Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

IN SILICO MOLECULAR PREDICTION OF ASCORBIC ACID, BETALAIN AND GALLIC ACID FROM *HYLOCEREUS UNDATUS* AGAINST APOPTOTIC PROTEINS (CASPASE-3, CASPASE-9 AND β -ACTIN)

Karthika S, Sivakumari K*, Rajesh S, Ashok K and Shyamala Devi K.
Department of Zoology, Presidency College, Chennai, India
*Corresponding Author Email: dr.sivakumari@rediffmail.com

DOI: 10.7897/2277-4572.076110

Received on: 25/07/18 Revised on: 27/11/18 Accepted on: 03/12/18

ABSTRACT

The dragon fruit *Hylocereus undatus* as an important source of polyphenols, it is a good natural resource of antitumor properties. Therefore, the present study was planned to predict the molecular interaction between the chemical compounds from *Hylocereus undatus* (Ascorbic acid, Betalain, and Gallic acid) with apoptotic proteins (Caspase-3, Caspase-3, and β-Actin) by ARGUSLAB docking software. The docking interaction was predicted for Ascorbic acid with Caspase-3 (-7.45), Betalain with Caspase-3 (-10.14), Gallic acid with Caspase-3 (-7.80); Ascorbic acid with Caspase-9 (-7.33), Betalain with Caspase-9 (-8.99), Gallic acid with Caspase-9 (-8.08); Ascorbic acid with β-Actin (-7.67), Betalain with β-Actin (-8.46), Gallic acid with β-Actin (-7.79). Among the three ligands, Ascorbic Acid recorded a less docking score with Caspase-3, Caspase-9 and β-Actin. Hence, Ascorbic acid can be taken as the best ligand among the three. The result of Lipinski rule suggests Ascorbic acid 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.

KEY WORDS: Ascorbic acid, Betalain, Gallic acid, Apoptotic proteins, ARGUSLAB.

INTRODUCTION

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecules. Hence, docking plays an important role in the rational design of drugs[1]. Molecular docking is a well-established computational technique, which predicts the interaction energy between two molecules and this technique mainly incorporates algorithms like molecular dynamics, Monte Carlo stimulation and fragment-based search methods[2]. The main aim of molecular docking is to computationally simulate the molecular identification process and accomplish an optimized conformation so that the free energy of overall system is minimized. The process of discovery of a new drug is a very difficult task. Modern drug discovery is mainly based on *in silico* chemico-biological approach. Use of computer aided techniques in drug discovery and development process is rapidly gaining popularity, implementation and appreciation[3]. Various docking programs have been formulated throughout the last twenty years. Some basic features such as endorsed platforms, license conditions, algorithms and scoring functions are currently available docking tools [4,5].

Dragon fruit is an important source of phytochemicals such as polyphenols, flavonoids and vitamin C, which are related to its antioxidant activity[6,7]. The red and white dragon fruits especially have recently drawn growing attention worldwide not only because of their economic values, but also for their health benefits[8]. Red dragon fruit consumption was reported to decrease total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) levels, while increasing the high-density lipoprotein cholesterol (HDL-C) level in type 2 diabetic[9]. Though both red and white dragon fruit are reported to be rich, natural and cost-effective source of bioactive nutrients, few studies focused on the beneficial effects of white dragon fruit on diabetes and NAFLD (Non-Alcoholics Fatty Liver Disease)[10]. Dragon fruit is believed to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen kidney function and bone, to strengthen the brain working capacity and to increase the sharpness of the eyes as well as it is used in cosmetic ingredients[11]. Modern research is confirming that many compounds are active at some molecular targets, which are being sought to find out potential newer generation "targeted" biological response modifier drugs[12,13,14,15].

Therefore, the present study was aimed to predict the mechanism of *in silico* molecular docking interaction using ascorbic acid, betalain and gallic acid that are present in dragon fruit against apoptotic proteins *viz.*, caspase-3, caspaes- 9 and β-Actin, to assess its docking potential.

MATERIALS AND METHODS

In silico Docking Studies

To predict the mode of action of the ligand, Ascorbic acid, Betalain and Gallic acid 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 as 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 was searched against SwissProt and PDB.

SwissProt

The proteins of **Caspase-3, Caspase-9 and β-Actin** were retrieved from SwissProt 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.**

ACD ChemSketch

The **3-D** structure of **Ascorbic Acid, Betalain** and **Gallic Acid,** (ligands) compounds were drawn using ACD ChemSketch.

