDF file format. The ligand preparation for additional chemical analysis was completed by converting all of the files to the“. pdbqt" format. This file type may be used as AutoDockVina's simple input format. In a similar manner, the control compound CID60750 is selected and prepared for comparison from the PubChem database.

AS prediction and receptor grid formation

The term "Active site" (AS) refers to the location on the receptor protein where the ligand binds. Strong affinity for the ligand is promoted by specific amino acid residues from the protein molecule's binding site, which aid in the ligand's chemical bonding. The target SRSF1 protein (serine/arginine-rich splicing factor 1) was shown to have an AS pocket. The optimum pockets containing the AS are anticipated based on the ideal pocket valve. Three AS pockets in total were predicted for SRSF1, with a best pocket score of 17.9 chosen. Their residues (A_107 A_109 A_111 A_117 A_120 A_124 A_145 A_163 A_165 A_166 A_168 A_169 A_171 A_217 A_218 A_220 A_496 A_497 A_499 A_86 A_87 A_88 A_89 A_90 A_91 A_92 A_94) were isolated for the selective docking, with the X, Y, and Z coordinates being 14.3354, 13.532, and 78.1959, respectively. Our ability to arrange the docking grid for subsequent steps is aided by these coordinates (Fig. 1 and Table 1).

Molecular docking analysis

One of the most important steps in any initial drug discovery process is the molecular docking technique.

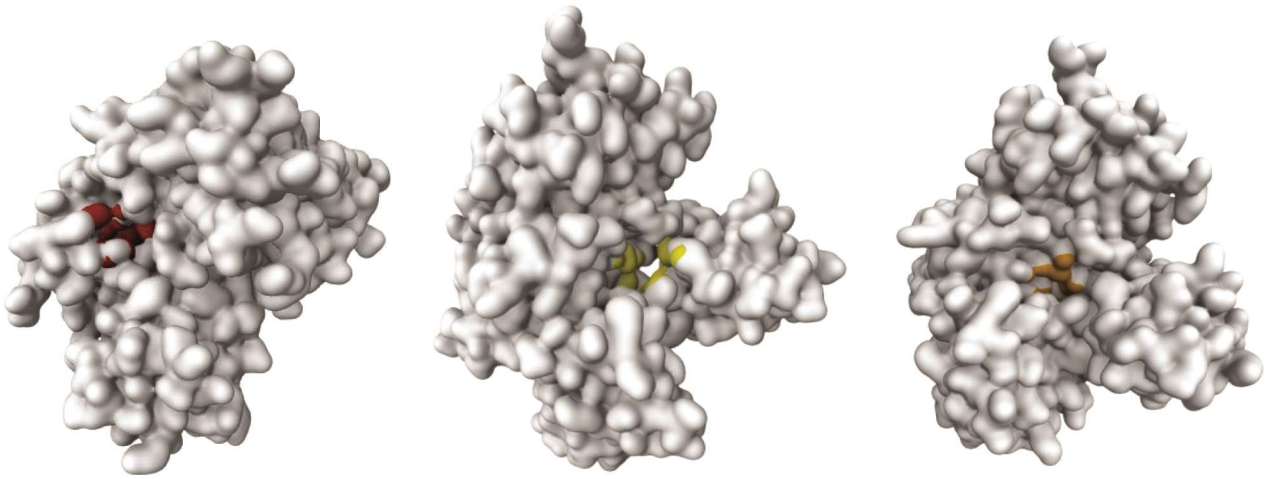

Fig. 1 — (Color Online) ThePrankweb tool is used to predict the binding pockets and correspondence binding sites of the SRSF1protein. Three binding pockets were predicted with different colors (Red, yellow and orange). The first binding pocket (Red color) is the highest score of all six pockets, with a pocket score of 17.90, 27 amino acids, and a probability score of 0.809 (a). The second binding pocket (Yellow color) with a pocket score of 6.29, 13 amino acids, and a probability score was 0.340 (b). The third binding pocket (Orange color) had the least pocket score of 1.73, 8 amino acids, and the probability score was 0.030 (c)

Table 1 — Prankweb tool used to prediction of binding pockets and correspondence binding sites of SRSF1 receptor. A total of three binding pockets were predicted with different color. The first pocket is coloured in red with pocket score of 17.9, 27 amino acids, and a probability score of 0.809.

