Eco. Env. & Cons. 27 (3) : 2021; pp. (1278-1283)
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ISSN 0971–765X

Docking studies on common phytochemicals present in *Punica granatum* peel and *Vitis vinifera* seeds with p53

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(Received 6 December, 2020; Accepted 3 February, 2021)

ABSTRACT

Plants are the major source of traditional medicines against many diseases across the globe. They contain important secondary metabolite known as phytochemicals which provides different bioactive potentials. In this study we have analyzed the interaction of phytochemicals that are present in both *Punica granatum* peel and *Vitis vinifera* seeds against tumour suppressor protein p53. Eight bioactive compounds commonly present in both the selected plant materials 2,3- Dihydro-3,5- dihydroxy-6-methyl-4H-pyran-4-one (DDMP), alpha.-Tocopherol-beta.-D-mannoside gamma-Sitosterol, Glycerin, Guanosine, Pyragallol, palmitic acid and Ethyl palmitate respectively were selected for this *in silico* study. The 3D protein structure of p53 was procured from protein data bank. The chemical structures of bioactive compounds were obtained from Chemspider and drawn using Chemsketch software. This study clearly shows that Pyrogallol interacts with target protein p53 with energy level of -7.52 kcal/mol (3 hydrogen bond). The p53-MDM2 (murine double minute 2) forms interaction with pyrogallol at lowest energy level with -7.52 kcal/mol (3 hydrogen bond). The interaction of anti-oxidant and anti-cancer potential pyro gallol with p53 may play major role in providing chemo preventive property for both *Pomegranate* peel and *Grape* seeds.

Key words : *Punicagranatum, Vitis vinifera, Pyrogallol, MDM2.*

Introduction

Plants were used as medicine in ancient cultures without knowledge of their active ingredients. In recent years interest in the use of medicinal plant is revived due to its high efficacy and less side effects. Plants continue to be an important resource material for preparation of many therapeutic Medicinal agents both in developing and developed countries (Prajapati and Purohit, 2010). A medicinal plant contains compounds which are used for the therapeutic purpose and can also be the precursor for synthesis

of useful drugs. The most important ingredients present in plants are alkaloids, flavonoids, glycosides terpenoids, phenols, tannins and steroids (Abayomi, 1993).

p53 a tumour suppressor gene plays amajor role in human cancer (Vogelstein *et al.*, 2000; Levine, 1997). p53 is aneffective transcription factor that is initiated in response to assorted stresses, leading to apoptosis. Nevertheless, many are thought to have. The inadequate functions of 50% of human tumors expressing wild-type p53 are due to abnormalities in p53 regulation or defective signaling in the p53 pathway (Vogelstein *et al.*, 2000). p53 uses its negative regulator gene product MDM2 as a mechanism for its suppression (Fakharzadeh *et al.*, 1993). In most of the human tumors over production of MDM2 is due to the magnification of a chromosome segment of *mdm2* gene (Oliner *et al.*, 1992; Freedman *et al.*, 1999; Momand *et al.*, 1998). MDM2 is a zinc finger on co-protein which acts as negative regulator of the p53 tumor suppressor protein (Cahilly-Snyder *et al.*, 1987; Fakharzadeh *et al.*, 1991; Oliner *et al.*, 1992; Momand *et al.*, 1992; Finlay *et al.*,1989). Under physiological conditions, MDM2 is destabilized which keeps p53 inactive and maintained very low level (Abayomi, 1993; Wu, *et al.*, 1993; Piette, *et al.*, 1997; Fakharzadeh *et al.*, 1993). The activity of p53 in cancer cells can be achieved back by inhibiting MDM2. It has been well proven the role of MDM2 in p53 regulation and they form an auto regulatory feedback loop and mutually control their cellular levels. As one of its transcription targets p53 binds to the promoter and regulates the expression of the *mdm*2 gene and the expression level rises, it binds and p53 is inactivated by inhibitingp53 transactivation domain and by steering p53 protein for ubiquitin-dependent degradation in proteasome (Freedman *et al.*, 1999; Michael and Oren, 2003).

Pomegranate (Punica granatum) and *Grapes (Vitis vinifera*) are universally important plants placed in *Vitaceae* and *Lythraceae families* respectively. The *Punica granatum peel* is a potent astringent and acts as a cure for diarrhea, used as a mouthwash. The *Vitis vinifera* seeds possess, antimicrobial, antioxidative, anti-inflammatory, cardio protective, hepatoprotective, and neuroprotective effects.

