Asian Journal of Research in Medical and Pharmaceutical Sciences

3(4): 1-14, 2018; Article no.AJRIMPS.41129
ISSN: 2457-0745

Target Level (Ligand/Receptor Based) Analysis of Anti-oxidant Enzymes Modulating Activity of Phytochemicals of *Dillenia pentagyna*

Mohammad Nadeem Khan[1*], Hena Kaushar[1], Jennifer Qureshi[1] and Nitesh Pusham[1]

[1]Department of Biotechnology, Bastar University, Jagdalpur (C.G.)-494001, Chhattisgarh, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MNK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors HK and JQ managed the analyses of the study. Author JQ managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2018/41129
Editor(s):
(1) Somdet Srichairatanakool, Professor, Department of Biochemistry, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200,Thailand.
Reviewers:
(1) Paula Mendonça Leite, Universidade Federal de Minas Gerais, Brazil.
(2) Fatma Kandemirli, Kastamonu University, Turkey.
(3) B. C. Revanasiddappa, Nitte Deemed to be University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/24689

Original Research Article

Received 7th March 2018
Accepted 16th May 2018
Published 19th May 2018

ABSTRACT

Antioxidants play a very important role in alleviating problems related to oxidative stress. They affect beneficial metabolic and cellular processes and also play key role in pathological conditions of the body. It is normally balanced by endogenous antioxidant system like a modulating in enzymatic activity of super oxide dismutase, catalase and Glutathione peroxidase. Present study was aimed at assessing the *in-silico* based drug development of modulating antioxidant enzyme activity with the help of known compounds of *Dillenia pentagyna.* Draw and energy minimization and simulation of phytochemicals of 2D, 3D structure of *Dillenia pentagyna* (Eleven) were docked with that of antioxidant enzymes by PyRx (AutoDock) and Discovery studio 3.1 version. All Phytochemicals were bound with all three anti-oxidant enzymes. The results show that all phytochemical compounds possessed potential agonist characteristics that is capable of enhance the anti-oxidant enzymes activity that is first line of defense against the reactive oxygen species. Among these two

**Corresponding author: E-mail: sahani.nadeem35@gmail.com;*

compounds that code was Dillnia-5 ,6 showed better affinity towards SOD and CAT with docking score -8.4, -7.6 and -8.0,-8.0 respectively. The phytocompound code dilinia-8 highest docking scores -7.0 respectively. Therefore, we can infer that Dillnia-5 and Dillnia -6 and Dillinia-8 have direct affinity towards SOD and hence these lead molecules activate SOD and CAT, GPx enzymes. Endogenous oxidant scavenging enzymes were act as oxidative stress balancing. Further studies are needed to prove its mechanisms *in vitro* condition.

Keywords: Antioxidant enzymes; dilligin; dipnoic acid; molecular docking; simulation.

1. INTRODUCTION

During the last few decades, there has been interest in identifying metabolites from plants that can exert beneficial effect some human health [1]. Among the some metabolites, the antioxidants or free radical scavengers have received special attention for their pharmacological potential [2]. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others [3]. Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems that include a variety of enzymatic scavengers, such as Superoxide dismutase (SOD), Glutathione peroxidase (GPX) and Catalase (CAT). Superoxide dismutase catalyzes the superoxide into oxygen and hydrogen peroxide [4]. This hydrogen peroxide will be decomposed as water and oxygen molecule, in presence of catalase and glutathione peroxidase (Fig. 1) [5]. The free radical neutralizing property of several plants was reported by previous studies. The extracts from number of medicinal plants which are known to have some biologically active principles are used in ayurvedic preparations and these extracts are prepared in bulk for commercial purpose [6] a wide range of pharmacological and biological activities was exhibited by the secondary metabolites isolated from plants belonging to *Dillenia pentagyna*. Among them terpenoids and flavonoids were the most well known, though a large amount of alkaloid was also searched [7] Betulinic acid was isolated from *Dillenia indica* & D. return, 4 which exhibited high antitumor activity [8]. Two new compounds dihydro-isorhamnetin from the stem bark and dillenetin from the pericarp of *Dillenia indica* have been isolated. 4-rhammentin 3-glucoside also isolated from the Dillenia pentagyna. 3 Two new flavonoid glycosides, naringenin 7- galactosyl and dihedral quercetin 5-glactoside were isolated from D. pentagyna have been found to exhibit cytotoxic and lymphocytic activity.6 A new diterpene, dipoloic acid isolated from the stem of Dillenia pentagyna exhibited cytotoxic activity [9]. Costunolide, a sesquiterpene lactone, present in most of the medicinal plants has been identified as an active principle responsible for the medicinal property of

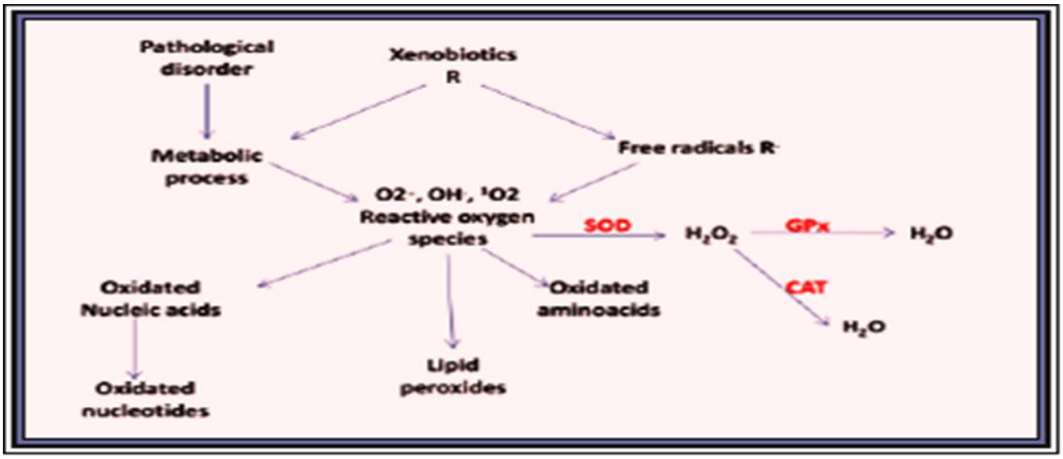


Fig. 1. Pathway in which SOD, CAT and Gpx are involved (Navrot, et al, 2007)

