IJBPAS, August, 2018, 7(8): 1551-1565

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

IN SILICO MOLECULAR DOCKING OF ALGINIC ACID AND FUCOIDAN COMPOUND PRESENT IN *S. WIGHTII* AGAINST APOPTOTIC PROTEINS (CASPASE-3, CASPASE-9 AND β -ACTIN)

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Received 20th Feb. 2018; Revised 27th March. 2018; Accepted 30th April 2018; Available online 1st August 2018

https://doi.org/10.31032/IJBPAS/2018/7.8.4513

ABSTRACT

The present molecular docking study stands useful for the design and development of novel drug algenic acid and fucoidan compound having better inhibitory activity against the selective western blot expressed apoptotic proteins (caspase-3, caspase-9 and β-actin) of human prostate cancer cell line. The docking scores were highest for when alginic acid was docked against β-actin giving a docking score of -8.9081 kcal/mol followed by caspase-3 (-8.5634 kcal/mol), and caspase-9 (-8.2513 kcal/mol). Likewise, when fucoidan was docked with caspase-9, it showed highest docking score of -8.5091 kcal/mol, followed by caspase-3 (-7.6683 kcal/mol), and β-actin (-7.6636 kcal/mol). Among the three docked proteins against algenic acid, caspase-9 gave a minimum docking score, which concludes that alginic acid controls caspase-9 expression. Likewise, fucoidan compound, gave a minimum docking score with control expression of β-actin. The Log P, lower hydrogen bond counts, confirming the capability of algenic acid and fucoidan for binding at the active site of the receptor. This potential drug candidate can further be validated by wet lab studies for its proper function.

Keywords: Algenic acid, Fucoidan, Apoptotic proteins, ARGUSLAB

1. INTRODUCTION

Computational drug discovery can help in identifying potential drug molecules and targets *via*. bioinformatics tools. They can also be used to evaluate the target structures for possible binding/active sites, generate active drug molecules, and check for their dynamic and kinetic properties. The docking studies of the drug molecules

with the target molecules will help us to know the affinity and efficacy of developed molecule and we rank them according to their binding affinities [1]. The molecules which are showing better activity can be modified and build to get good activity towards the target molecules, and further optimize the molecules to improve binding characteristics. The use *in silico* methods will help us in all aspects of drug discovery today and forms the importance of structure-based drug design. There are plenty of programs which are helping us to build an active drug molecule. Meanwhile, high-performance computing, data management software and internet are helping us to generate high quality data and also transformation of huge complex biological data into accessible knowledge in current trends to discover novel drug molecules [2].

Seaweeds, especially brown seaweeds, are rich in biologically active polysaccharides that exhibit a broad spectrum of biological activities. Examples of these polysaccharides include fucoidans, laminarins and alginic acids [3]. *Sargassum wightii* is one of the important species belonging to the genus *Sargassum* and a wide range of bioactive properties have been reported from this species [4, 5, 6, 7, 8, 9, 10]. It is widely distributed on the southern coasts of Tamil Nadu, India, and many parts of Asia and it is reported to be used as animal feed, food ingredients and fertilizer. Since *Sargassum wightii* is available in large quantities, it appears to be the most suitable raw material for commercial exploitation. *Sargassum wightii* also shows a good amount of flavonoids in support and its antioxidant activity [11] indicating that this species is an ideal target for investigating the activity of the biomolecules present in *Sargassum wightii* for various medical and industrial applications [7].

Alginic acid, a chemical constituent of the cell wall of most brown seaweeds and a linear polymer of high molecular weight consisting of β-(1—>4) linked D-*mannuronic* acid and L-*guluronic* acid units in the *pyranose* ring form, is the only extract presently obtained from the brown seaweeds [12]. Of the many commercially important chemicals derived from seaweeds, alginic acid and its salt find their application in food, pharmaceuticals, cosmetics, papers and textile industries [13].

The biological activity of fucoidans is related to a molecular structure, which include fucose linkage, the sugar type, sulphate content; molecular weight being the most important determinant. Fucoidan from *Fucus vesiculosus* (Phaeophyceae) is mainly composed of α-(1-3) linked sulphated L-fucose. The core region of the fucan is composed primarily of a polymer

of α (1-3) linked fucose with sulphate groups substituted at the 4 position on some of the fucose residues [14]. Fucoidan has also been shown to have cyto-protective properties. Chemotherapeutic anticancer drugs are effective against cancer cells, but because of a lack of selectivity, they also attack normal immune cells. It has been demonstrated that fucoidan can protect dendritic cells from the effect of 5-Fluorouracil (a representative cancer drug) [15].

Fucoidan extracted from various types of sea weeds like *Undaria pinnatifida Saccharina cichorioides, Fucus evanescens* have shown promising antitumour effects on various types of cancer cells like PC-3, HeLa, A549 and HepG2 cell lines (Vishchuk *et al*., 2011) mouse epidemial cells (JB6 C141), human colon cancer cells (DLD-1), breast cancer cells (T-47D) and melanoma (RPMI-7951) (Lee *et al*., 2013). Likewise, the anticancer effect of fucoidan (AcFu) nanoparticles loaded with the chemotherapy drug doxorubicin against HCT-116 and HCT-8 cell lines [15].

