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In silico identification of potential target protein for fucoidan against colon cancer in rats

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Abstract
The present study was designed to find potential target protein for fucoidan against Colon cancer in rats. The sequence of all the seven target proteins were retrieved from UniProt database. As the 3D structure is not available for these target proteins, the sequence of all the seven target proteins were modelled using Swiss-Model and the results were evaluated using Ramachandran Plot. The 3D structure of the compound fucoidan was obtained from PubChem database. All the target proteins were subjected to perform docking studies with fucoidan. The PyRx software was used to carry out this docking studies. Discovery studio 2021 was used to analyse the results. From results, the target protein RAC-alpha serine/threonine-protein kinase (Akt1), Vascular endothelial growth factor receptor 2 (Kdr) and Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k) showed very good binding affinity of -6.1, -5.8, -5.6 Kcal/mol, respectively with the compound fucoidan. Hence, the present study concludes that the best binding affinity was obtained between RAC-alpha serine/threonine-protein kinase and fucoidan and finally this study suggests that RAC-alpha serine/threonine-protein kinase belongs to Akt1 gene may act as a potential target protein for fucoidan.

Keywords: colon cancer, rat, modelling, fucoidan, docking study, Akt1 gene

Introduction
Globally, the second reason for people's death is Cancer. In 2018, one in six deaths and around 9.6 million people are died because of this cancer. The common types of cancer in men are lung, prostate, colorectal, stomach and liver cancer and in women breast, colorectal, lung, cervical and thyroid cancer are the most common (WHO, 2021) [44]. The report of WHO states that out of 183 countries in the world, 112 countries are having the rank of first or second in the death of people by cancer before the age of 70 years (WHO, 2020) [43] and 23 countries are in third and fourth place (Sung *et al.*, 2021) [34].
Colorectal cancer (CRC) is the most common cancer globally and it is the third most general cancer in males and the second in females, 1.4 million new cancer patients are diagnosed every year due to this cancer (Torre *et al.*, 2015) [35]. Mortality rates are very low in many Western countries due to its various diagnostic methods like early detection, screening and enhanced treatment (Center *et al.*, 2010) [6].
Fucoidan or fucoidans is a family of sulfated fucose-rich polysaccharides and mainly made up of L-fucose, identified in many marine sources like marine cucumbers (Mansour *et al.*, 2019) [21] or brown algae (Zhao *et al.*, 2018) [49]. Fucoidans have many therapeutic properties such as anti-inflammatory, anti-coagulant and anti-proliferative properties on cancer cells (Ale and Meyer, 2013) [3]. Structural make-up, monosaccharide composition, sulfate content, the position of sulfate ester groups and molecular weight determines the therapeutic property of fucoidans (Li *et al.*, 2008) [15]. Researchers found that sulfate is essential for the antiviral property (Ponce *et al.*, 2003; Hemmingson *et al.*, 2006) [24, 10]. Further, Mandal *et al.* (2007) [20] reported that sulfate located at *C*-4 of (1→3)-linked fucopyranosyl units are important for the anti-herpetic activity of fucoidan.
When compared to other sulfated polysaccharides, as fucoidans are commonly available from various kinds of easy sources, the research people are used fucoidans to find new drugs and functional foods (Li *et al.*, 2008) [15]. Commercially available fucoidan was obtained from *Fucus vesiculosus.* It has 44.1% fucose, 26.3% sulfate, 31.1% ash and a small amount of amino glucose (Black *et al.*, 1952; Nishino *et al.*, 1994) [5, 22].
Li *et al.* (1995) [18] reported that fucoidan from *Laminaria japonica* has the property against RNA and DNA virus. The disease caused by herpes simplex virus (HSV) is called Herpes.