the plants. There are no relative studies on the effect of costunolide as antioxidant agent on human breast cancer. Hence, the present study was attempted to determine the antioxidant enzymes potential to phytochemicals of *Dillenia pentagyna*, through analyzing the key antioxidant enzymes involved in scavenging free radicals. To best of our knowledge, the receptor-level mechanism behind this process is no where mentioned. Present study was aimed at the analysis of receptor-level binding affinity of Dillenia phytochemicals with SOD, CAT and GPx through in-*silico* approaches.

2. MATERIALS AND METHODS

2.1 PDB or SWISS-PORT

PDB (Protein Data Bank) is the single worldwide archive of structural data of biological macro molecules, established in Brookhaven National Laboratories. It contains structural information of the macromolecules determined by the X-ray crystallographic and NMR methods. 3D structure of SOD, CAT and GPX were taken from PDB, whose PDB ids are 1CB4, 2CAG, 2P31 respectively [10].

2.2 ACD LABS (Chem Sketch)

Three dimensional structure of inhibitors-CHEMSKETCH: The Chemical structure of *Dillenia pentagyna* phytochemicals were drawn using Chem Sketch (Chem Sketch–www.acdlabs.com/download/), a quite powerful chemical structure drawing program [11].

2.3 Argus Lab

Ligand drug potential ability was done with the ARGUSLAB software (ArgusLab–www.arguslab.com/), in which the result is being obtained on the basis of pose energy. Before docking a molecule, first it is needed to define the atoms that make up the Ligand like drug, inhibitor, etc., and the Binding Site on the protein where the drug binds. The final results are based on the type of calculation we run such as Geometry optimization-search for 'Final Geometry' and Electronic spectra-search for 'Excited state properties' [12].

2.4 Discovery Studio

Structure of Dillenia phytochemicals retrieve with saved MOL file format were upload in Discovery studio 3.1 version. Hydrogen bonds were added and energy was minimized using CHARMm force field. Further, docking analysis studies also carried out using Discovery studio 3.1 versions [13].

2.5 Docking

The protein atoms were typed using the CHARMm force field. The active site of the protein was first identified and defined using an eraser size 10.0 Aº. Then the ligands were docked into the active site using Pyrx (Autodock) dock procedure. Affinity of binding energy score, absolute energy were obtained from the study [14].

3. RESULTS

Fig. 2 shows the 3D (different) x-ray crystallographic model of SOD, CAT and GPx receptors, which were downloaded from PDB. Dillenia phytocompounds 2D & 3D model structure draws and optimized stchiometric and totameric with using of *ACD labs* software. The proposed Dillenia phytocompounds structure organized in Fig. 3. Ligand (phytocompounds) physical specification and its IUPAC name were given in Table 2. The energy simulation model of phytochemicals like lower occupied, highest occupied and ESP model mapping and its potential geometry, depicted in Figure-4. After docking, the highest docking score given phytocompounds selected and studied their binding pocket and binding domain with compounds bound exactly at the active site of SOD, CAT and GPx anti-oxidant enzymes, which was shown in Fig. 5a,5b,5c. A careful inspection of the binding pocket indicated that the compounds at the Cu-Zn domain of SOD heme domain of catalase and selenium domain and GPx metal binding domain. A close view of hydrogen bond interaction has been viewed in 2D view. Docking details for *Dillenia pentagyna* Phytochemicals were tabulated in Table 3 respectively.

4. DISCUSSION

Discovery of pharmacological active ingredients (API) from natural products have gained enormous importance in the field of drug discovery. Demand for natural antioxidant has been increasing due to concerns about safety of synthetic antioxidants [15]. Drug discovery from plants involves a multidisciplinary approach combining botanical, ethno-botanical,

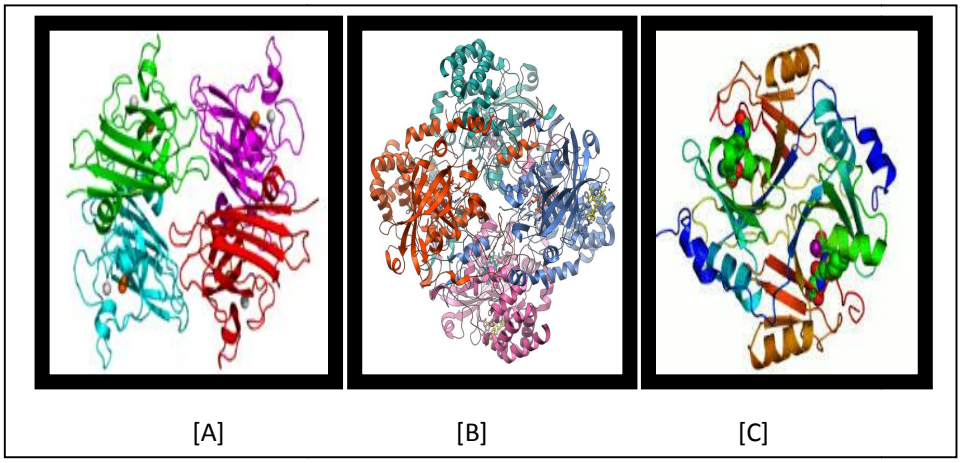


Fig. 2. X-ray crystallographic structure of (A) Superoxide dismutase (SOD), B) Catalase (CAT), C) Glutathione peroxidase (GPx)