Name	Rank	Score	Probability	X axis	Y axis	Z axis	Amino acid residues
Pocket1	1	17.9	0.809	14.3354	13.532	78.1959	A_107 A_109 A_111 A_117 A_120 A_124 A_145 A_163 A_165 A_166 A_168 A_169 A_171 A_217 A_218 A_220 A_496 A_497 A_499 A_86 A_87 A_88 A_89 A_90 A_91 A_92 A_94
Pocket2	2	6.29	0.34	7.0872	14.5883	68.0011	A_171 A_173 A_174 A_215 A_217 A_89 A_90 B_133 B_134 B_135 B_149 B_151 B_152
Pocket3	3	1.73	0.03	12.9041	13.1298	68.3181	A_213 A_215 A_217 A_218 A_497 A_500 A_514 A_91

The atomic interaction between the target protein and the tiny ligand molecule was modelled by this molecular docking procedure. It also describes the basic metabolic mechanisms that underlie all diseases and their circumstances. Molecular docking of the 34 phytochemicals of *Withania somnifera* with the target SRSF1 was carried out using the PyRx tool. The docking results indicated that the region of –10.4 kcal × mol^{-1} to –8.4 kcal × mol^{-1} is where the binding affinity for SRSF1 is identified. The phytochemicals exhibiting the highest binding affinity were chosen, and their binding affinities were contrasted with those of the medication under control. The binding affinities of the control molecule and the highest-ranking compound were determined. The phytochemicals exhibiting the highest binding affinity were chosen, and their binding affinities were contrasted with those of the control molecule. The binding affinities of the control molecule and the highest-ranking compound were determined. Somniferine (–10.4 kcal × mol^{-1}), physagulin-D (–10.2 kcal × mol^{-1}) and 27-Deoxywithaferin - A (–10.1 kcal × mol^{-1}), were shown to be the top compounds from *Withania somnifera* when compared with the control molecule CID60750 (–6.7 kcal × mol^{-1}) against SRSF1 (Table 2).

Interpretation of protein – ligand interaction

The chosen ligands and the intended protein targets were interpreted using the BIOVIA Discovery Studio Visualizer tool. Numerous carbon-hydrogen bonds were established between the target protein "SRSF1" and the drugs "Somniferine", "physagulin-D" and "27-Deoxywithaferin - A" which are the top phytoconstituents that generate scores with the SRSF1 protein, as shown in both 2D and 3D pictures. Based on the PLIP Profiler in (Table 3), the SRSF1 - Somniferine complex was found to have two hydrophobic interactions in (TYR153B (3.67Å), GLU184B (3.90Å) and five hydrogen bond (TYR153B (3.16Å), ARG561A (2.94Å),TRP88A (3.34Å), HIS171A (2.06Å), GLU217A (2.21Å)).The SRSF1-physagulin-Dcomplex was found to have sixhydrophobic interactions (TYR153B (3.67Å), GLN135B (3.72Å), VAL145A (4.00Å), PHE165A (3.77Å), LEU220A (3.59Å), ASP497A (3.45Å)) and twelve hydrogen bonds (TYR153B (3.16Å), ARG561A (2.94Å), TRP88A (2.79Å), TRP88A (3.02Å), SER133B (2.09Å), GLN135B (2.74Å), HIS171A (2.26Å), GLU184B (3.10Å), GLU217A (2.10Å), ASN218A (3.38Å), ASP497A (3.01Å), ASP497A (2.16Å)).The SRSF1-27-Deoxywithaferin - A complex was found to have three hydrophobic interactions (TYR153B (3.67Å), TYR181A (3.89Å), GLU552A (3.96Å)) and nine hydrogen bonds (TYR153B (3.16Å), ARG561A (2.94Å), GLN182A (2.71Å), SER207B (2.80Å), SER207B (2.34Å), SER207B (2.25Å), ARG210B (3.09Å), GLU552A (3.03Å), GLU552A (3.20Å)). This relationship facilitates comprehension of the stability of the protein–ligand interaction (Fig. 2).