Drug designing is a process of choosing of novel drug candidates in which many necessary steps are taken to banish such drug molecules that have side effects and also represent interaction with other drug candidates. There are vast numbers of softwares which play a crucial role in *in silico* pharmaceutical research. The *in silico* drug designing softwares are used to inspect molecular modeling of gene, protein sequence analysis and 3D structure of proteins [16]. In view of this, *in silico* molecular docking studies were done using alginic acid and fucoidan compounds that are present in brown algae *S. wightii,* to assess its docking potential.

2. MATERIALS AND METHODS

In silico docking studies

To predict the mode of action of the ligands, Alginic Acid and Fucoidan compound against Caspase-3, Caspase-9 and β-Actin protein *in silico* molecular docking studies were carried out. Following databases and tools are used in the present investigation such us SwissProt, Protein Data Bank and RasMol, ChemSketch, ARGUSLAB, and PyMol Viewer.

Structure Retrieval

Database similarly searches are one of the most important steps in analyzing a sequence. If the query sequence has a similar copy already in the database, a search will quickly reveal this fact. If a similarly of sequence or structure is found from another species, then they may be homologous (*i.e*., sequence that descended from common ancestral). This will pave a way for further analysis of the query sequence. The structure homologues for a given protein sequence query is searched against SwissProt and PDB.

SwissProt

The proteins of Caspase-3, Caspase-9 and β-Actin were retrieved from Swiss-Prot database. The accession numbers are: P42574, P55211, and P60709.

PDBSum

The structure of Caspase-3, Caspase-9 and β-Actin were downloaded from PDBSum database and the PDB IDs are: 1CP3, 1JXQ and 3BYH.

Docking: ARGUSLAB

In this present docking study analyzed using ArgusLab docking software. ArgusLab is a program to build graphic representations of molecular models. Using this program, will be able to show molecular models to pupils, or even design matters by combining different elements. That will be able to include in model several atoms, residues, groups and calculations.

Visualization of Protein using PyMol Viewer

The PyMol software interactively displays molecular models and creates publication quality images. A `ribbon drawing' is featured here. Space-filling, ball-and-stick representations, molecular surfaces, density map contours, and crystal packing diagrams, and movies are also supported.

The docked structures were then visualized using the PyMol Viewer software and the results were predicted.

3. RESULTS

***In Silico* Molecular Docking Studies**

To study the interaction between ligands (alginic acid and fucoidan) and apoptotic proteins (caspase-3, caspase-9 and β-actin) and to explore their binding mode, docking study was performed by using ArgusLab software.

Retrieval of Protein Sequence from SWISS-PROT Database

Caspase -3

Protein Name: Caspase-3

Alternative name(s): Cysteine protease CPP32

SWISS-PROT ID: P42574

Organism: *Homo sapiens* (Human)

FASTA Sequence:

>sp|P42574|CASP3_HUMAN Caspase-3 OS=Homo sapiens GN=CASP3 PE=1 SV=2
MENTENSVDSKSIKNLEPKIIHGSESM
DSGISLDNSYKMDYPEMGLCIIINNKN
FHKSTG
MTSRSGTDVDAANLRETFRNLKYEVR
NKNDLTREEIVELMRDVSKEDHSKRS
SFVCVLLS
HGEEGIIFGTNGPVDLKKITNFFRGDR
CRSLTGKPKLFIIQACRGTELDCGIETD
SGVDD
DMACHKIPVEADFLYAYSTAPGYYSW
RNSKDGSWFIQSLCAMLKQYADKLEF
MHILTRVN
RKVATEFESFSFDATFHAKKQIPCIVS
MLTKELYFYH

Caspase – 9

Protein Name : Caspase-9

Alternative name(s) : Apoptotic protease Mch-6; Apoptotic protease-activating factor 3

SWISS-PROT ID : P55211

Organism : *Homo sapiens* (Human)

FASTA Sequence:

>sp|P55211|CASP9_HUMAN Caspase-9 OS=Homo sapiens GN=CASP9 PE=1 SV=3
MDEADRRLLRRCRLRLVEELQVDQL
WDALLSRELFRPHMIEDIQRAGSGSRR
DQARQLII
DLETRGSQALPLFISCLEDTGQDMLAS
FLRTNRQAAKLSKPTLENLTPVVLRPE
IRKPEV
LRPETPRPVDIGSGGFGDVGALESLRG
NADLAYILSMEPCGHCLIINNVNFCRE
SGLRTR
TGSNIDCEKLRRRFSSLHFMVEVKGDL
TAKKMVLALLELAQQDHGALDCCVV
VILSHGCQ
ASHLQFPGAVYGTDGCPVSVEKIVNIF
NGTSCPSLGGKPKLFFIQACGGEQKDH
GFEVAS
TSPEDESPGSNPEPDATPFQEGLRTFDQ
LDAISSLPTPSDIFVSYSTFPGFVSWRD
PKSG
SWYVETLDDIFEQWAHSEDLQSLLLR
VANAVSVKGIYKQMPGCFNFLRKKLF
FKTS

β-actin.