phytochemical, and biological techniques. Drug discovery typically starts with an analysis of binding sites in target proteins, or an identification of structural motifs common to active compounds. I*n silico* molecular docking is one of the most powerful techniques to discover novel ligand for receptors of known structure and thus play a key role in structure based drug design. Molecular docking continues to hold great promise in the field of computer based drug designing which screens small molecules by orienting and scoring them in the binding site of a protein [16]. The docking process involves the prediction of ligand confirmation and orientation (posing) within targeted binding site and their interaction energies were calculated using the scoring functions. Oxidative stress, defined as an imbalance between oxidants and antioxidants, leads to any biochemical changes and acts as the causative factor for many diseases like diabetes, atherosclerosis, cardiovascular problems etc. In order to find the mechanism of action behind this process, we have taken these receptors and compounds and carried out docking process [17]. It was clear from Fig. 1 that in the free radical scavenging cascade SOD comes in the first position. Its activation will further activate CAT and GPx. Here, docking studies demonstrated that Dillnia pentagyna phytochemicals docked with SOD, CAT and GPx. As a result of docking, different conformations were generated for several compounds with SOD CAT and GPx. But only for the top ranked docked complex, the scores were copied from the table browser view of Discovery studio for binding affinity analysis. To correlate the biological activity of the receptor and site-directed docking of ligands, here we used binding affinity (-kcal/mol) as dock score (which is PLP like score (stericand H-bonding intermolecular functions, Higher PLP scores indicate stronger receptor-ligand binding)) [18]. The docked results further more explains that the ligands were fit with a specific optimal orientation exactly to the active site cavities of the receptor. In this study, docking score of phytochemicals were -7.9 and -8.0 respectively. A higher score indicates a stronger receptor- ligand binding affinity. The scoring functions have been used to estimate ligand-binding affinity to screen out active and inactive compounds during the process of virtual screening with *Argus LAB* software [19], when biological activities were compared the scoring functions, dillnia-8 showed good binding affinity to the receptor SOD than Dillnia-6. Previous work has also shown that a correlation does exist between binding affinities and dock scores [20]. To ensure that the ligand orientation obtained from the docking studies were likely to represent valid and reasonable binding modes of the inhibitors, the PyRx (Autodock) program parameters had to be first validated for the crystal structure's active site. Protein utilities and health protocol of Discovery studio3.1 version have used to find out the active sites in the structure and it was found that the active site contains amino acids such as His 48, His 46, SO41, His 61, His 63 and His 120. Docking results showed that binding affinity (-ΔG) dock determined the optimal orientation of the docked inhibitor, exactly to the active site. It is also reported that His 46, His 48 and his 120 are important residues for the activity of Superoxide dismutase to function as an antioxidant [21].