Pharmacokinetics properties

The Swiss ADME and pkCSM servers were used to examine the compound's drug-likeness, lipophilicity, and toxicity properties; all of the data are represented in (Table 4). The Lipinski's rule of five was utilized to examine both of the top-scoring ligands, Somniferine (molecular weight: 608.68 g × mol^{-1}), physagulin-D (molecular weight: 620.77 g × mol^{-1}) and 27-Deoxywithaferin A (molecular weight: 470.60 g × mol^{-1}). The ligand Somniferine and physagulin-Dare found to have a molecular weight greater than 500, meaning that although it can be utilized as a medication, it violates one of the five Lipinski rules. However, the Lipinski rule of five is not broken by the ligand 27-Deoxywithaferin A. The increased molecular weight of Somniferine and physagulin-D and increased polar surface area of physagulin (166.14 $Å^2$) were the only factor that led to the observed violation. It is discovered that the leads Somniferine and 27-Deoxywithaferin-A have improved gastrointestinal absorption. The reduced polar surface

Table 2 — Binding affinity of compounds from *Withania somnifera* and Gemcitabine (control) against the target protein SRSF1

S No	Compound ID (CID)	Ligand Name	Binding affinity (kcal × mol–1)
1	CID: 14106343	Somniferine	-10.4
2	CID: 10100412	Physagulin-d	-10.2
3	CID: 16680447	27-Deoxywithaferin A	-10.1
4	CID: 189586	Sitoindoside IX	-10
5	CID: 162623730	Coagulin Q	-10
6	CID: 161671	Withanolide D	-9.9
7	CID: 23266161	17alpha-hydroxywithanolide D	-9.9
8	CID: 179575	Withanolide L	-9.8
9	CID: 57403080	Viscosalactone B	-9.8
10	CID: 21679027	Withanone	-9.7
11	CID: 11049407	Withanolide S	-9.7
12	CID: 101559583	Withanolide C	-9.7
13	CID: 70684083	2,3-Didehydrosomnifericin	-9.6
14	CID: 442985	Solasodine	-9.5
15	CID: 23266167	24,25-dihydrowithanolide D	-9.5
16	CID: 44562998	Withanolide K	-9.4
17	CID: 193567	24-Methyldesmosterol	-9.4
18	CID: 301751	Withanolide E	-9.2
19	CID: 265237	Withaferin A	-9
20	CID: 222284	beta-Sitosterol	-8.5
21	CID: 23694214	Hydrocortisone sodium succinate	-8.2
22	CID: 14807783	Stigmasterone	-8.2
23	CID: 442877	Withasomnine	-7.4
24	CID: 5280450	Linoleic acid	-5.9
25	CID: 89594	Nicotine	-5.8
26	CID: 445639	Oleic acid	-5.8
27	CID: 985	Palmitic acid	-5.6
28	CID: 1201543	Cuscohygrine	-5.5
29	CID: 5793	D-Glucose	-5.5
30	CID: 11005	Myristic acid	-5.3
31	CID: 92987	Pelletierine	-5
32	CID: 8424	Tropine	-4.8
33	CID: 11850	Galactitol	-4.7
34	CID: 440933	Hygrine	-4.6
Reference Standard drug			
35	CID: 60750	Gemcitabine (Standard)	-6.7

Table 3 — Details of bonding interactions between selected bioactive compounds and control drug Gemcitabine with SRSF1

Ligand	Residues	Amino acids	Distance (Å)	Bond category
Somniferine	153B	TYR	3.67	Hydrophobic interactions
	184B	GLU	3.90	
	153B	TYR	3.16	Hydrogen bonds
	561A	ARG	2.94	
	88A	TRP	3.34	
	171A	HIS	2.06	
	217A	GLU	2.21	
Physagulin-D	153B	TYR	3.67	Hydrophobic interactions
	135B	GLN	3.72	
	145A	VAL	4.00	
	165A	PHE	3.77	
	220A	LEU	3.59	
	497A	ASP	3.45	
	153B	TYR	3.16	Hydrogen bonds
	561A	ARG	2.94	
	88A	TRP	2.79	

(*Contd*.)