Docking: ARGUSLAB

In the present study, docking was analyzed by using ArgusLab docking software. ArgusLab is a program to build graphic representations of molecular models. Using this program, we can show molecular models to pupils, or even design matters by combining different elements, which 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.

RESULT AND DISCUSSION

Molecular Docking Studies

For molecular docking studies of proteins *viz*., Caspase-3, Caspase-9 and β-Actin against the ligands Ascorbic acid, Betalain and Gallic acid the proteins were downloaded from SwissProt database and the details are given below:

Retrival of Protein Structure from PDB Database

The 3-D structures of Caspase-3 **(**PDB ID-1CP3), Caspase-9 (PDB ID-1JXQ) and β-Actin (PDB ID-3BYH) were downloaded from PDB database and are given in Fig. 1.

Retrieval of Protein Sequence from SwissProt Database

The sequence of Caspase-3 (**P42574**), Caspase-9 (**P55211**), β-Actin (**P60709**) were retrieved from Swissprot database. 3-D structure of Caspase-3 (**1CP3**), Caspase-9 (**1JXQ**), and B-Actin (**3BYH**) were downloaded from PDB database.

Protein Name: CASPASE-3
Alternative name(s): Cysteine protease CPP32
Swissprot ID: **P42574**
Organism: ***Homo sapiens*** **(Human)**

FASTA Sequence:
>sp|P42574|CASP3_HUMAN Caspase-3 OS=Homo sapiens GN=CASP3 PE=1 SV=2
MENTENSVDSKSIKNLEPKIIHGSESMDSGISLDNSYKMD
YPEMGLCIIINNKNFHKSTG
MTSRSGTDVDAANLRETFRNLKYEVRNKNDLTREEIVEL
MRDVSKEDHSKRSSFVCVLLS
HGEEGIIFGTNGPVDLKKITNFFRGDRCRSLTGKPKLFIIQ
ACRGTELDCGIETDSGVDD
DMACHKIPVEADFLYAYSTAPGYYSWRNSKDGSWFIQSL
CAMLKQYADKLEFMHILTRVN
RKVATEFESFSFDATFHAKKQIPCIVSMLTKELYFYH

Protein Name: CASPASE-9
Alternative name(s): Apoptotic protease Mch-6; Apoptotic protease- activating factor 3
Swissprot ID: P55211
Organism: ***Homo sapiens*** **(Human)**

FASTA Sequence:
>sp|P55211|CASP9_HUMAN Caspase-9 OS=Homo sapiens GN=CASP9 PE=1 SV=3
MDEADRRLLRRCRLRLVEELQVDQLWDALLSRELFRPH
MIEDIQRAGSGSRRDQARQLII
DLETRGSQALPLFISCLEDTGQDMLASFLRTNRQAAKLSK
PTLENLTPVVLRPEIRKPEV
LRPETPRPVDIGSGGFGDVGALESLRGNADLAYILSMEPC
GHCLIINNVNFCRESGLRTR
TGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLALLEL
AQQDHGALDCCVVVILSHGCQ
ASHLQFPGAVYGTDGCPVSVEKIVNIFNGTSCPSLGGKPK
LFFIQACGGEQKDHGFEVAS
TSPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIF
VSYSTFPGFVSWRDPKSG
SWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQ
MPGCFNFLRKKLFFKTS

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
MDDDIAALVVDNGSGMCKAGFAGDDAPRAVFPSIVGRP
RHQGVMVGMGQKDSYVGDEAQS
KRGILTLKYPIEHGIVTNWDDMEKIWHHTFYNELRVAPE
EHPVLLTEAPLNPKANREKMT
QIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDSGDGV
THTVPIYEGYALPHAILRLDL
AGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVA
LDFEQEMATAASSSSLEKSY
ELPDGQVITIGNERFRCPEALFQPSFLGMESCGIHETTFNSI
MKCDVDIRKDLYANTVLS
GGTTMYPGIADRMQKEITALAPSTMKIKIIAPPERKYSVW
IGGSILASLSTFQQMWISKQ
EYDESGPSIVHRKCF

Retrieval of Ligands 3-D Structure ACD ChemSketch

The **Ascorbic Acid, Betalain** and **Gallic Acid,** (ligands) compounds structure was drawn using ACD ChemSketch and are given in fig. 2.