Protein Name : β -actin

Alternative name : Actin, cytoplasmic 1

SWISSPROT ID : P60709

Organism : *Homo sapiens* (Human)

FASTA Sequence:

>sp|P60709|ACTB_HUMAN Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1
MDDDIAALVVDNGSGMCKAGFAGDD
APRAVFPSIVGRPRHQGVMVGMGQK
DSYVGDEAQS
KRGILTLKYPIEHGIVTNWDDMEKIW
HHTFYNELRVAPEEHPVLLTEAPLNPK
ANREKMT
QIMFETFNTPAMYVAIQAVLSLYASGR
TTGIVMDSGDGVTHTVPIYEGYALPH
AILRLDL
AGRDLTDYLMKILTERGYSFTTTAERE
IVRDIKEKLCYVALDFEQEMATAASSS
SLEKSY
ELPDGQVITIGNERFRCPEALFQPSFLG
MESCGIHETTFNSIMKCDVDIRKDLYA
NTVLS
GGTTMYPGIADRMQKEITALAPSTMKI
KIIAPPERKYSVWIGGSILASLSTFQQM
WISKQ
EYDESGPSIVHRKCF

Retrieval of Protein Structure:

The 3D structure of Caspase-3, Caspase-9 and β-actin were derived from SWISS-PROT, PDB and RasMol for using them as a target for docking simulation (Fig. 1, Fig. 2 and Fig. 3).

Retrieval of ligands structure:

The 3D structure Ligands were created and prepared for the docking procedure using ChemSketch, and the structure of the ligands obtained from the ChemSketch are shown in the Fig. 4 and Fig. 5.

Docking:

The 3D structure of caspase-3, caspase-9 and β-actin were docked with alginic acid and fucoidan using ArgusLab software (Fig. 6). The docking results were analyzed using PyMol visualization tool (Fig. 7, Fig. 8 and Fig. 9).

When alginic acid was docked against caspase-3, a docking score of -

8.5634kcal/mol was recorded and it formed 9 hydrogen bond. Likewise, when alginic was docked against caspase-9, a docking score of -8.2513 kcal/mol with the 8 hydrogen bond formation was observed. β-actin also gave a docking score of -8.9081 kcal/mol with 9 hydrogen bond formation. Of the three proteins docked caspase-9 gave a minimum docking score, which concludes that alginic acid controls the caspase-9 expression. Similarly, when fucoidan was docked with caspase-3 it showed a docking score of -7.6683 kcal/mol with 3 hydrogen bond formation. Likewise, caspase-9 showed docking score of -8.5091 kcal/mol with 6 hydrogen bond formation and the least score was found in the β-actin with the scores of -7.6636 kcal/mol with 12 hydrogen bond formation. Among the three docked proteins against fucoidan compound, β-actin alone gave a minimum docking score with the control expression of β-actin when docked with fucoidan compound.

In toto, alginic acid docked with caspase-9 alone confirming the ability of the ligand for binding at the active site of receptor was determined by this *in silico* docking method and funcoidan compound also showed the ligand binding interaction against β-actin (Table 1).

The results show that there is a presence of binding site between these three proteins and two ligands. The docking is also valid by the formation of hydrogen bond between them. The result of Lipinski rule suggests the analyzed compound as best therapeutic drug. The *in silico* docking studies proves the application of these two compounds present in *S. wightii* as potential and natural therapeutic agent to treat prostate cancer.

Table 1: Docking score and number of H_2 bonds formed between the protein and ligands

S. No.	Protein	Name of the ligand	Docking score Kcal/mol.	H-Bond
1	Caspase-3	Alginic acid	-8.5634	9
2	Caspase-9	Alginic acid	-8.2513	8
3	β –actin	Alginic acid	-8.9081	9
4	Caspase-3	Fucoidan	-7.6683	3
5	Caspase-9	Fucoidan	-8.5091	6
6	β –actin	Fucoidan	-7.6636	12