Fucoidans of *Adenocystis utricularis* (Ponce *et al.*, 2003) [24], *Undaria pinnatifida* (Mekabu) (Lee *et al.*, 2004) [14], *Stoechospermum marginatum* (Adhikaria *et al.*, 2006) [1], *Undaria pinnatifida* (Hemmingson *et al.*, 2006) [10], *Cystoseira indica* (Mandal *et al.*, 2007) [20] and *Undaria pinnatifida* (Hayashi *et al.*, 2008) [9] have potential antiviral property mainly for HSV-1 and HSV-2 (Mandal *et al.*, 2007) [20]. Besides, the study of Ponce *et al.* (2003) [24] reported that fucoidans have potential antiviral properties against some enveloped viruses like human immune deficiency and cytomegalovirus.

Nowadays, antitumor activity was reported in several polysaccharides. Fucoidans found from *Eisenia bicyclics* and *L. japonica* are having a potential effects against sarcoma 180 (Usui, 1980; Song *et al.*, 2000) [39, 32]. Fucoidan persuades apoptosis in HT-29 colon cancer cells (Kim *et al.*, 2010) [12], MCF-7 human breast cancer cells (Yamasaki-Miyamoto *et al.*, 2009) [47] and HS-Sultan human lymphoma cells (Aisa *et al.*, 2005) [2].

In the *in vitro* environment, fucoidan enhances the production of interleukin-1 (IL-1) and interferon-γ (IFN-γ) and stimulate the functions of T lymphocyte, B cell, macrophage and natural killer cell (NK cell) and in the *in vivo* condition, it increases the main antibody response in the sheep red blood cell (SRBC) (Yang *et al.*, 1995) [48]. Fucoidan from *L. japonica* reduces the total cholesterol, triglyceride and LDL-C and increases HDL-C in the serum of mice with hypercholesterolemia and rats with hyperlipidaemia and proficiently prohibited the development of experimental hypercholesterolemia in mice (Li *et al.*, 1999; Li *et al.*, 2001) [17, 16]. Furthermore, fucoidan decreases the cholesterol content and triglyceride in serum of patients with hyperlipidaemia, without any side-effects like damaging of liver and kidney (Wang and Bi, 1994) [41].

Fucoidan of *Cladosiphon okamuranus* tokida is useful for gastric protection (Shibata *et al.*, 2000) [31]. While developing an antiulcer agent and inhibitor for *Helicobacter pylori*, fucoidan was added as a major ingredient. This novel drug is useful to treat and prevent ulcers on the gastric mucosa and to inhibit the *Helicobacter pylori* on the gastric (Itsuko, 1995) [11]. Moreover, fucoidan is used to reduce osteoarthritis, liver damage and controls liver fibrosis. In addition, Fucoidan is also used against malaria, snake venoms and radiation exposure (Ramakrishnan *et al.*, 2021) [26]. Further, due to the water - soluble nature of fucoidans, it is used in the cosmetic industry to make beauty products like lotions, creams, etc. (Ale and Meyer, 2013) [3].

Viruses are not only infecting humans, but it also infects household animals like canines (Williams and Barker, 2001) [45]. The canine distemper virus (CDV), belongs to the genus of morbillivirus which causes the infection in many aquatic carnivores (Beineke *et al.*, 2009) [4]. CDV replication of 90.4% was inhibited by fucoidan of *C. okamuranus* and molecular weight, fucose, sulfate and other sugars of this fucoidan were 92.1 kDa, 38.6%, 15.9% and 23 %, respectively (Trejo-Avila *et. al.*, 2014) [36].

The excretion of calcium oxalate monohydrate crystals is increased in the urine of hyperoxaluric rats and these crystals were deposited in renal tissues which were stopped by fucoidan treatment (Veena *et al.*, 2007) [40]. Fucoidan oligosaccharides have very good antihypertensive effects on renovascular hypertensive rats (Fu *et al.*, 2004) [7].

Akt is essential for the cell signaling pathway which controls cell growth and survival and PI3K is useful for intracellular signaling (Xue *et al.*, 2017; Kim and Nam, 2018) [46, 13]. Pettersson *et al.* (1992) [23] reported that Bcl-2 expression is associated with many tumors. Ravi and Bedi (2002) [27] found that the BAX persuades programmed cell death. The genes Pten, Pcna Kdr are involved in tumor suppression, cell division and apoptosis (Salvatore *et al.*, 2019; Zhou *et al.*, 2018; Loaiza-Bonilla *et al.*, 2016) [28, 50, 19].