Docking: ARGUSLAB

The 3-D structure of Caspase-3, Caspase-9, β-Actin were docked with **Ascorbic Acid, Betalain** and **Gallic Acid,** (ligands)

inhibitors using ArgusLab software. The docking results were analyzed using PyMol visualization tool.

In silico docking study revealed the interactions between ligands *viz*., Ascorbic acid, Betalain and Gallic acid with Caspase-3, Caspase-9, and β-Actin protein, respectively by *in silico* molecular docking method in order to calculate the minimum binding energy (kcal/mol) between them (table 1).

The interaction of Ascorbic acid with Caspase-3 forms 4 hydrogen bond with docking score of -7.45; Betalain with Caspase-3 forms 4 hydrogen bond with docking score of -10.14; Gallic acid with Caspase-3 forms 5 hydrogen bond with docking score of -7.80 (Fig. 3).

Table 1: Interactions between ligands *viz*., Ascorbic acid, Betalain and Gallic acid Caspase-3, Caspase-9 and β-Actin protein.

Ligands	Docking score of protein interaction		
	Caspase-3	Caspase-9	B-Actin
Ascorbic acid	-7.45	-7.33	-7.67
Betalain	-10.14	-8.99	-8.46
Gallic acid	-7.80	-8.08	-7.79

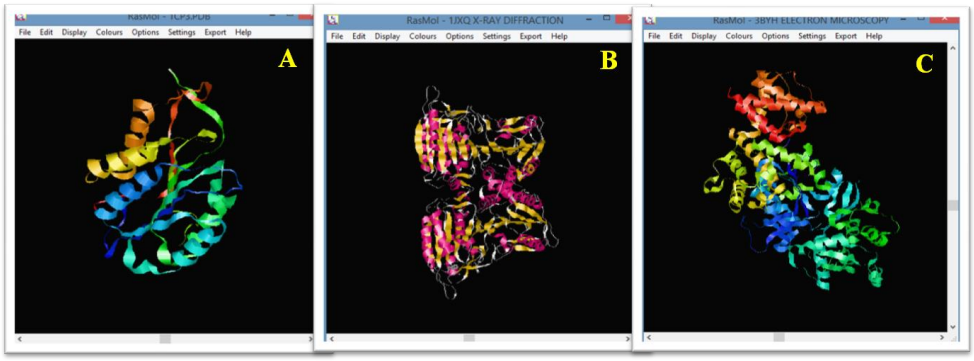

Fig. 1: 3-D structure of proteins (A - Caspase-3, B – Caspase-9, C - β-Actin)

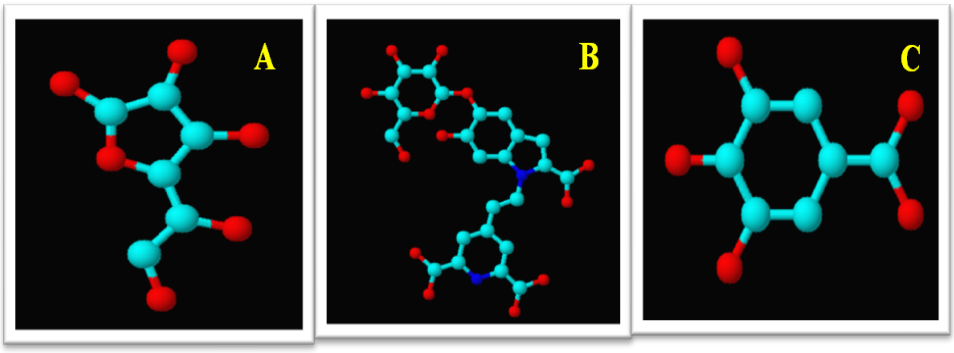

Fig. 2: 3-D structure of proteins (A – Ascorbic Acid, B – Betalain, C – Gallic Acid)