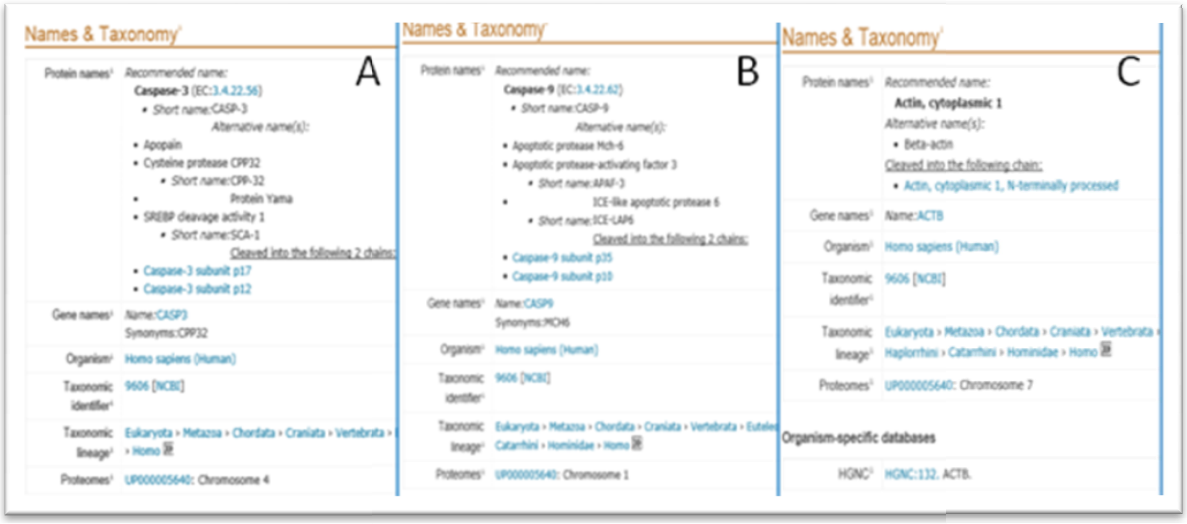

Fig. 1: SWISS-PROT Homepage (A) caspase-3 (B) caspase-9 (C) β-actin

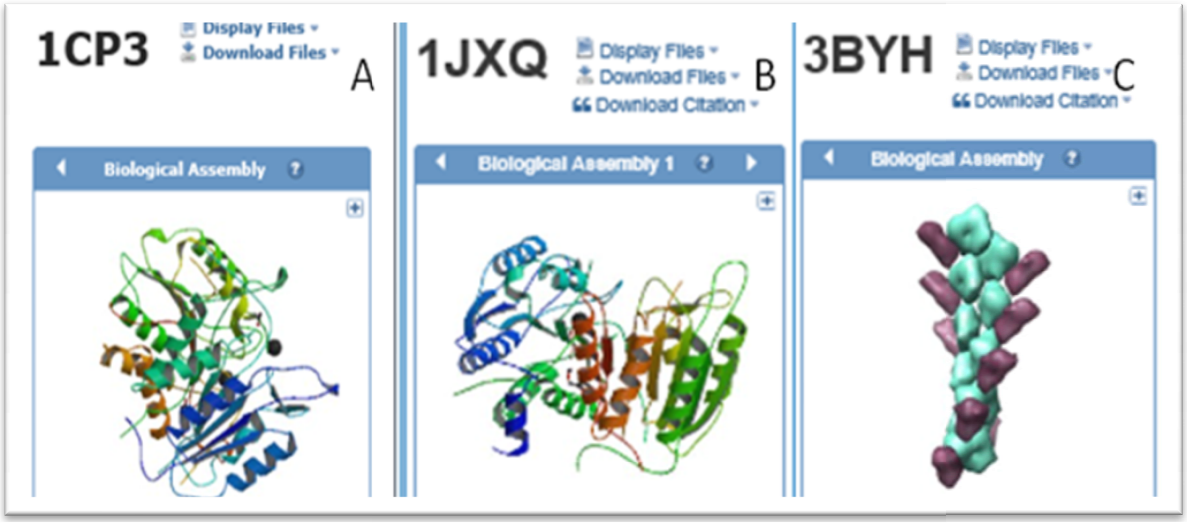

Fig. 2: PDB Homepage (A) caspase-3 (B) caspase-9 (C) β-actin

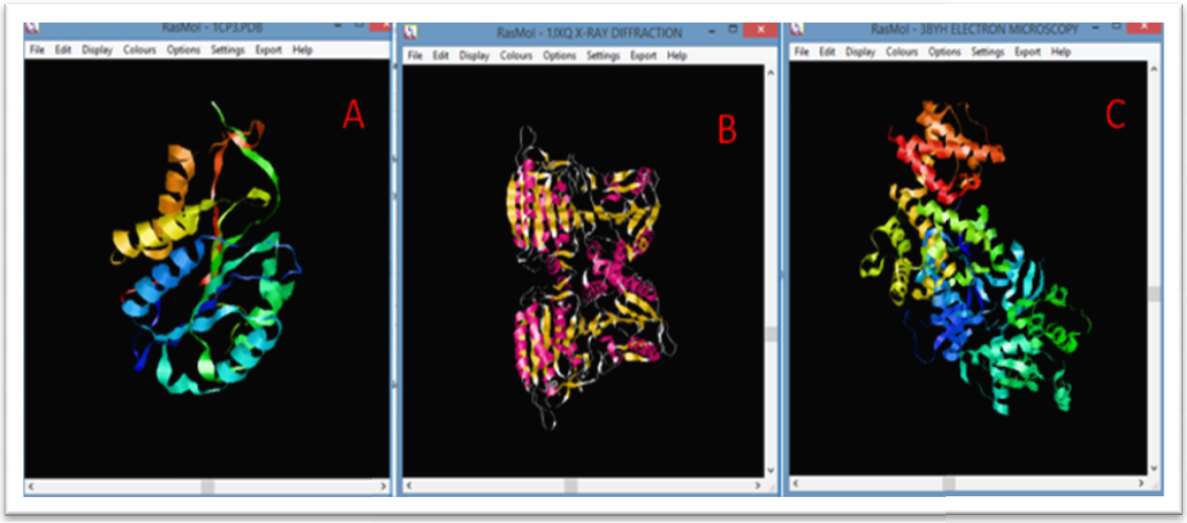