In this study, the genes of rats (*Rattus norvegicus)* which include Akt1, Kdr, Pi3k, Bcl2, Pcna, Pten and Bax was taken to perform *in silico* studies. The main objective of the present study was to find potential target protein for fucoidan against Colon cancer in rats using Homology modeling and docking studies.

Materials and methods

Ligand selection

The 3D structure of the compound fucoidan was retrieved from the PubChem database (PubChem database, 2021) [25] and used in this study.

Target protein selection

The sequence of target protein for the genes of rat against colon cancer were taken from the UniProt database (UniProt database, 2021) [38]. The selected target protein and the genes were RAC-alpha serine/threonine-protein kinase (Akt1), Vascular endothelial growth factor receptor 2 (Kdr), Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k), Apoptosis regulator Bcl-2 (Bcl2), Proliferating cell nuclear antigen (Pcna), Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase (Pten) and Apoptosis regulator BAX (Bax).

‘Homology modelling

As the 3D structure is not available for the target protein, the sequence of the target proteins were modelled using Swiss-Model (Waterhouse *et al.*, 2018) [42]. Modelled 3D structure of the target protein was evaluated using SAVES server (SAVES server, 2021) [29] and the Ramachandran plot was analysed.

Docking studies

Docking studies were performed for the ligand fucoidan with different target proteins of rat against colon cancer using PyRx software (Trott and Olson, 2010) [37]. The ligand was prepared using Open Babel option in the PyRx software and all the target proteins were prepared using Discovery Studio 2021. The results were also analysed using Discovery Studio 2021. In the results, the lowest binding affinity indicates good result.

Results and Discussion

Ligand and Protein Selection

The 3D structure of the ligand fucoidan (SID: 402346915) was retrieved from PubChem database. The sequence of all the seven target proteins were taken from UniProt databases and their UniProt ID was tabulated in table 1.

Homology Modeling

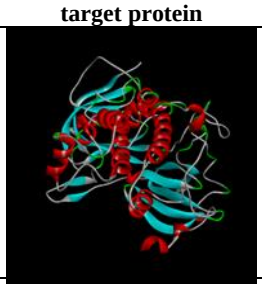
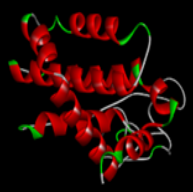
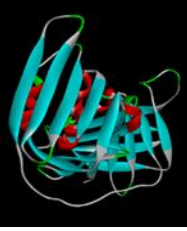
The sequence of all the target proteins were modeled using Swiss-Model and the sequence identity and template details of all the target proteins were noted. Modeled 3D structure of all the target proteins was evaluated using SAVES server and the value of Ramachandran plot was noted. All the results were tabulated in table 1 and the modeled 3D structure of all the target proteins were tabulated in table 2. From the results of

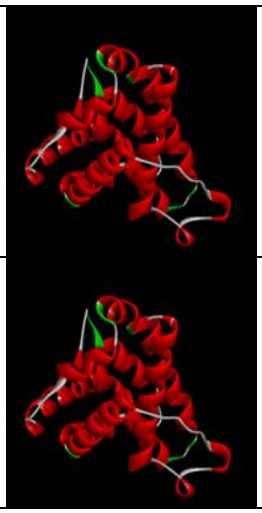
the homology modeling (table 1), the most of the sequence identity of the target proteins were above 85% and the Ramachandran plot value (Residues in the most favored regions) of all the target proteins were above 80 % which confirms the modeled 3D structure was good.