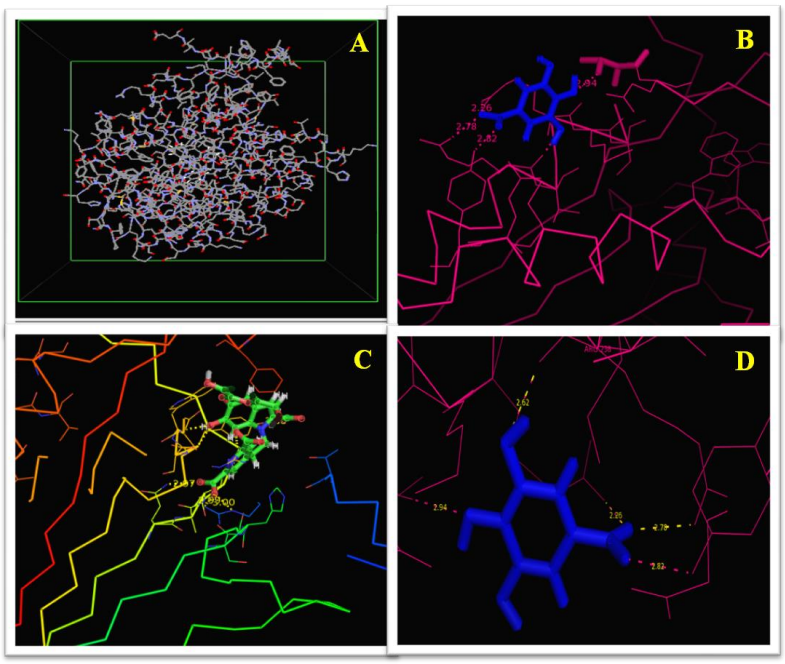

Fig. 3: Visualization of docked complex in PyMol tool Caspase-3 docks with Ascorbic Acid, Betalain and Galic Acid.
A – Caspase-3 Grid setting B – Caspase-3 docks with Ascorbic Acid
C – Caspase-3 docks with Betalain D – Caspase-3 docks with Gallic Acid

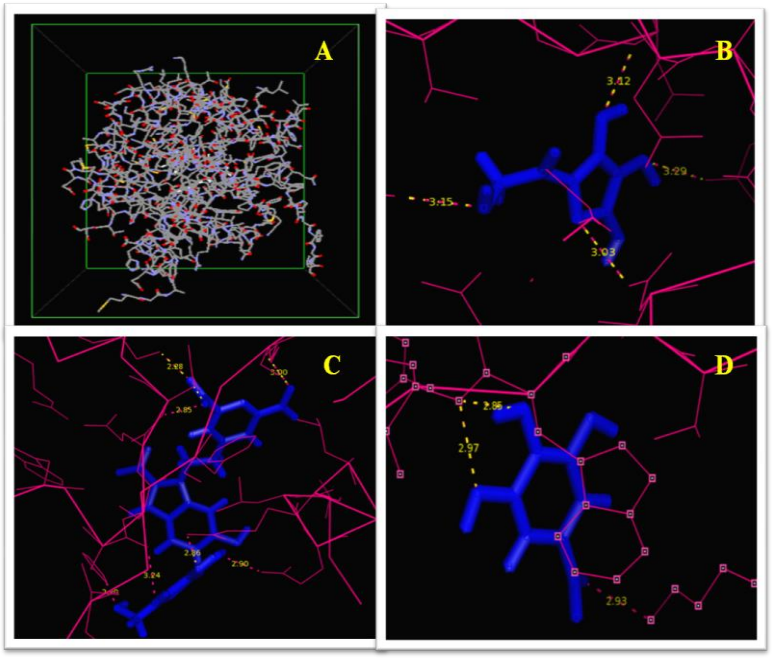

Fig. 4: Visualization of docked complex in PyMol tool Caspase-9 docks with Ascorbic Acid, Betalain and Galic Acid.
A – Caspase-9 Grid setting B – Caspase-9 docks with Ascorbic Acid
C – Caspase-9 docks with Betalain D – Caspase-9 docks with Gallic Acid