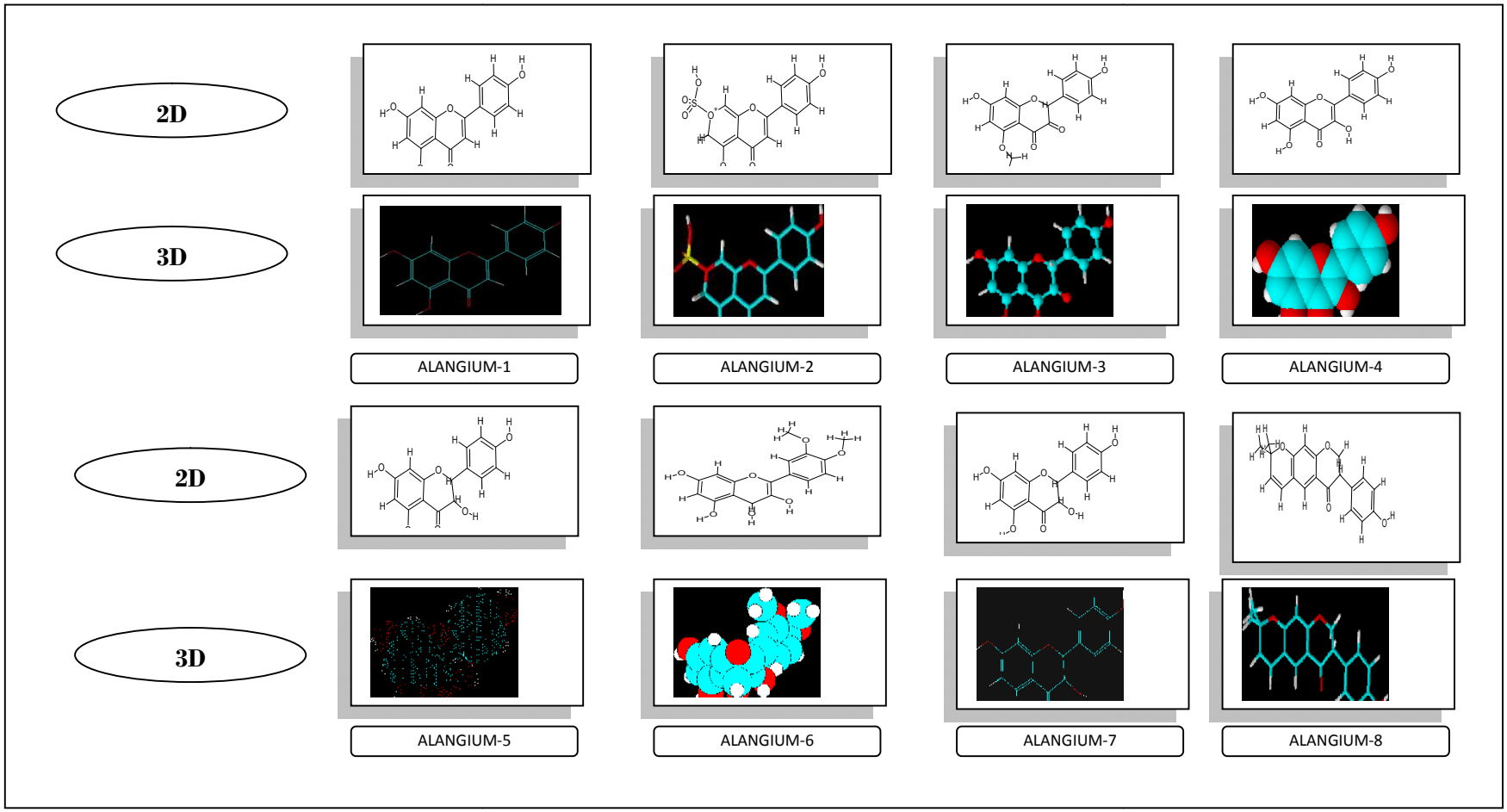


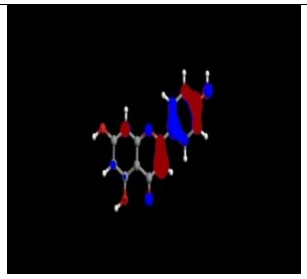
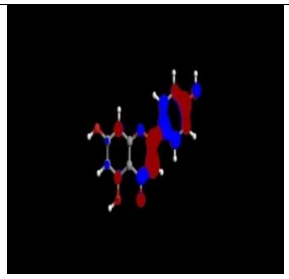
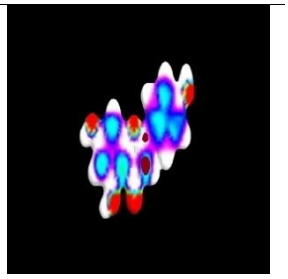
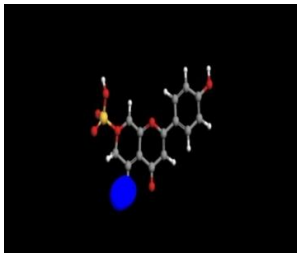
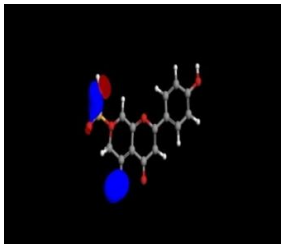
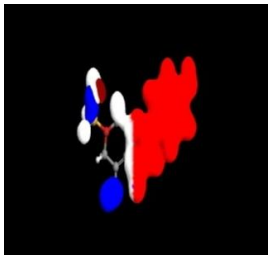
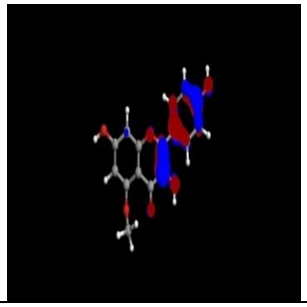
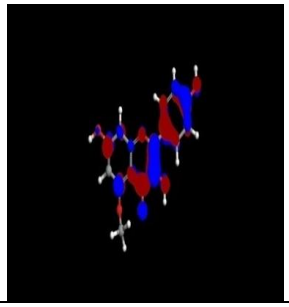
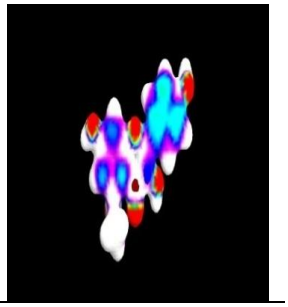
Fig. 3. 2D and 3D diagram of *Dillinia pentagyna*

Draw and optimization of active molecular structure of phytochemicals of *Dillnia pentagyna* by ACD labs *(Chemsketch)*

In Fig. 5a the space-fill portion represented the functional part of the receptor which was known as Cu-Zn domain. Both Dillnia-5 and Dillnia-6 (Fig. 5a) bound at the Cu-Zn domain of the receptor [21], Stated that the most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy poses predicted by the docking score. By binding these two active phytocompounds with SOD receptor, the energy values were found to be minimum in dillnia5 (-7.6) and maximum in Dillnia-6 (-8.0). We analyzed the hydrogen bond interaction of the receptor with Dillnia-5 and Dillnia-6, 8. A close view of the binding interactions of the receptor with compounds was shown in Fig. 5a, 5b, 5c. As shown in Fig. 5a, there are two hydrogen bonds (shown in green dotted lines) formed between the receptor and Dillnia-5,6. The residue involved in forming hydrogen bonds with the compound was His61. Dillnia-8 did not form any hydrogen bond interaction with the receptor. The detailed atoms, which forming the hydrogen bonds are given Table 1, which may provide useful information for in- depth understanding in binding mechanism of the compound to the active site(pocket pose) of the protein. Hydrogen bond formation also makes important contributions to the interaction between ligand and the receptors [22].