Table 1: Modelling results of seven target proteins

S. No.	Uni Prot ID	Name of the target protein	Gene Name	Sequence Identity	Template PDB ID	Ramachandran plot value (Residues in most favoured regions)
1	P47196	RAC-alpha serine/threonine-protein kinase	Akt1	97.3 %	6hhg	88.6 %
2	O08775	Vascular endothelial growth factor receptor 2	Kdr	95.08 %	3vhk	87.2 %
3	O88763	Phosphatidylinositol 3-kinase catalytic subunit type 3	Pi3k	96.9 %	6i3u	90.9 %
4	P49950	Apoptosis regulator Bcl-2	Bcl2	86.76 %	5jsn	86.9 %
5	P04961	Proliferating cell nuclear antigen	Pcna	70.66 %	4hk1	91.0 %
6	O54857	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase	Pten	99.68 %	5bug	82.6 %
7	Q63690	Apoptosis regulator BAX	Bax	92.71 %	4S0o	95.3 %

Table 2: The 3D structure of modelled target proteins

S. No.	Uni Prot ID	Name of the target protein & Gene	3D Structure of modelled target protein
1	P47196	RAC-alpha serine/threonine-protein kinase (Akt1)	
2	O08775	Vascular endothelial growth factor receptor 2 (Kdr)	
3	O88763	Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k)	
4	P49950	Apoptosis regulator Bcl-2 (Bcl2)	
5	P04961	Proliferating cell nuclear antigen (Pcna)	

6	O54857	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase (Pten)		
7	Q63690	Apoptosis regulator BAX (Bax)		

Docking studies

Docking studies were performed for the ligand fucoidan with 7 different target proteins of rat against colon cancer using PyRx software. The interaction of fucoidan with 7 different target proteins were tabulated in table 3 and the 2D & 3D interaction of the best 3 docked complex was shown in figure 1 – 6.

Table 3: Interaction of Fucoidan with Seven different target proteins

S. No.	Uni Prot ID	Name of the target protein & Gene	Binding Affinity (Kcal/mol)	No. of bonds	Interacting Residues	Bond Length (Å)
1	P47196	RAC-alpha serine/threonine-protein kinase (Akt1)	-6.1	4	ASN 54 TYR 326 ARG 273 ARG 273	2.15 2.48 2.76 3.46
2	O08775	Vascular endothelial growth factor receptor 2 (Kdr)	-5.8	5	ARG 925 ARG 925 GLY 1098 GLY 1098 SER 921	2.44 2.58 2.87 2.99 2.68
3	O88763	Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k)	-5.6	5	ALA 459 LYS 530 ARG 523 ARG 523 THR 477	4.40 2.44 2.15 1.54 2.78
4	P49950	Apoptosis regulator Bcl-2 (Bcl2)	-5.4	5	GLU 157 ARG 26 ARG 26 ARG 26 SER 102	2.19 2.13 2.44 3.00 2.70
5	P04961	Proliferating cell nuclear antigen (Pcna)	-5.3	4	SER 161 SER 161 LYS 181 LYS 181	2.48 2.82 2.07 2.31
6	O54857	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase (Pten)	-5.1	3	ARG 173 ARG 173 TYR 177	1.27 1.31 5.21
7	Q63690	Apoptosis regulator BAX (Bax)	-5.0	3	GLN 52 GLN 52 SER 60	2.36 2.80 3.33

From the results (table 3), among the interaction of 7 different target proteins with fucoidan, the target protein RAC-alpha serine/threonine-protein kinase belongs to Akt1 gene showed very good binding affinity (-6.1 Kcal/mol) with fucoidan and their interacting amino acid residues were ASN 54, TYR 326 and ARG 273. The amino acid residues such as ARG 925, GLY 1098 and SER 921 of Vascular endothelial growth factor receptor 2 belongs to Kdr gene also showed good binding affinity of -5.8 Kcal/mol with fucoidan. The binding affinity of Phosphatidylinositol 3-kinase catalytic subunit type 3 belongs to Pi3k gene with fucoidan was -5.6 Kcal/mol and the ligand showed interaction with the amino acid residues like ALA 459, LYS 530, ARG 523 and THR 477 of this target protein.