Total of eight compounds were present common in *P.granatum* peel and *V.vinifera* seed extracts (Ashok Kumar and Vijayalakshmi, 2011). and they were selected for docking studies. Glycerin, Pyrogallol, DDMP, Guanosine, Palmitic acid, alpha.-Tocopherol-beta.-D-mannoside, gamma.-Sitosterol and Ethyl palmitate were the eight compounds selected.

Materials and Methods

Database

Protein data bank (PDB)

The 3D structures of target protein p53 (1YCR) was procured from PDB (Figure 1). The 3D structure of p53 transactivation domain bound with MDM2 N-terminal domain was selected for docking studies.

Fig. 1. Structure of p53-MDM2 (1YCR)

Chemspider

The bioactive compounds DDMP, alpha.-Tocopherol-beta.-D-mannoside and gamma.-Sitosterol structures were obtained from chemspider. These compounds (ligands) and their interaction with selected target proteins were analyzed.

Tools used for the studies

Chemsketch

Chem Sketch an online drawing interface that allows to draw almost any chemical structure. The five compounds present in both the extracts namely Glycerin, Guanosine, Pyragallol, palmitic acid and Ethyl palmitate structures were prepared using chemsketch.

Argus Lab

Argus Lab is an online molecular modeling, graphics, and drug design program. This software helps in studying interaction between the target protein and the selected ligands. In this study the selected target p53 docking (interaction) with eight ligands were analyzed.

Pymol

PyMOL an open-source, molecular visualization system is used to visualize the interaction selected ligands with the target protein.

Steps involved in Molecular docking

The 3D structure of the selected target (p53) were procured from PDB. The selected ligands structure was obtained from Chemspider or drawn using Chemsketch. Target proteins interaction with the selected ligands was studied using Argus Lab. The docking analyze was visualized using pymol software. Number of interaction between the Ligand and target protein, Minimal energy for the interaction and atoms involved in the interactions were criteria's used to predict the docking of specific ligand with the target protein.

Results and Discussion

Total of eight compounds present common in both the selected extracts were selected for docking studies.Docking was performed using Argus lab for the eight ligands with both the selected target proteins. The dockingresults were displayed in Table 1.

The interaction of the ligands with the target proteins visualized through pymol was presented in Figures 2 - 9.

The docking studies showed the pyrogallol, guanosine, ethyl acetate, DDMP and glycerin interacts with p53 –MDM2 complex with the hydrogen interaction. Other ligands selected for the docking didn't show any interaction with p53 –MDM2 complex. Out of above five ligands showing interaction, pyro-

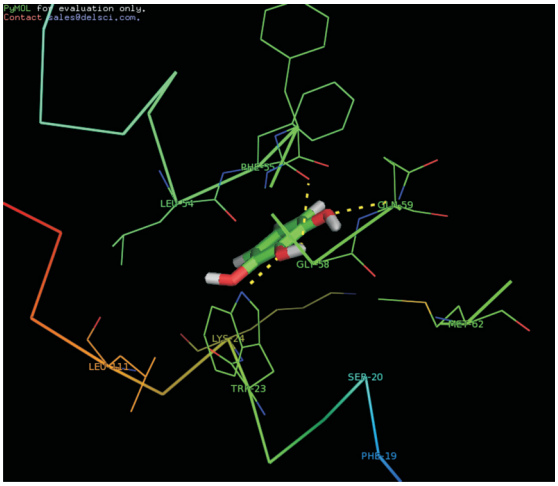

Fig. 2. Interaction of pyrogallol with p53

Table 1. Results of p53-MDM2 docking with the selected ligands

S. No.	Ligand name	Protein residue atom	Ligand atom	Hydrogen bond distance (^{0}A)	No. of Hydrogen bonds	Energy value (Kcal/mol)
1	Pyrogallol	Phe 55 O – H –Trp 23	H	2.8	3	-7.52
		N – H –Gln 59 N – H –	H	2.9		
			O	3.0		
2	Guanosine	Ser 40 N – H –	O	3.0		
		Ser 40 OG– H –	O	2.8	4	-6.58
		Tyr60 OH– H –Lys	O	2.6		
		64 N – H –	O	3.0		
3	Ethyl palmitate	Glu 25 OE1 – H –	O	2.57		
		Glu 52 N – H –	O	2.63	2	-6.31
4	Glycerin	Tyr 76 OH – H	O	2.5	2	
		Tyr 76 OH – H	O	2.6		-6.23
5	2,3-Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one	Arg 97 N – H	O	2.4	2	-5.65
		His 96 N – H	O	2.96		
6	gamma.-Sitosterol	No inter action	No inter action	Nil	Nil	-10.77
7	alpha.-Tocopherol-beta-D-mannoside	No Interactions	No Interactions	Nil	Nil	-9.20
8	Palmitic acid	No inter action	No inter action	Nil	Nil	-7.75

gallol forms interaction with energy value -7.52 kcal/mol with 3 hydrogen bonds and guanosine forms 4 hydrogen bonds with energy value of -6.58 kcal/mol. The docking study clearly shows that pyrogallol forms interaction with the selected target proteins at lower energy level. Guanosine also shows interaction p53.