Table 1. Specification related Ligand (Argus Lab)]

S. no	Specification	Dillenia 1	Dillenia 2	Dillenia 3	Dillenia 4	Dillenia 5	Dillenia 6
1	SCF	-134.35	-103.06	-151.81	-146.11	-147.15	-170.45
2	GEOMETRY	-134.44	-103.08	-151.92	-146.12	-147.26	149
3	UV	-151.75		-171.10	-163.95	-165.66	

S. no	Ligand	Highest	Lowest	ESP
1	Dillenia 1			
2	Dillenia 2			
3	Dillenia 3			

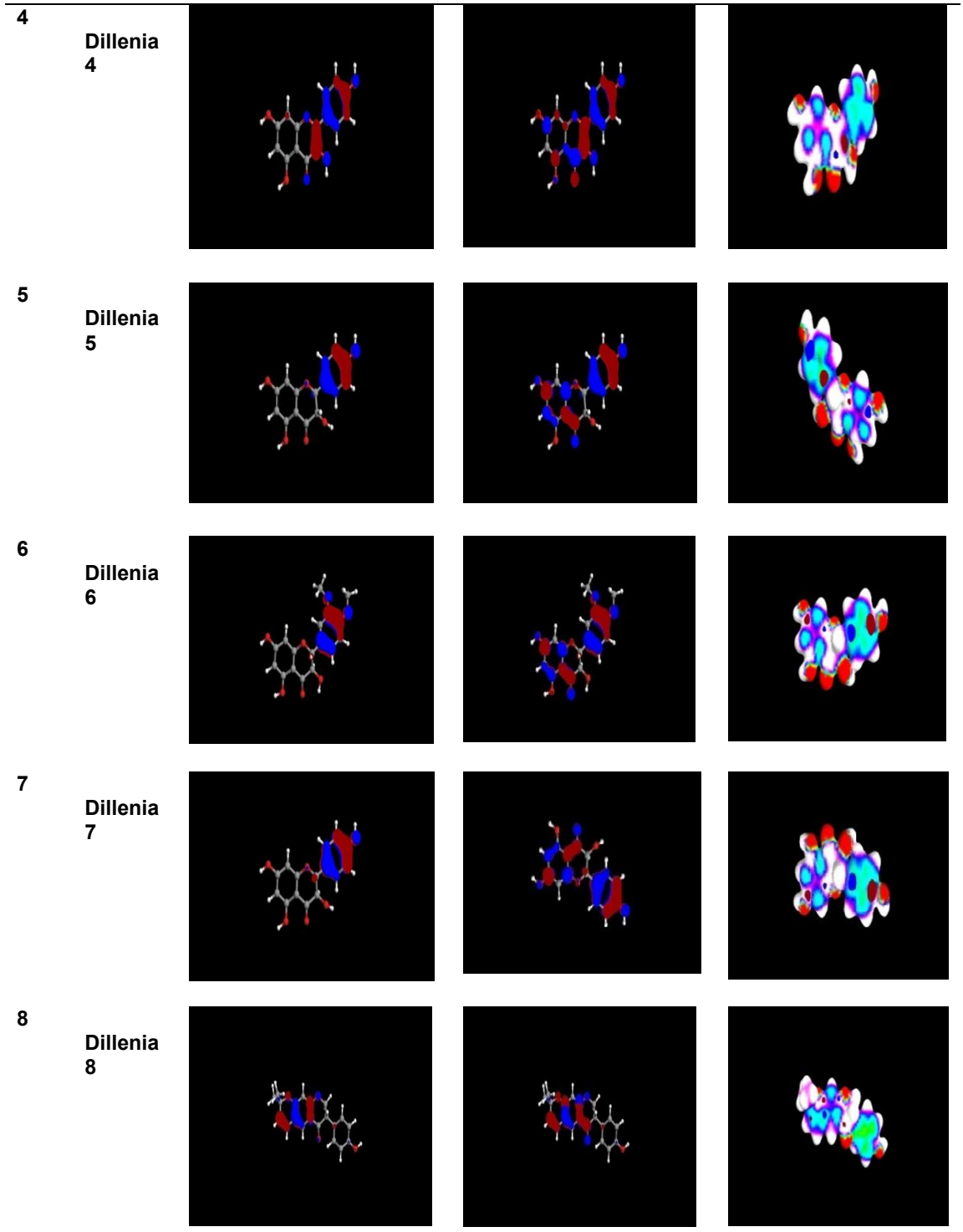


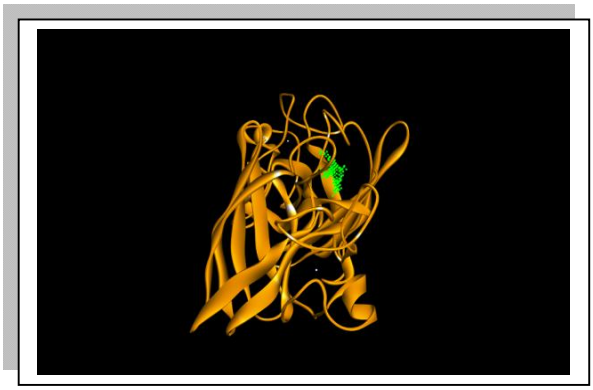
Fig. 4. lead molecule energy simulation and drug like molecule (pharmacophore structure) originated with help of Argus lab (www.Arguslabs.org)