The lowest binding affinity (-5.0 Kcal/mol) was observed between the target protein Apoptosis regulator BAX belongs to Bax gene and the ligand fucoidan and their interacting amino acid residues were GLN 52 and SER 60. In the previous study, *in silico* docking studies were performed for fucoidan with 5 EMT proteins PLEKHA7, β-catenin, E-

cadherin, PI3K and Snail1. Among these five the proteins PLEKHA7 and PI3K showed very good interaction with fucoidan (Ganapathy *et al.*, 2020) [8]. The report of Senthil *et al.* (2019) [30] stated that fucoidan is a potent inhibitor for both α-amylase and α-D-glucosidase in the study type 2 diabetes mellitus. The effect of fucoidan on different target proteins of Canine breast cancer was evaluated using computational docking studies (Sujatha *et al.*, 2016) [33]. Moreover, the present study was carried out to find the potential target protein for fucoidan in the seven different target proteins of Colon cancer in rats and found that RAC-alpha serine/threonine-protein kinase belongs to Akt1 gene may act as a potential target protein for fucoidan to treat colon cancer in the rats.

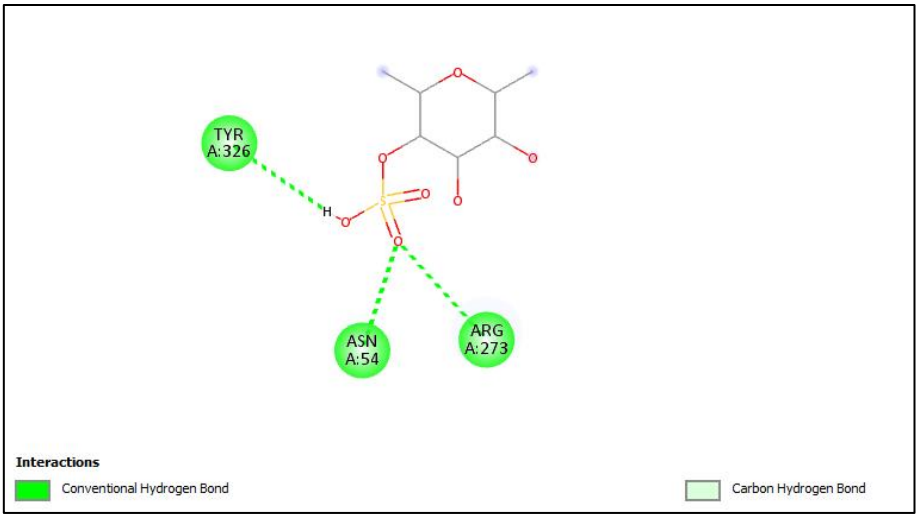


Fig 1: The 2D interaction of fucoidan with RAC-alpha serine/threonine-protein kinase (Akt1)

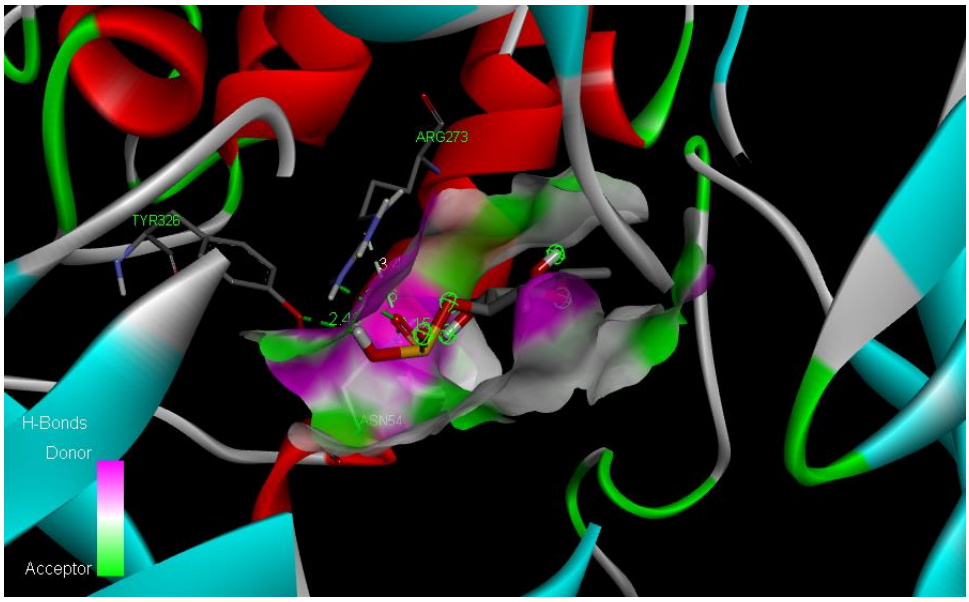


Fig 2: The 3D interaction of fucoidan with RAC-alpha serine/threonine-protein kinase (Akt1)