Fig. 3: RasMol Toolpage (A) caspase-3 (B) caspase-9 (C) β-actin

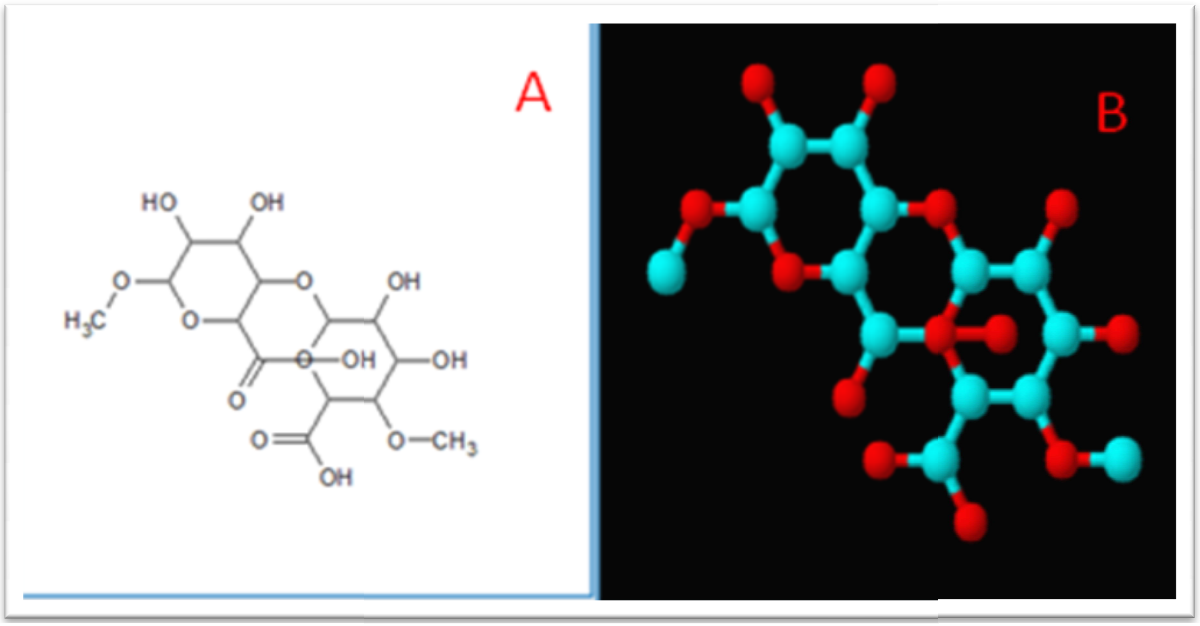

Fig. 4: ChemSketch Homepage of alginic acid(A) 2D structure (B) 3D structure

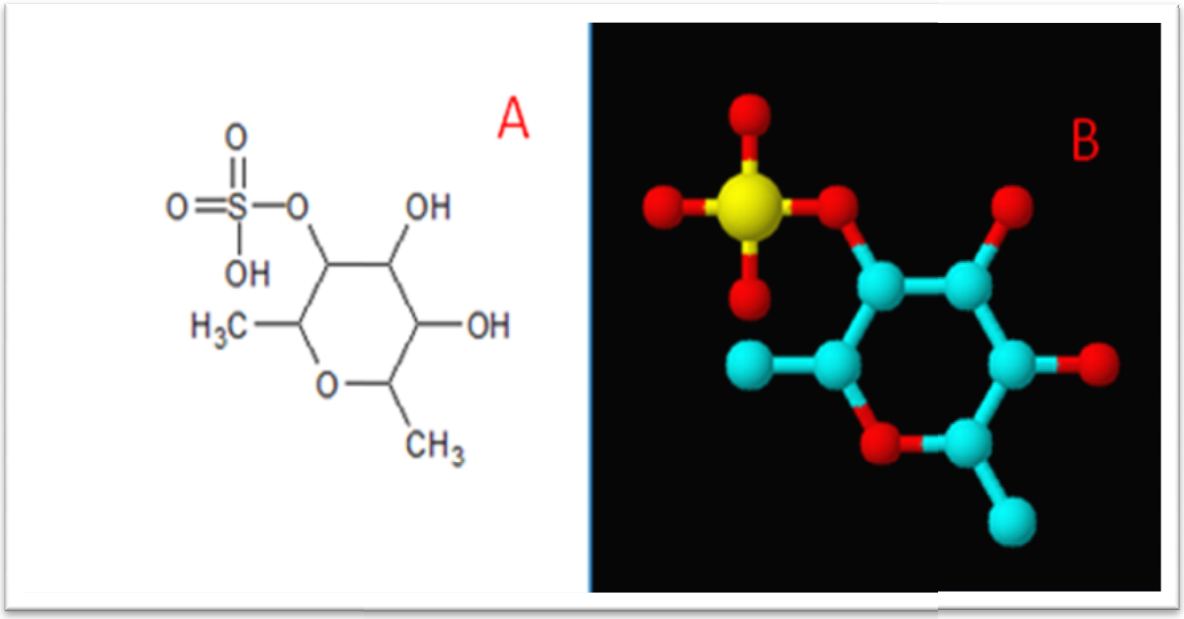