Table 3 — Details of bonding interactions between selected bioactive compounds and control drug Gemcitabine with SRSF1 (*Contd*.)

Ligand	Residues	Amino acids	Distance (Å)	Bond category
	88A	TRP	3.02	
	133B	SER	2.09	
	135B	GLN	2.74	
	171A	HIS	2.26	
	184B	GLU	3.10	
	217A	GLU	2.10	
	218A	ASN	3.38	
	497A	ASP	3.01	
	467A	ASP	2.16	
27 -Deoxywithaferin - A	153B	TYR	3.67	Hydrophobic interactions
	181A	TYR	3.89	
	552A	GLU	3.96	
	153B	TYR	3.16	Hydrogen bonds
	561A	ARG	2.94	Hydrogen bonds
	182A	GLN	2.71	
	207B	SER	2.80	
	207B	SER	2.34	
	207B	SER	2.25	
	210B	ARG	3.09	
	552A	GLU	3.03	
	552A	GLU	3.20	
CID 60750 (Gemcitabine)	2A	ALA	2.52	Hydrophobic interactions
	86A	LEU	3.68	
	91A	PHE	3.66	
	220A	LEU	3.53	
	220A	LEU	3.57	
	497A	ASP	3.85	
	561A	ARG	2.91	Hydrogen bonds
	134B	TRP	2.94	
	135B	GLN	2.46	
	218A	ASN	3.22	
	227A	TYR	2.87	
	497A	ASP	2.72	
	497A	ASP	2.55	
	513A	GLN	2.15	

areas of 27-Deoxywithaferin A (96.36 Å²) and Somniferine (100.93 Å²) are the cause of this outcome. Among three of the chosen leads only Somniferine and 27-Deoxywithaferin-Ahad positive bioavailability +0.55, indicating that the lead compounds have strong drug-like qualities, whereas physagulin-D had a bioavailability of +0.17, which indicating that this lead compound have reduced drug-like qualities. Thus, by comparing all the drug-likeness properties among the three selected lead compounds, only Somniferine and 27-Deoxywithaferin-A have desired properties, whereas physagulin-Dhas more than 2 violations in the Lipinski's rule of five. The two desired bioactive compounds' synthetic accessibility was found to be >3, indicating that it can be produced under laboratory conditions. The boiled-egg model, which is crucial to the development of any pharmacological molecule, is shown in Figure 3 and predicts the lead compounds' gastrointestinal absorption and blood-brain barrier perforation. The two desired bioactive molecules (Somniferine and 27-Deoxywithaferin-A) exhibit superior activity since the boiled-egg region contains all of these components. Furthermore, all of the bioactive chemicals are inside the boiled-egg zone, indicating these compounds have the desired bioactivity against pancreatic cancer. The radar plot in Figure 4 illustrates the drug-likeness characteristics of the chosen compounds. The pink hexagonal zone contains the best range of bioactive compounds; under this region, the saturation (SATU) region has a minimum of 0.25, a maximum of 6 log S, and a maximum of 9 rotatable bonds, all of which are indicative of increased drug-likeness. The resulting compounds all exhibited improved ADME characteristics and are all pharmacologically soluble. To verify that the lead compound was appropriate, a QSAR analysis was performed using the