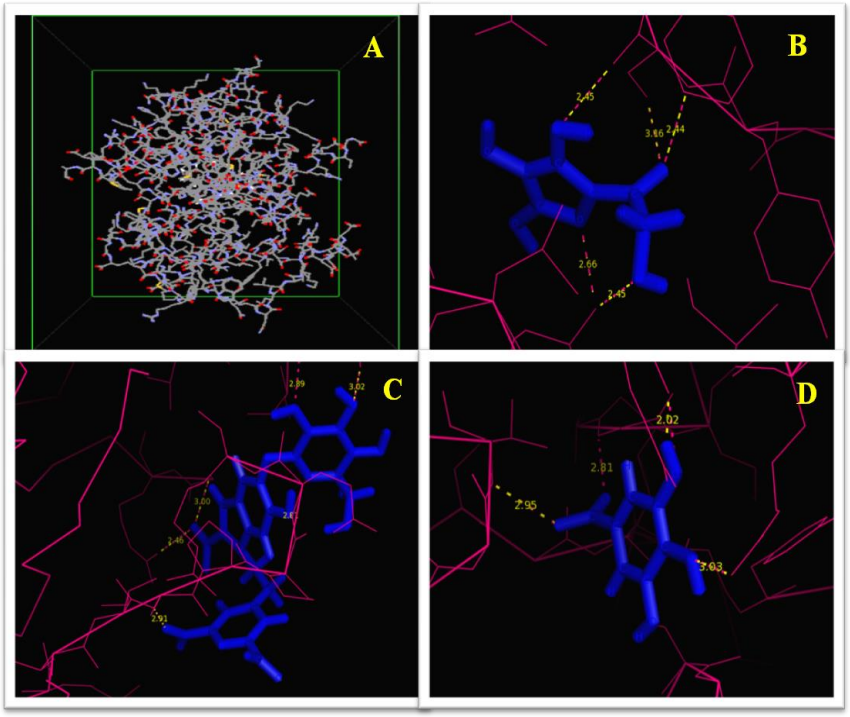

Fig. 5: Visualization of docked complex in PyMol tool β-Actin docks with Ascorbic Acid, Betalain and Galic Acid.
A – β-Actin Grid setting **B – β-Actin docks with Ascorbic Acid**
C – β-Actin docks with Betalain **D – β-Actin docks with Gallic Acid**

Likewise, the interaction of Ascorbic acid with Caspase-9 forms 4 hydrogen bond with docking score of -7.33; Betalain with Caspase-9 forms 7 hydrogen bond with docking score of -8.99; Gallic acid with Caspase-9 forms 3 hydrogen bond with docking score of -8.08 (Fig. 4).

Similarly, the interaction of Ascorbic acid with β-Actin forms 5 hydrogen bond with docking score of -7.67; Betalain with β-Actin 6 forms hydrogen bond with docking score of -8.46; Gallic acid with β-Actin forms 4 hydrogen bond with docking score of -7.79 (Fig. 5).

This result shows that there is a presence of binding site between these three proteins and three ligands. The docking is also valid by the formation of hydrogen bond between them. From the above docking results, it is pragmatic that the ligands docks well to these proteins responsible for disease. Among the three ligands, Ascorbic Acid recorded a less docking score with Caspase-3, Caspase-9 and β-Actin. Hence, Ascorbic acid can be taken as the best ligand among the three. 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.

The exact mechanism should be further investigated in future studies and to elucidate the medicinal properties of *H. undatus*, especially the active component *viz.*, ascorbic acid, betalains and gallic acid, there is a need for further investigation that will pave a way for finding this herbal resource as a medicine to control hepatocellular carcinoma as well as for different types of cancers in future. *In silico* docking study in our case revealed the interactions between ligands *viz.*, Ascorbic acid, Betalain and Gallic acid, and Caspase-3, Caspase-9, β-Actin protein, respectively by *in silico* molecular docking method in order to calculate the minimum binding energy (kcal/mol) between them. This result showed that there was a presence of binding site between these three proteins and three ligands. The docking was also valid by the formation of hydrogen bond between them. Similar docking studies were also done by Wellington and Hayden, (2000)[16], Mohr and Zwacka (2007)[17], Jhansi Lakshmi *et al*. (2009)[18], Manimaran *et al*. (2015)[19], Muthukala *et al*. (2015)[20], Rajesh *et al*. (2016)[21], Jayameena *et al*. (2018)[22] and Flora Priyadarshini *et al*. (2018)[23], which supports the results if the present work.

CONCLUSION

The results reveal that the *H. undatus* fruit has a promising potential anticancer and anti-apoptotic agent for cancer therapy. The potential drug candidate can further be validated by wet lab studies for its proper function.

ACKNOWLEDGEMENTS

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

REFERENCES

1. Kitchen DB, Decornez H, Furr JR, Bajorath J. Docking and scoring in virtual screening for drug discovery: Methods and applications. Nat. Rev. Drug. Discov, 2004; 3: 935-949.
2. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr. Opin. Stru. Biol, 1996; 6: 402-406.
3. Ferreira LC, dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. Mol, 2015; 20: 13384-13421.
4. Jain AN. Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. J. Med. Chem, 2003; 46: 499-511.
5. Kellenberger E, Rodrigo J, Muller P, Rognan D. Comparative evaluation of eight docking tools for docking and virtual screening accuracy. Prot. Stru. Func. Bioinform, 2004; 57(2): 225-242.