The use of small molecule inhibitors for disruption of the p53-MDM2 interaction has been confirmed as afeasible and remarkable cancer therapy (Buolamwini *et al.*, 2005). Therefore, this interaction has been potent step on MDM2targeted anticancer drug discovery. The molecules that have potential to disrupt the p53-MDM2 interaction includes synthetic chalcones, norbornane derivatives, *cis*-imidazoline derivatives, apyrazolidinedione sulfonamide and 1,4-benzodiazepine-2,5-diones.

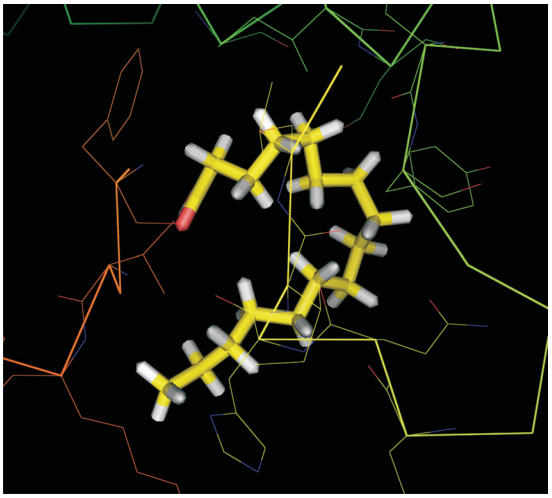

Fig. 3. Interaction of palmitic acid with p53

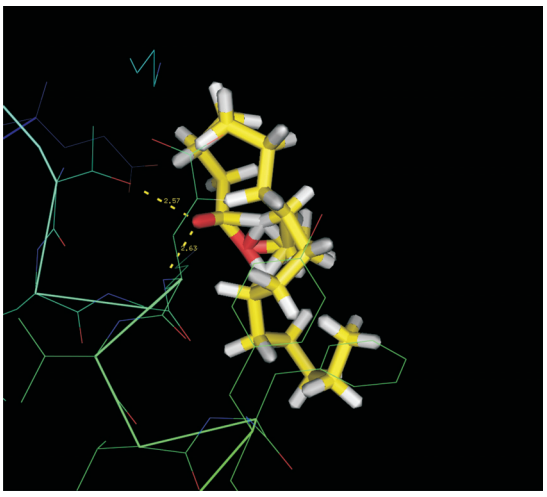

Fig. 4. Interaction of ethyl palmitate with p53

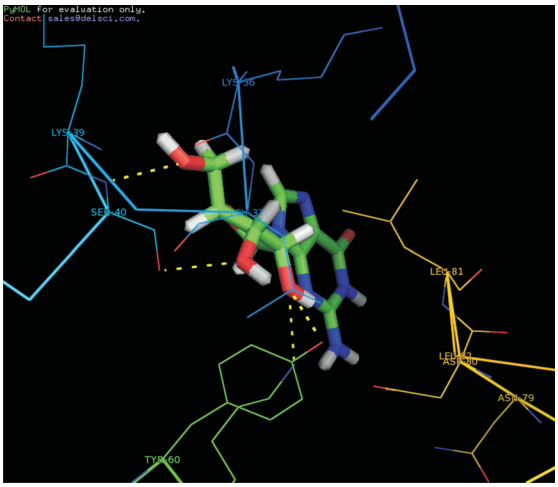

Fig. 5. Interaction of guanosine with p53

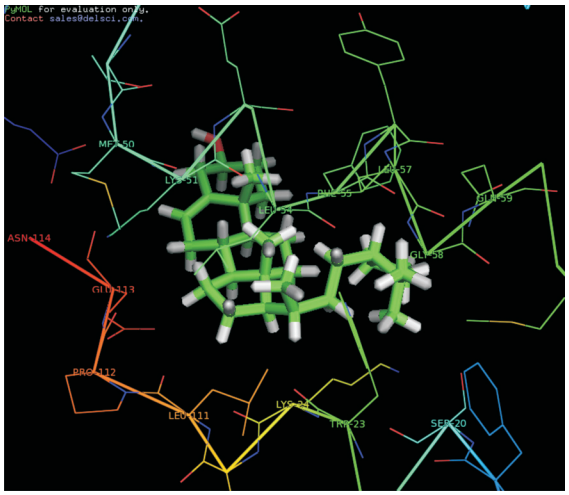

Fig. 6. Interaction of gamma-Sitosterol with p53

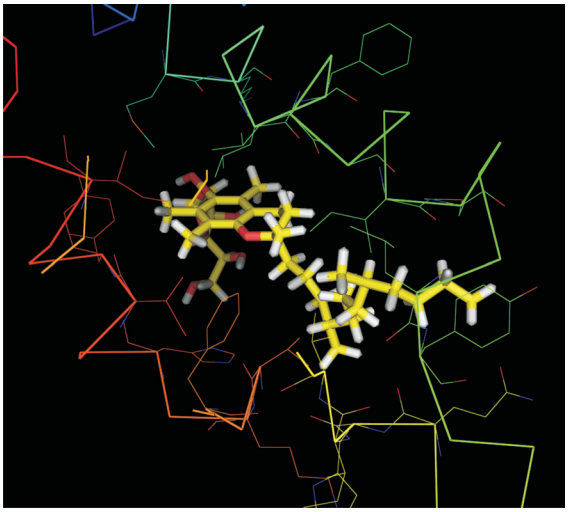

Fig. 7. Interaction of alpha-tocopherol-beta.-D-mannoside with p53

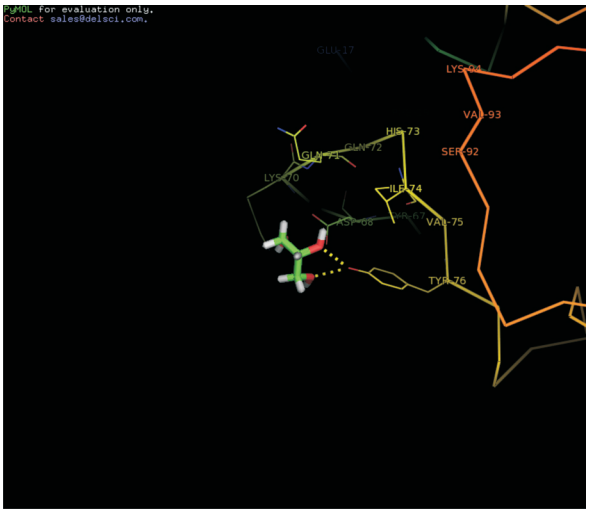

Fig. 8. Interaction of glycerin with p53

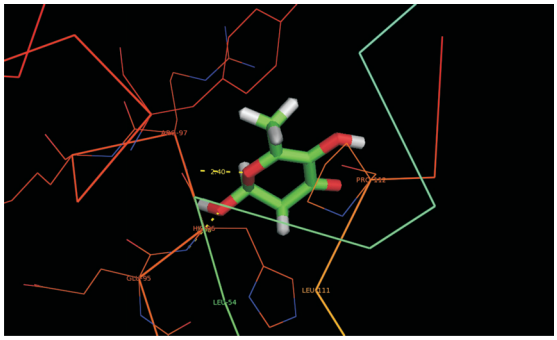

Fig. 9. Interaction of 2,3- Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one with p53

From the above docking study the selected plant materials contains pyrogallol, guanosinepossess antioxidant, antimutaganic and antiproliferative properties (Duke's Phytochemical and Ethnobotanical Databases database) also interacts with the transcriptional factors p53-MDM2 complex and may play role in regulation of this proteins and may provide chemo preventive potential for *Punica granatum* peel and *Vitis vinifera* seeds.

This clearly shows that both the extracts maypossess anticancer potential. Both the extracts contain bioactive constituents whose anticancer potential has been reported and presence of this might be the reason for its chemo preventive activity. The *P. granatum* peel and *V. vinifera* seeds showed protective effect on DEN Induced Hepatocellular damage induced by oxidative stress in rats (Ashok Kumar and Vijayalakshmi, 2015). The *in vitro* studies showed both the EPGP and EVVS possess anticancer potential against Hep G2 cell lines (Ashok Kumar *et al.*, 2019). As both the extracts possess anticancer activity, in this study the common phytochemicals in both extract were selected. p53, the DNA binding protein were selected as target protein for *in silico* docking study. They were selected based on their role in Cancer regulation especially HCC as evidenced by the previous reports.

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