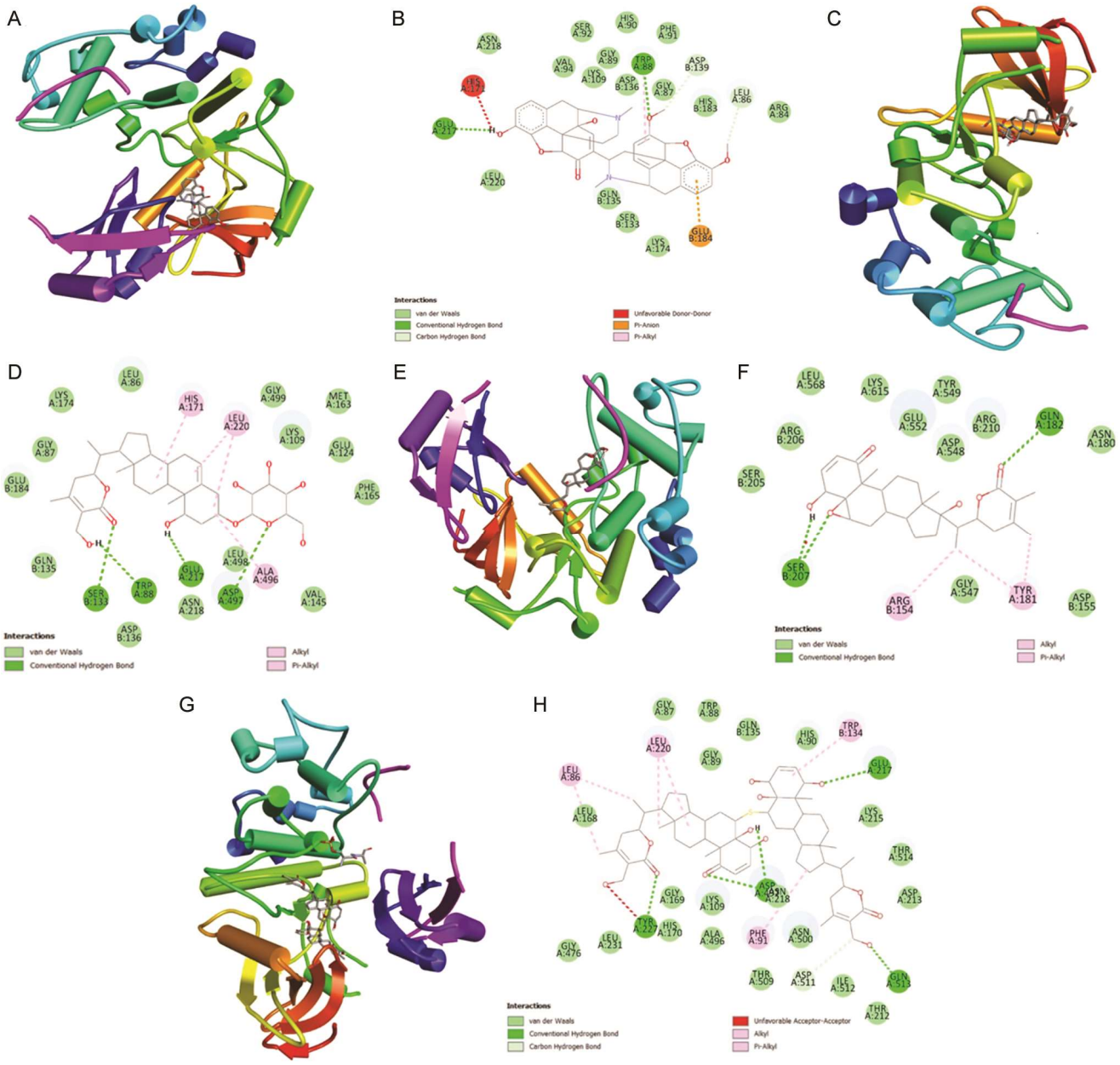

Fig. 2 — (Color Online) Representation of intermolecular interaction between the bioactive compound Somniferine with SRSF1 protein complex. The left side representing 3D (A); and the right side represents the 2D complex protein–ligand interaction (B); the interaction between the compound physagulin-D with SRSF1 protein complex. Left side representing 3D (C); and the right side represents the 2D complex protein–ligand interaction (D); the interaction between the control drug 27-Deoxywithaferin-A with SRSF1 protein.Left side representing 3D (E); and the right side represents the 2D complex protein–ligand interaction (F); the interaction between the control drug CID60750 (Gemcitabine) with SRSF1 protein. The left side represents 3D (G); and the right side represents the 2D complex protein–ligand interaction (H)

PASS Server (http://www.way2drug.com/passonline/). Supplementary Table S1 shows the major bioactivities of specific ligands, Somniferine, 27-Deoxywithaferin A and physagulin-D, linked to pancreatic cancer. Their respective Pa values ranged from 0.178–0.924, 0.141–0.919and 0.064-0.975.