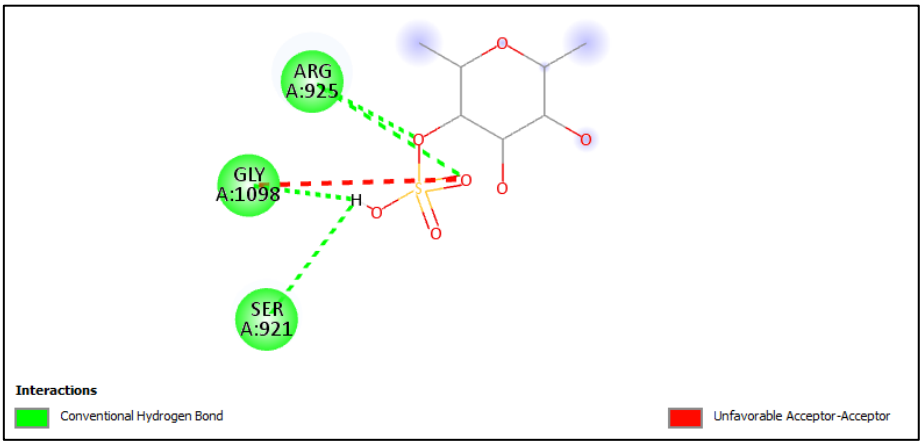


Fig 3: The 2D interaction of fucoidan with Vascular endothelial growth factor receptor 2 (Kdr)

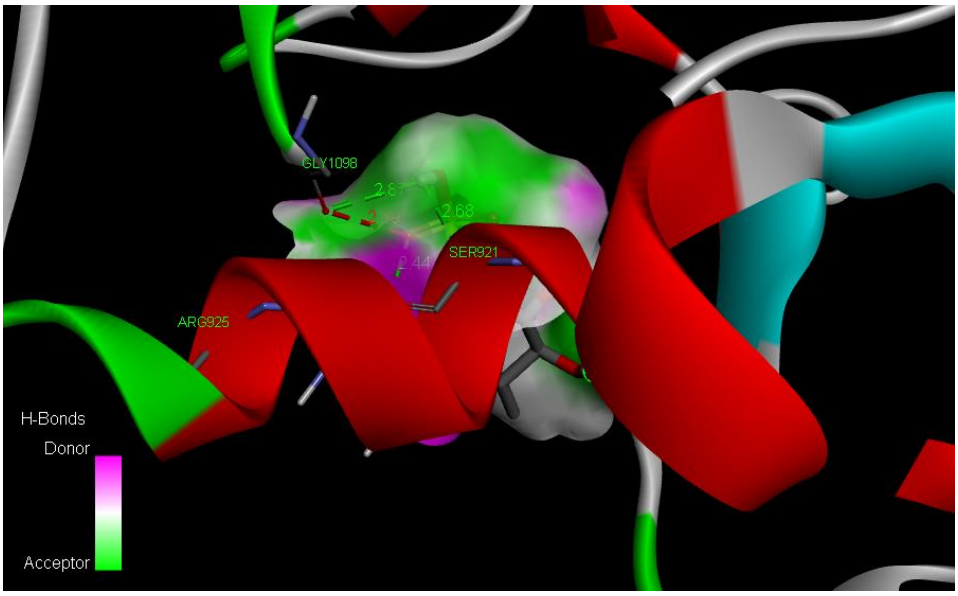


Fig 4: The 3D interaction of fucoidan with Vascular endothelial growth factor receptor 2 (Kdr)