Table 2. General Properties of phytochemicals obtained from DILLENIA

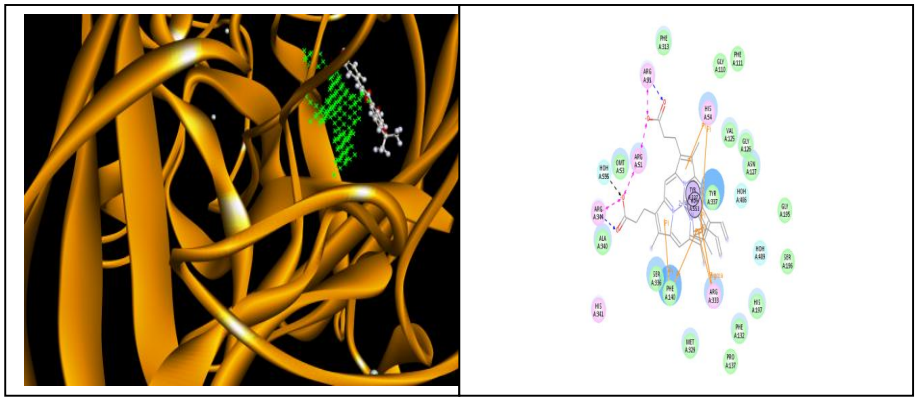
S.no	Properties	Dillenia 1	Dillenia 2	Dillenia 3	Dillenia 4	Dillenia 5	Dillenia 6	Dillenia 7	Dillenia 8
1	Name of chemicals	5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one	5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-7-sulfo-4,6-dihydropyrano[3,4-*b*]pyran-7-ium	(2*R*)-7-hydroxy-2-(4-hydroxyphenyl)-5-methoxy-2*H*-1-benzopyran-3,4-dione	3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one	(2*S*,3*S*)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one	(4*R*)-2-(3,4-dimethoxyphenyl)-4*H*-1-benzopyran-3,4,5,7-tetrol	(2*R*,3*R*)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)-2,3-dihydro-4*H*-1-benzopyran-4-one	(7*S*)-7-(4-hydroxyphenyl)-2,2-dimethyl-7,8-dihydro-2*H*,6*H*-benzo[1,2-*b*:5,4-*b'*]dipyran-6-one
2	Molecular formula	$C_{15}H_{10}O_5$	$C_{14}H_{11}O_8S+$	$C_{16}H_{12}O_6$	$C_{15}H_{10}O_6$	$C_{15}H_{12}O_6$	$C_{16}H_{12}O_6$	$C_{20}H_{18}O_4$	$C_{20}H_{16}O_5$
3	molecular weight	270.23	339.29	300.26	286.23	288.25	300.26	322.30	336.33
4	Composition	-	-	-	-	-	-	-	-
5	Molar refractivity	-73.99	79.36	76.24	76.01	72.73	85.16	72.73	91.75

Table 3. Mean values of docking energies (kcal/mol) and standard deviation for each skeletal type of *Dillinia pentagyna* phytochemicals as liagands with anti-oxidant enzymes enzyme targets

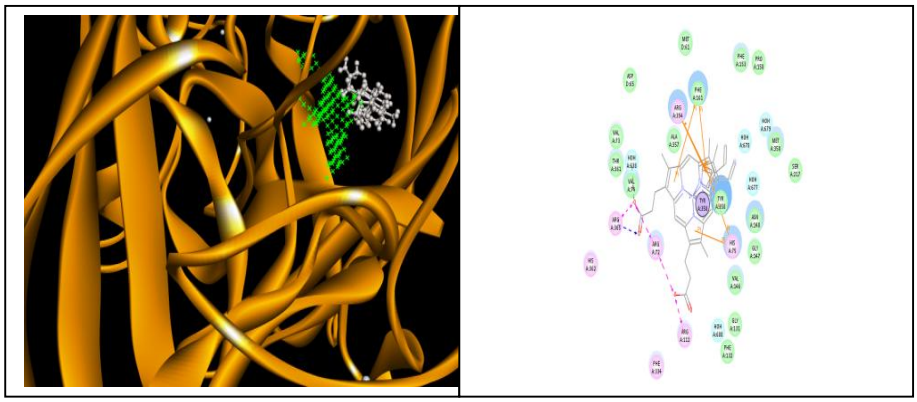
Target	Ligands	Dimession Centre(x=25Ay=25z=25	No of pose	RSD %lower	RSD %upper	Mean binding energy
SOD	Dillinia1	X=48.8446,Y=101.718, Z=38.2861	9	153.21%	106.28%	-7.1
	Dillinia2		9	46.86%	58.47%	-7.7
	Dillinia3	X=48.8446,Y=101.718, Z=38.2861	9	55.13%	68.88%	-7.8
	Dillinia4		9	95.46%	89.70%	-6.5
	Dillinia5		9	139.47%	113.931%	-8.0
	Dillinia6		9	140.19%	81.65%	-7.6
	Dillinia7		9	130.98%	79.14%	-7.2
	Dillinia8		5	59.26%	65.16%	-6.7
CAT	Dillinia1	X=48.8446,Y=101.718, Z=38.2861	9	54.11%	49.87%	-8.0
	Dillinia2		9	58.71%	48.14%	-7.9
	Dillinia3		9	52.21%	47.02%	-7.7
	Dillinia4		9	95.46%	89.70%	-6.5
	Dillinia5		9	48.98%	40.70%	-8.0
	Dillinia6		9	40.96%	66.24%	-8.0
	Dillinia7		9	130.98%	79.14%	-7.2
	Dillinia8		9	59.26%	42.85%	-8.3
GPx	Dillinia1	X=-12.041, y=22.8816, Z=9.2389	7	60.99%	68.44%	-5.3
	Dillinia2		5	64.21%	72.28%	-5.3
	Dillinia3		9	45.89%	52.95%	-5.1
	Dillinia4		5	60.57%	83.28%	-5.4
	Dillinia5		9	91.51%	65.87%	-6.4
	Dillinia6		9	79.47%	74.74%	-6.5
	Dillinia7		9	77.69%	66.89%	-6.7
	Dillinia8		9	67.20%	58.09%	-7.0