Toxicity properties

Table 5 denotes the carcinogens, hERG inhibition induced by drugs, P-glycoprotein inhibitor (PGI) activity of selected ligands, Somniferine, Physagulin-D and 27-Deoxywithaferin-A, rat acute toxicity (LD50 in mol × kg^{-1}), and toxicity of

Table 4 — Pharmacokinetics and physicochemical parameters of selected top binding scored bioactive molecules and control drug Gemcitabine

Properties	Somniferine (CID:14106343)	Physagulin-D (CID:10100412)	27-Deoxywithaferin –A (CID:16680447)	Gemcitabine (CID:60750)
Formula	$C_{36}H_{36}N_2O_7$	$C_34H_{52}O_{10}$	$C_{28}H_{38}O_6$	$C_9H_{11}F_2N_3O_4$
Num. heavy atoms	45	44	34	18
MW (g/mol)	608.68	620.77	470.60	263.20
Num. aromatic Heavy atoms	12	0	0	6
Fraction Csp3	0.47	0.85	0.79	0.56
Num. rotatable bonds	3	6	2	2
Num. H-bond acceptors	9	10	6	7
Num. H-bond donors	2	6	2	3
Molar refractivity	171.39	161.83	127.53	54.83
0TPSA (Å²)	100.93	166.14	96.36	110.60
Solubility class	Moderately soluble	Moderately soluble	Moderately soluble	Very soluble
GI absorption	High	Low	High	High
BBB permeation	No	No	No	No
Violation of Lipinski's rule of five	1	2	0	0
Violation of Veber rule	0	1	0	0
Bioavailability Score	0.55	0.17	0.55	0.55
Synth. accessibility	7.32	7.78	6.84	3.71

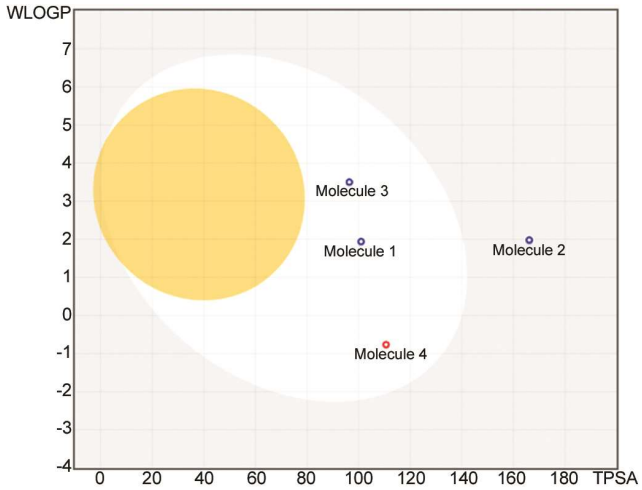

Fig. 3 — (Color Online) The EGG-BOILED model for the bioactive molecule Somniferine (molecule 1), physagulin-D (molecule 2), 27 – Deoxywithaferin – A (molecule 3), and control drug CID60750 (Gemcitabine – molecule 4). The EGG-BOILED represents for intuitive evaluation of passive gastrointestinal absorption (HIA) white part and blood brain penetration (BBB) yellow part as well as substrates (PGP+) and non-substrates (PGP–) of the permeability glycoprotein (PGP) are represented by blue and red color circles, respectively, of the selected top binding scored bioactive molecule Somniferine, physagulin-D, 27-Deoxywithaferin – A and control drug CID60750 (Gemcitabine) in the WLOGP-versus-TPSA graph. The grey region is the physicochemical space of compounds predicted to exhibit high intestinal absorption

Tetrahymenapyriformis (TP) and honeybees (HB). This makes these toxicity tests an efficient method for the drug development process because they shorten the duration of the physical drug test and increase accuracy. As part of an *in silico*-based toxicity assessment procedure, the pkCSM webserver was utilized to examine the ligand molecule's toxicity profile.