6. Esquivel P, Stintzing F, Carle R. Phenolic compound profiles and their corresponding antioxidant capacity of purple pitaya (*Hylocereus sp*.) genotypes. Nat. Sci, 2007; 62: 636-644.
7. Ruzlan N, Kamarudin KR, Idid SO, Idid SZ, Mohamed Rehan A, Koya MS. Antioxidant study of pulp and peel of dragon fruits: A comparative study. Phytother. Res, 2008; 22: 512-518.
8. Wybraniec S, Mizrahi Y. Fruit flesh betacyanin pigments in *H. cacti*. J. Agr. F. Chem, 2002; 50(21): 6086-6089.
9. Abd Hadi N, Mohamad M, Rohini MAK, Mohd Yusof R. Effects of red pitaya fruit (*H. polyrhizus*) consumption on blood glucose level and lipid profile in type 2 diabetic subjects. Borneo Sci. J, 2012; 31(2): 113-129.
10. Song H, Zheng Z, Wu J, Lai J, Chu Q, Zheng X. White pitaya (*H. undatus*) juice attenuates insulin resistance and hepatic steatosis in diet-induced obese mice. Plos One, 2016; 11(2): 312-328.
11. Suryono J. Consuming dragon fruit to treat various diseases. Sinar. Tain, 2006; 15-21.
12. Greenwald P, Milner JA, Anderson DE, McDonald SS. Micronutrients in cancer chemoprevention. Can. Metast. Rev, 2002; 21(3, Supply 4): 217-230.
13. Milner JA. Molecular targets for bioactive food components. J. Nut, 2004; 134(9): 2492-2498.
14. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. Digest. Liver Dis, 2007; 39(4): 293-304.
15. Lopez-Lazaro M. Anticancer and carcinogenic properties of curcumin: Considerations for its clinical development as a cancer chemopreventive and chemotherapeutic agent. Mol. Nut. F. Res, 2008; 52(1): 103-127.
16. Wellington CL, Hayden MR. Caspases and neurodegeneration: On the cutting edge of new therapeutic approaches. Clin. Gen, 2000; 57(1): 1-10.
17. Mohr A, Zwacka RM. *In situ* trapping of initiator caspases reveals intermediate surprises. Cell Biol. Int, 2007; 31(5): 526-530.
18. Jhansi Lakshmi P, Suneel Kumar BVS, Nayana RS, Srinivas Mohan M, Bolligarla R, Das SK, et al. Design, synthesis, and discovery of novel non-peptide inhibitor of Caspase-3 using ligand based and structure based virtual screening approach. Bioorg. Med. Chem, 2009; 17(16): 6040-6047.
19. Manimaran M, Sivakumari K, Ashok K. Molecular docking studies of resveratrol against the human oral cancer cell line proteins (KB cells). Int. J. Curr. Ad. Res, 2015; 4(10): 275-280.
20. Muthukala B, Sivakumari K, Ashok K. *In silico* docking of Quercertin compound against HeLa cell line proteins. Int. J. Curr. Pharmaceu. Res, 2015; 7(1): 13-16.
21. Rajesh S, Sivakumari K, Ashok K. *In silico* docking of selected compound from *C. halicacabum* Linn. leaf against human hepatocellular carcinoma (Hep-G2) cell line. Int. J. Comp. Bioin. In Silico Model, 2016; 5(2): 780-786.
22. Jayameena P, Sivakumari K, Ashok K, Rajesh S. *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). JCRT. 2018; 6(2): 28-33. doi: 10.12691/jcrt-6-2-1.
23. Flora Priyadarshini J, Sivakumari K, Ashok K, Jayaprakash P, Rajesh S. GC-MS analysis for identification of active compounds in propolis and molecular docking studies of selected compounds against apoptotic proteins (Caspase-3, Caspase-9 and B-Actin). J. Biol. Chem. Research, 2018; 35(2): 349-358.

How to cite this article:

Karthika S *et al*. In silico molecular prediction of ascorbic acid, betalain and gallic acid from *Hylocereus undatus* against apoptotic proteins (Caspase-3, Caspase-9 and β -actin). J Pharm Sci Innov. 2018;7(6): 215-220.
http://dx.doi.org/10.7897/2277-4572.076110

Source of support: Nil, Conflict of interest: None Declared