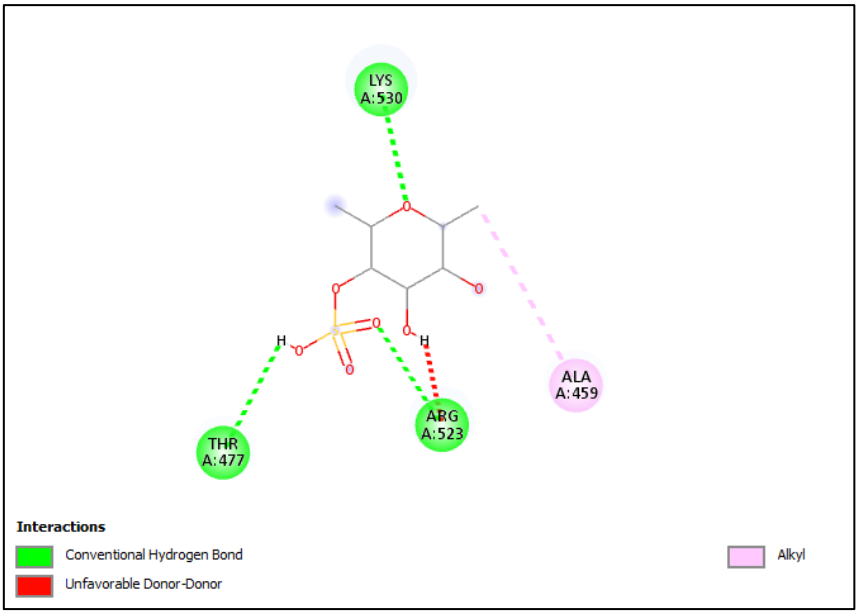


Fig 5: The 2D interaction of fucoidan with Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k)

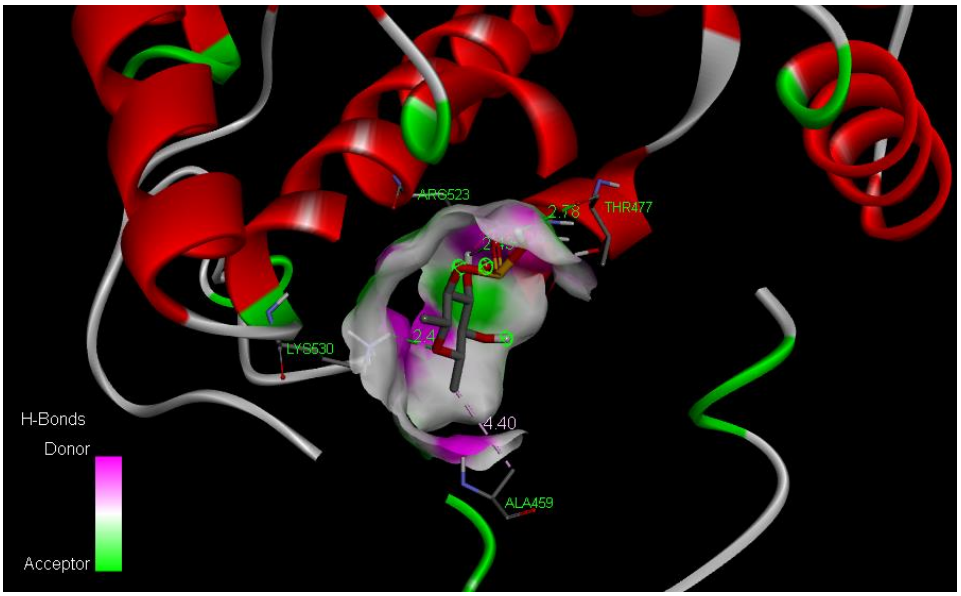


Fig 6: The 3D interaction of fucoidan with Phosphatidylinositol 3-kinase catalytic subunit type 3 (Pi3k)

Conclusion

In the present study, the best binding affinity was observed between RAC-alpha serine/threonine-protein kinase and fucoidan. Hence, this study concludes that RAC-alpha serine/threonine-protein kinase belongs to Akt1 gene may act as a potential target protein for fucoidan to treat colon cancer in the rats.

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