Fig. 5: ChemSketch Homepage of fucoidan (A) 2D structure (B) 3D structure

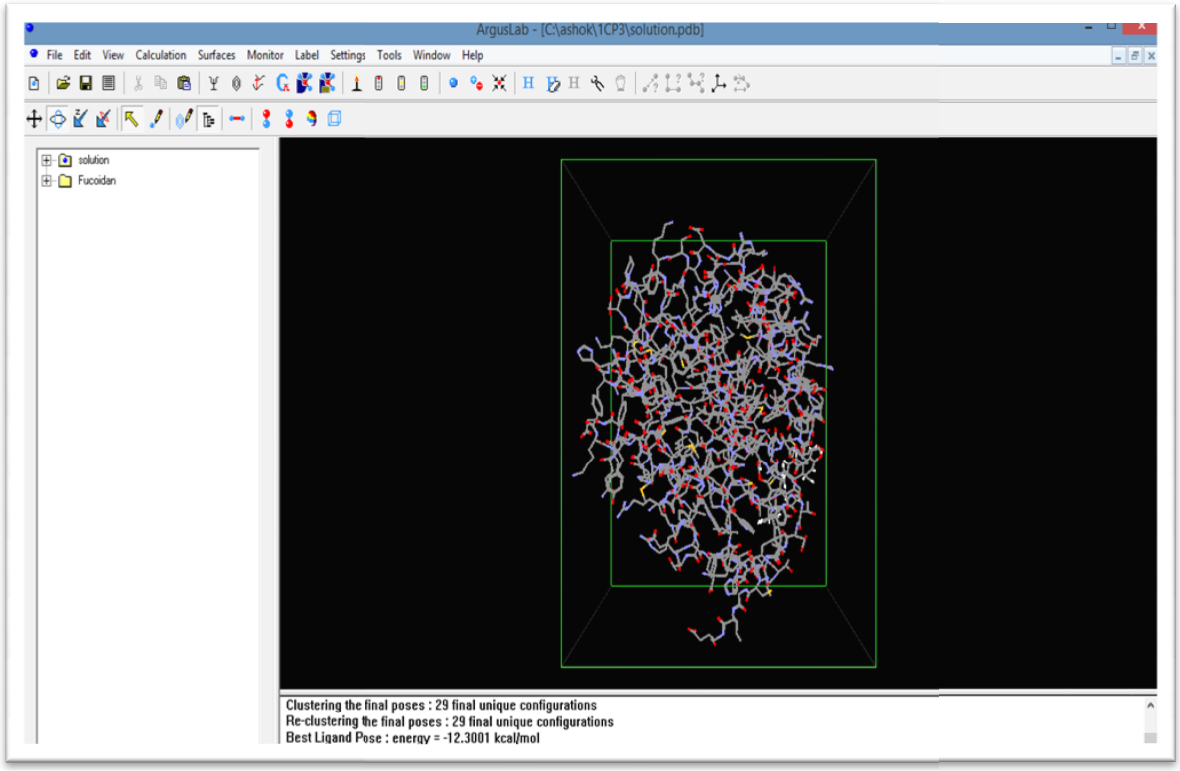

Fig. 6: ArgusLab Toolpage

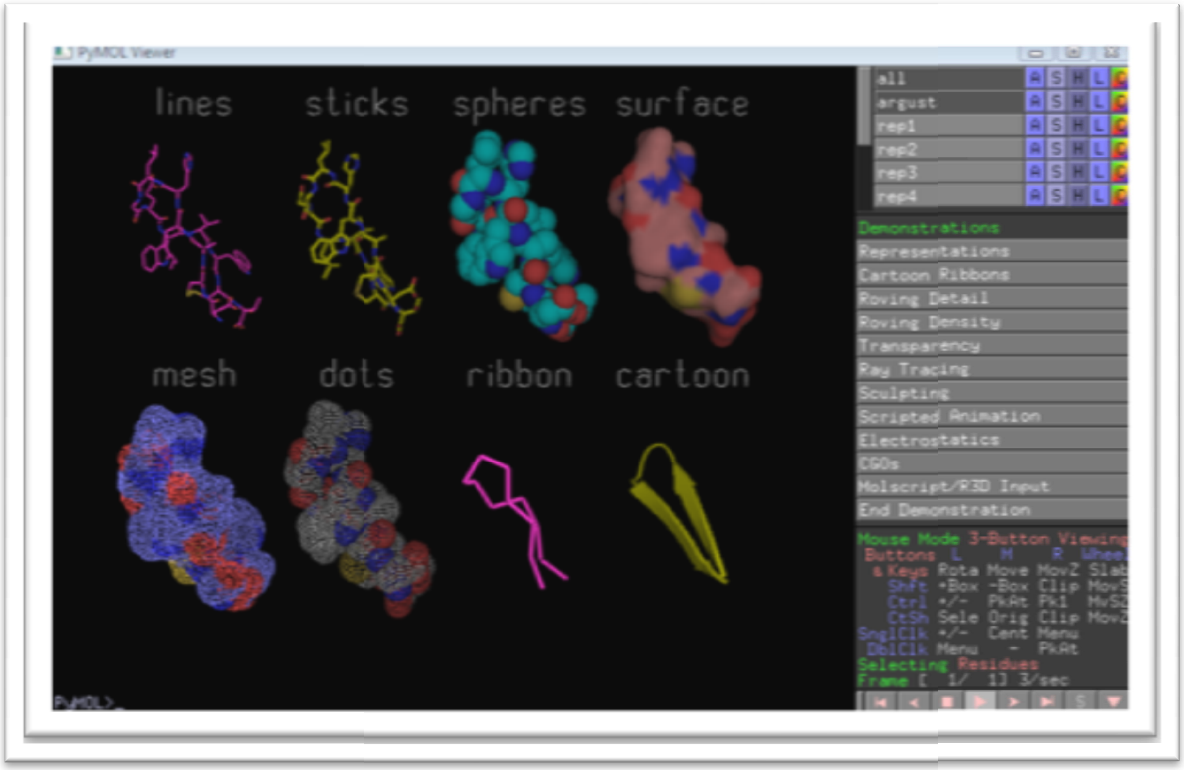

Fig. 7: PyMol Homepage

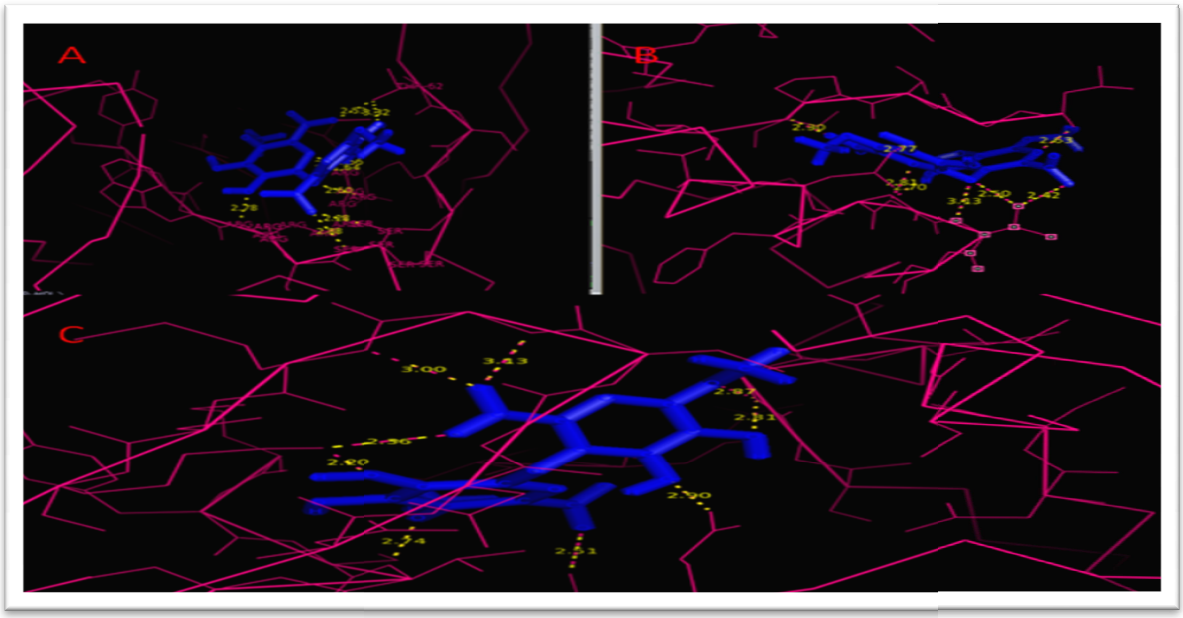