Superoxide dismutase

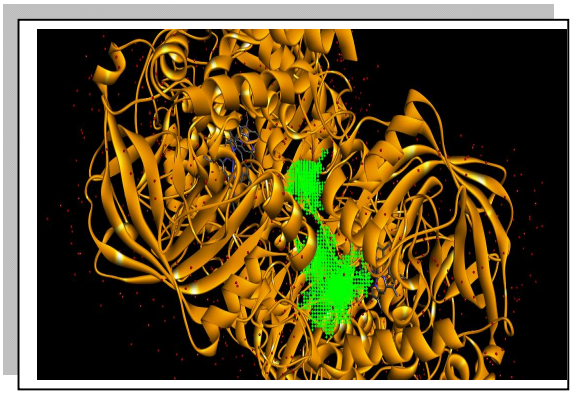


Dillnia phytocompound-5

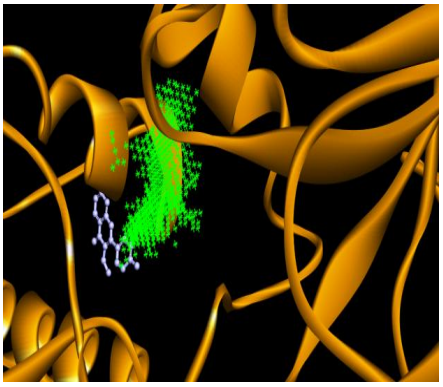


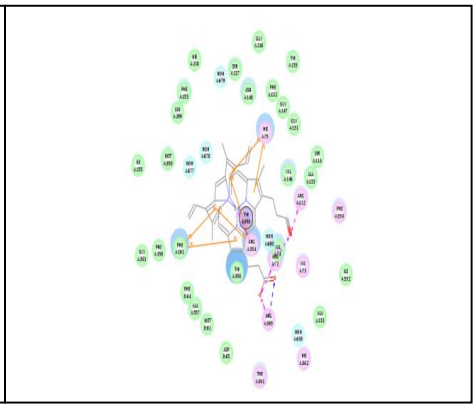
Dillnia phytocompound-6

Fig. 5a. Visualize binding modes of ligand molecules within the binding pocket of receptor and interactions of top two ligand molecules of Dillenia phytochemicals with super oxide dismutase (SOD) receptor