Discussion

The primary goal of this study is to identify drug-like bioactive compounds from Indian medicinal herbs that may be effective against pancreatic cancer. Tumours, which are masses of healthy cells in the pancreas that grow out of control and stop functioning normally, are the first sign of pancreatic cancer. While the current pancreatic cancer medicine helps to lessen symptoms, long-term use of the prescription creates a variety of negative effects. Consequently, the application of herbal medicine as a means of lowering pancreatic cancer is the main emphasis of this study. And hence, *Withania somnifera*, an herbaceous plant, was chosen. While *Withania somnifera* is known to contain a variety of phytochemicals, including cuscohygrine, anahygrine, pseudotropine, choline, dl-isopelletierine, anaferine, and others, 34 phytochemicals have been identified within the *Withania somnifera* family. The prospective targets against pancreatic cancer were identified in this study through the application of the literature analysis method. The Serine/arginine-rich splicing factor 1 (SRSF1) was the best candidate chosen because of its activity and location. Pre-mRNA splicing, an essential stage in the expression of genes, is mediated by a protein called SRSF1 (serine/arginine-rich splicing factor 1). Numerous malignancies, including pancreatic cancer, have been

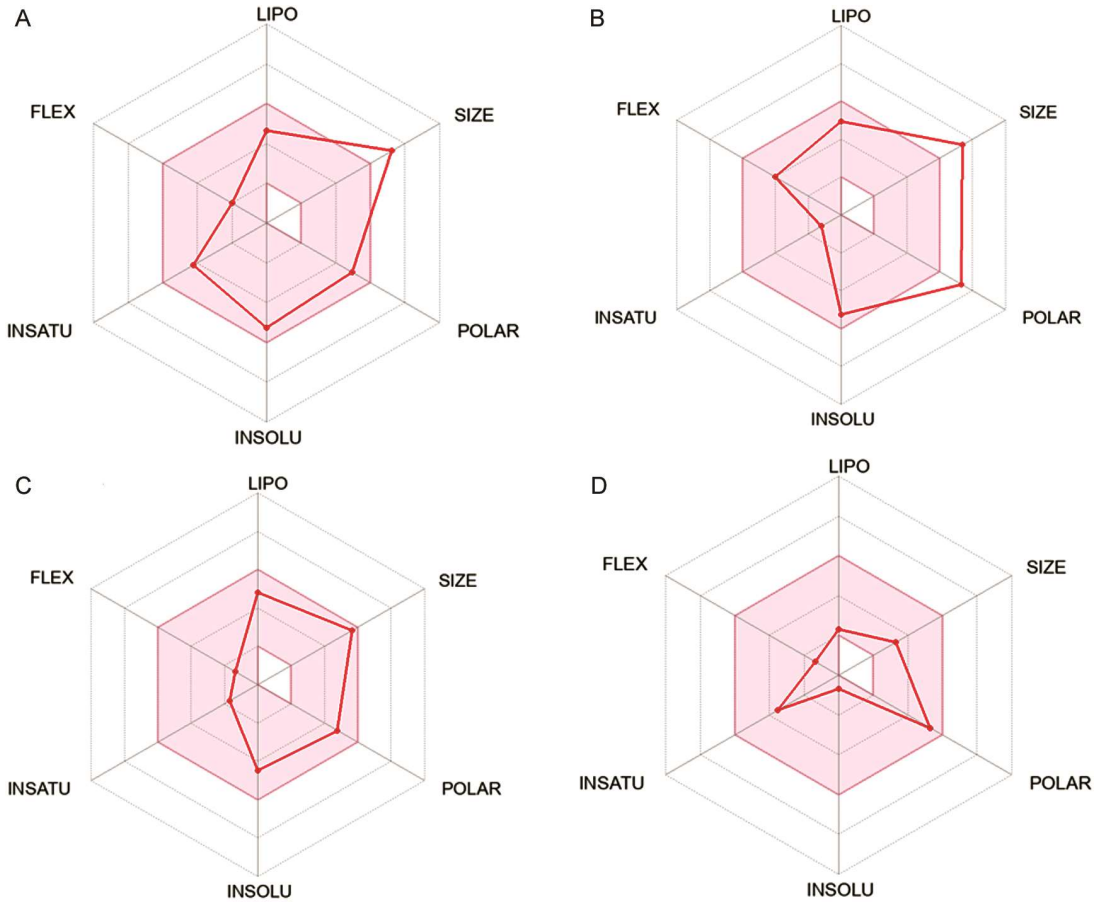

Fig. 4 — (Color Online) Bioavailability radar plot for oral bioavailability of top binding scored bioactive molecules. Somniferine (A), physagulin-D (B), 27- Deoxywithaferin - A (C) and control drug CID60750 (Gemcitabine) (D). The pink area exhibits the optimal range for each property (Lipophilicity as XLOGP3 between −0.7 and +5.0; Size as molecular weight between 150 g mol^{-1} and 500 g mol^{-1}; Polarity as TPSA (topological polar surface area) between 20 Å and 130 Å2; Insolubility in water by log S scale not higher than 6; Insaturation as per fraction of carbons in the sp3 hybridization not less than 0.25 and Flexibility as per rotatable bonds no more than 9)