Fig. 8: Visualization of docked complex of (A) caspase-3 (B) caspase-9 (C) β-actin with alginic acid using PyMol tool

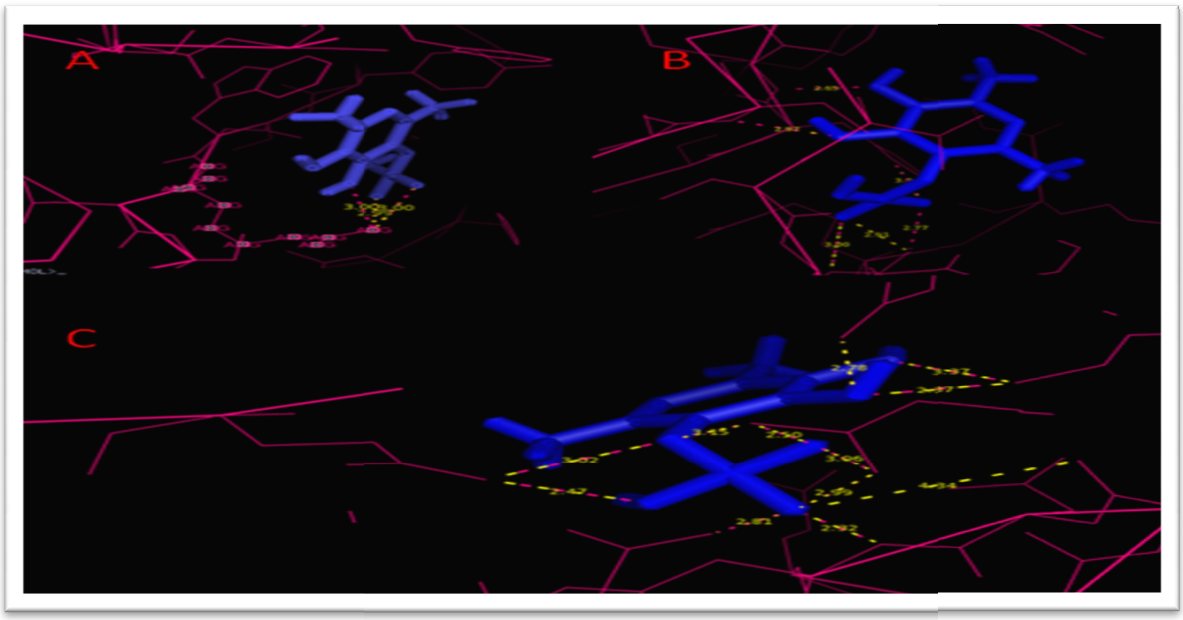

Fig. 9: Visualization of docked complex of (A) caspase-3 (B) caspase-9 (C) β-actin with fucoidan using PyMol tool