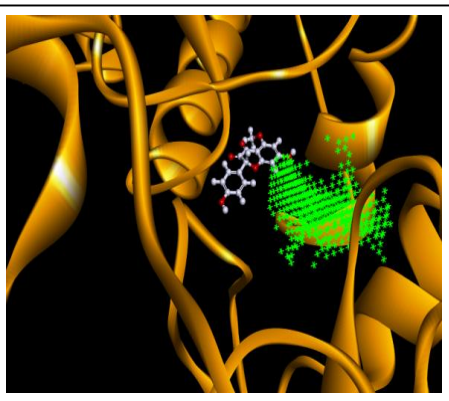


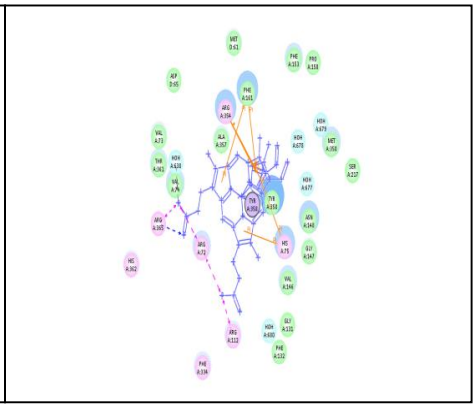
Catalase





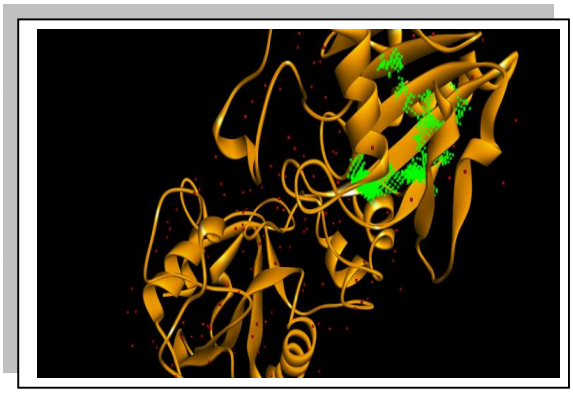
Dillnia phytocompound-5



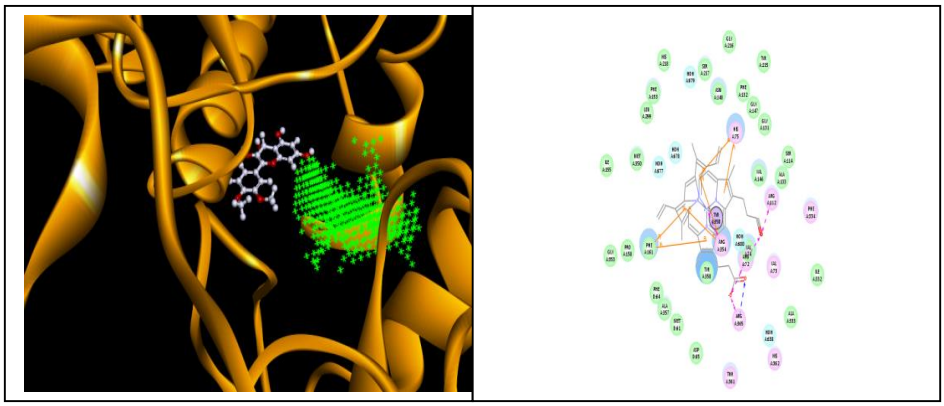


Dillnia phytocompound-6

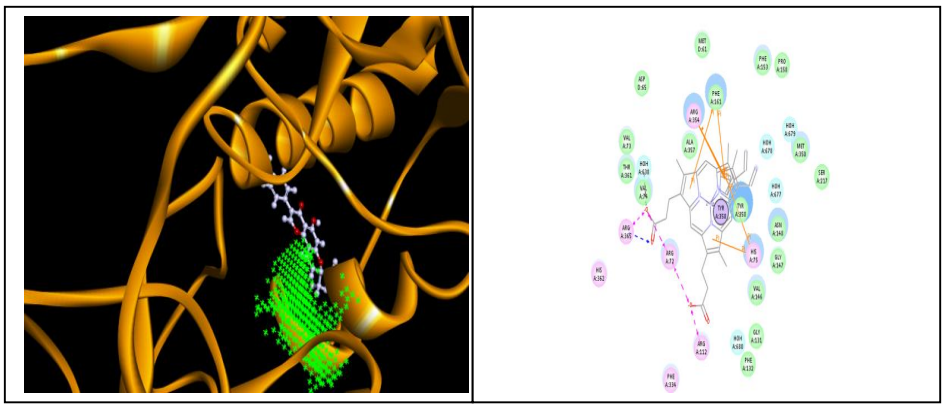
Fig. 5b. Visualize binding modes of ligand molecules within the binding pocket of receptor and interactions of top two ligand molecules of Dillenia phytochemicals with Catalase(CAT) receptor



Glutathione peroxidase



Dillnia phytocompound-7



Dillnia phytocompound-8

Fig. 5c. visualize binding modes of ligand molecules within the binding pocket of receptor and interactions of top two ligand molecules of Dillenia phytochemicals with GPx receptor

5. CONCLUSION

The protein- ligand interaction plays a significant role in structural based drug designing. Based on dock score values it was predicted that both Dillnia-5 and Dillnia-6 have good binding affinity towards superoxide dismutase and catalase also among the all compounds, Dillnia-8 showed better activity with glutathione peroxidase. From these docking studies, hence we conclude that binding of phytochemicals to the metal binding domain of the antioxidant enzymes receptor may lead to increase its activity and reduce oxidative stress. However, this mechanism of prediction requires further *in- vitro* analysis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 2006;30:177-183.
2. Imran M, Khan H, Shah M, Khan R, Khan F. Chemical composition and antioxidant activity of certainmorus species. Journal of Zhejiang University Science B11. 2010;973–980.
3. Brooijmans N, Kuntz ID. Molecular recognition and docking algorithms. Annu rev. Biophys Biomol Struct. 2003; 32:335–373.
4. Del Rio A, Sandalio LM, Corpas FJ, Palma JM, Barroso JB. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol. 2006;141:330e335.
5. Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, et al. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. Biomed Res Int; 2015.
   DOI: 10.1155/2015/265425
6. Douglas B Kitchen, Hélène Decornez, John R Furr, Jürgen Bajorath. Docking and scoring in virtual screening for drug discovery. Methods and Applications. 2004;4:935-949.
7. Dubey PC, Sikarwar RLS, Khanna KK, Tiwari AP. Ethnobotany of *Dillenia pentagyna* Roxb. In Vindhya region of Madhya Pradesh, India. Nat Prod Radiance. 2009;8(5):546-8.
8. Gehlhaar DK, Verkhivker GM, Rejto PA, Sherman CJ, Fogel DB, Fogel LJ, Freer ST. Chem. Biol. 1995;2:317-324.
9. Gohlke H, Klebe G. Curr. Opin. Struct. Biol. 2001;11:231-235.
10. Ha S, Andreani R, Robbins A, Mueggel. J. Comput-Aided Mol. Des. 2000;14:435-448.
11. Hua P, L Lu, L Han-Xing and L Li-Gong. Sporogenesisand gametogenesis in *Sladenia* and their systematic implication. Botanical Journal of the Linnean Society. 2003;143:305-314.
12. John Hart P, Melinda M, Balbirnie, Nancy L, Ogihara, Aram M Nersissian, Manfred S Weiss, et al. A structure based mechanism for Copper-Zinc superoxide dismutase. Biochemistry. 1999;38:2167-2178.
13. Muegge I, Martin YC. J. Med. Chem. 2001;44:2333-2343.
14. Navrot N, Rouhier N, Gelhaye E, Jaquot JP. Reactive oxygen species generation and antioxidant systems in plant mitochondria, Physiol. Plant. 2007; 129.185e195.
15. Rosangkima G, Prasad SB. Anti tumour activity of some plants from Meghalaya and Mizoram against murine ascites Dalton's lymphoma. Ind. J. Exp. Biol. 2004;42:981-8.
16. Eliza J, Daisy P, Ignacimuthu S. Antioxidant activity of costunolide and eremanthin isolated from Costus speciosus (Koen ex. Retz) Sm. Chemico-Biological Interactions. 2010;188:467–472.
17. Sen T, Samanta, SK. Medicinalplants, human health and biodiversity: Abroad review. Adv. Biochem. Eng. Biotechnol. 2015;147:59–110.
18. Srinivasan P, Sudha A, Shahul Hameed A, Prasanth Kumar S, Karthikeyan M. Screening of medicinal plant compounds against NS5B polymerase of hepatitis C virus (HCV) using molecular docking studies. Journal of Pharmacy Research. 2011;4(1):136-140.

19. Sundarapandian SM, Swamy PS. Litter production and leaf-litter decomposition of selected tree species in tropical forests at Kodayar in the Western Ghats, India. Forest Ecology and Management. 1999; 123:231-244.
20. Venkatachalam CM, Jiang X, Oldfield T, Waldman M. J. Mol. Graph. Modell. 2003;21:289-307.
21. Young IS, Woodside JV. Antioxidants in health and disease. J. Clin. Pathol. 2001;54:176-186.
22. Langer T, Hoffmann RD. Virtual screening: An effective tool for lead structure discovery? Curr. Pharm. Des. 2001;7(7):509-27.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24689