Table 5 — Toxicity parameters of selected top binding scored bioactive molecules and control drug Gemcitabine

Compound Name	Somniferine (CID:14106343)	Physagulin-D (CID:10100412)	27-Deoxywithaferin A (CID:16680447)	Gemcitabine (CID:60750)
AMES Toxicity	No	No	No	Yes
Max. tolerated dose (human)	-0.496	-0.932	-0.813	0.701
hERG 1 inhibitor	No	No	No	No
hERG 11 inhibitor	Yes	Yes	No	No
Oral rat Acute Toxicity (LD50)	2.718	2.075	2.357	2.065
Oral Rat Chronic Toxicity (LOAEL)	2.451	2.29	1.622	0.96
T. Pyriformis toxicity	0.285	0.286	0.334	0.256
Hepatotoxicity	No	No	No	Yes
Skin sensation	No	No	No	No
Minnow toxicity	0.889	0.862	0.399	4.146

linked to the dysregulation of splicing factors like SRSF1. When compared to normal pancreatic tissues, pancreatic cancer tissues have been found to have higher amounts of SRSF1. Genes implicated in the development and spread of cancer may have abnormal splicing as a result of this overexpression. To ascertain whether the chosen compounds can be used for drug discovery, it is essential to comprehend the protein-ligand interaction. When the binding affinity value was compared to the control chemical, which has a binding affinity of -6.7 kcal mol^{-1}, it was found to be suitable within the range of –10.4 kcal mol^{-1} to –10.1 kcal mol^{-1}. Upon analysing the molecular docking of 34 phytochemicals with SRSF1, it was

observed that some phytochemicals, including Somniferine, Physagulin-D and 27-Deoxywithaferin-A, exhibited the binding affinity range previously indicated. The complex's stability can be enhanced by a larger hydrophobic interaction and higher drug binding when combined with a hydrogen bond, according to the analysis of hydrophobic and hydrogen bond interactions. In order to comprehend the drug's mechanism, pharmacokinetics and physicochemical qualities are crucial. It explains how the body responds to a certain substance that enters it. An ADMET analysis can be performed on it. Understanding the characteristics and potential adverse effects of a new medication requires this analysis. This investigation examined the following factors: molecular weight, BBB penetration, violation of Veber's Rule, and Lipinski Rule of 5. Drug distribution to the targeted region of the target is limited by the increased molecular weight. Parallel to this, the Lipinski Rule aids in the comprehension of how similar drugs are to human bodies. Reducing drug rejection and increasing drug penetration can be achieved by being aware of certain PK characteristics of a medicine. There was no evidence of cytotoxicity or carcinogenicity in the chosen bioactive chemicals. Lower levels of toxicity in the body are indicated by greater LD_{50} values. This is important to the process of finding and developing new drugs.

Conclusion

A multitude of variables connected to dysfunctions in the digestive, endocrine, biliary, and cardiovascular systems can lead to pancreatic cancer. Due to the fact that the precise origin of this condition varies to some degree, there is currently no suitable medication or treatment for it. This *in silico* study has been conducted in an attempt to find a suitable treatment for this condition. Using the databases, ADMET experiments were conducted and analyses were conducted on the possible ligands identified through screening of natural phytochemicals, and identification of effective sites for ligand binding. Ultimately, there has been improved activity demonstrated by the phytochemicals from *Withania somnifera*, namely Somniferine and 27-Deoxywithaferin - A, towards the chosen target protein, SRSF1. Knowing this makes it easier for them to combat pancreatic cancer.

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Conflict of interest

The author declares no conflict of interest.

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