DISCUSSION

Recently there are a few reports regarding the ligand-protein interactions of bioactive substances. Nguyen-Vu *et al*. (2013) [17] have reported that the liver X receptor ligands disrupt breast cancer cell proliferation through an E2F-mediated mechanism. *In silico* docking of fucoidan compound against the selective proteins of HepG-2 cell line has been done by Ashok and Sivakumari (2015) [18]. Manimaran *et al*. (2015) [19] have done the molecular docking studies of resveratrol against the human oral cancer cell line proteins (KB cells). Antimycobaterial activity of *Kappaphycus alvarezii* against

Mycobacterim tuberculosis and *in silico* molecular docking of kappa-carrageenan against InhA enzyme was done by Mayakrishnan *et al*. (2015) [20]. Likewise, *in silico* docking of Quercetin compound against the HeLa cell line protein was carried out by Muthukala *et al.* (2015) [21]. Similarly, Rajesh *et al*. (2016) [22] carried out *in silico* docking of stearic acid present in *Cardiospermum halicacabum* leaf against plasminogen and transferrin proteins present in HepG-2 cell line to predict its anticancer property. *In silico* molecular docking studies of Rutin compound against apoptotic proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53, Collagenase, Nitric Oxide Synthase and Cytochrome C) is carried out by Jayammena *et al*. (2018) [23]. Flora Priyadarshini *et al.* (2018) [24], reported, molecular docking studies of selected compounds in propolis against apototic proteins (Caspase-3, Caspase-9, B-Actin).

Senapati *et al*. (2005) [25] envisaged that chemical synthesis may still lead to the presence of some toxic chemical species adsorbed on the surface that may have adverse effects in medical applications. On this basis, green synthesis of AgNPs has been carried out in the present study. Although therapeutic potential of *S. wightii* is promising, the phytocompounds responsible for its therapeutic activity is yet to be exploited. There are good possibilities for the biomedical and biotechnological applications of AgNPs of *S. wightii*, since they are synthesized extracellularly, quite stable and eco-friendly in nature. In this context, *in silico* molecular docking studies on the phytocompounds *viz*., alginic acid and fucoidan compounds present in *S. wightii* against apoptotic proteins may throw more light on the activity of these phytocompounds in combating cancer.

According to Syad *et al.* (2013) [26], *S. wightii* is a major source of alginic acid used widely in food and drug industries. Successive extraction of *S. wightii* having antioxidant properties and the free radical scavenging activity has been done by taking number of parameters like 2,2-diphenyl-1-picrylhydrazyl, OH•, H_2O_2 radical scavenging assay. Syad *et al.* (2013) [26] evaluated the reducing power of the seaweed by ferric reducing antioxidant power and reducing power assay. Cholinesterase (ChE) inhibitory activity was evaluated by the Km, Vmax and Ki value. Thenon-polar extracts were found to possess significant antioxidant activity. The presence of high amount of terpenoids could be the possible reason for its potential antioxidant and ChE inhibitory activity.

It is well documented that major seaweed components such as fucoidan and fucoxanthin have effective anticancer

properties. However, the importance of screening crude seaweed extracts should not be overlooked, as minor components may also harbor potent biological activities. The sporophyll of *Undariapinnatifida* is considered to have lower utility value compared to other parts of the plant and is usually discarded as waste. An ethanol extract of the sporophyll was prepared and shown to reduce the viability of colorectal cancer HCT116 cells when tested by Nishibori *et al.* (2012) [27]. In view of this, *in silico* molecular docking was carried out using alginic acid and fucoidan ligands against three apoptotic proteins.

It is interesting to know that docking studies of compounds present in *S. wightii* against apoptotic proteins has not carried out and this is the first report that is recorded. *In silico* docking study revealed that caspase-9 showed a minimum docking score with alginic acid. On the other hand, β-actin showed minimum docking score with fucoidan. Hydrogen bond formation was good in all the three proteins, when docked with both alginic acid and fucoidan compound. This result shows that there is a presence of binding site between these three proteins and two ligands. The docking is also valid by the formation of hydrogen bond between them. The result of Lipinski rule suggests the analysed compound as best therapeutic drug. Docking study and *in silico* toxicity results proves the application of compounds as potential and natural therapeutic agents to treat disease.

Further, the active principle compounds present in *S. wightii* are to be isolated and their efficacy should be investigated in future to unravel the actual mechanism involved in the antioxidant and anticancer activity of *S. wightii*.

5. CONCLUSION

In the present study, *in silico* molecular docking study of alignic acid and fucoidan compound against apoptotic proteins proves the application of compounds as potential and natural therapeutic agent to treat disease.

6. ACKNOWLEDGEMENTS

The authors are thankful to Mrs. K. Shyamala Devi for the technical support.

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