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Computational Molecular Docking Analysis and Visualisation of Anthocyanins for Anticancer Activity

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ABSTRACT

Various *invitro* and computational methods were implemented to evaluate the anticancer potential of anthocyanidins, namely cyanidin, malvidin, delphinidin, peonidin, pelargonidin, and petunidin. These anthocyanidins were docked with CDK-2, CDK-6 and IGF-1R kinase proteins. Additionally, known inhibitors (KIs) such as SU9516, Palbociclib, OSI-906 are compared with their respective macromolecules, including, CDK-2, CDK-6 and IGF-1R kinase, in to compare results of the study based on Lipinski rule of 5. The Auto Dock Tool (Autodock 4) was used for molecular docking, and the docked complex compounds were visualised and interpreted using the Bio via Discovery Studio 2020 client. The Docking results obtained showed a very good inhibitory binding to almost all the selected cancer proteins, and these compounds might be a potential drug molecule.

Keywords- Molecular Docking, Anthocyanidins, Anti-cancer.

I. INTRODUCTION

Cancer is a biological condition in which any mutation occurs in the genetic material (DNA) such that it results in uncontrolled cell division leading to formation of tumour mass. There are certain types of cancers like lymphoma and myeloma that do not form tumours. Cancer growth includes certain phases like initiation, promotion, progression, invasion, and metastasis [1]. Scientist is researching more on the drugs for the treatment of cancer. Various anticancer compounds were identified that act as a potent anticancer agent [2]. Cancer mechanistical influence in the body through mitochondrial pathway and the External death receptor pathway. Various cancer molecules play an important role in this pathway. Thus anticancer macromolecules are now discovered targeting cancerous molecules for the treatment of cancer [3].

Phytotherapy is a growing medicinal treatment method for cancer which gained the attention of many pharmacist. The several phytocompounds have been extracted, isolated and purified from the plant source and have been potentially used in the treatment of cancer. Many researchers are keen on identifying and evaluating the anticancer potential of various phytocompounds [4]. Some of phytocompounds that were identified as a potent anticancer agent were Vincristine and Vinblastine (Catharanthus roseus), Paclitaxel (Taxus brevifolia), and Topotecan and Irinotecan (Camptotheca acuminate). Various plant pigments were also identified as anticancerous agent, and one among them was [5].

Anthocyanins is a plant pigment found in flowers, fruits and tubers that are blue, red or purple in colour. They are most abundant in fruits like in cherries, grapes, pomegranate, blueberry, etc. Naturally, anthocyanins were present in the form of Heterosides [6]. Anthocyanins have been reported to supress angiotenesis in epidermal keratinocytes by inhibiting H2O2 and tumour necrosis factor alpha (TNF- alpha) induced VEGF (Vascular endothelial growth factor). There are six most common anthocyanins - delphinidin, peonidin, petunidin, pelargonidin, malvidin and cyanidin. Among which satin in the poorest. Malvidin – 3-O glucoside was reported to be abundant in grapes. Non-acylated cyanidin 3-Sophoroside-5-glucoside was reported to possess anticancer activity. Similarly, various in vitro studies on anthocyanidins have proved it to be anti- cancerous agents [7,8].

Thus, with these *invitro* research works as background, the present study deals with drug-likeliness and docking studies to understand their inhibition on cancer causing proteins. Some important examples of cancer macromolecules are B-cell lymphoma 2 (Bcl-2), vascular endothelial growth factor receptor 2 (VEGFR-2), cyclin-dependent protein kinase 6 (CDK-6), CDK-2, IGF-1R kinase (insulin-like growth factor 1 receptor).

II. MATERIALS AND METHODS

Software Used

The software used in this study were AutoDock Tool (Auto-dock 4) [9] and Biovia Discovery Studio 2020 client[10].

Ligand Preparation

The anthocyanins namely, delphinidin (PubChem CID: 68245), peonidin (PubChem CID: 441773), petunidin (PubChem CID: 73386),

pelargonidin (PubChem CID: 440832), malvidin (PubChem CID: 159287), and cyanidin (PubChem CID: 128861) were downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in sdf format. These were converted to pdbqt format for docking using a software called Open Babel. Their individual properties were noted and tabulated to check whether they satisfy Lipinski rule of 5 [11].

Prediction of Drug likeness of Anthocyanidins

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) property and the druglikness of α- mangostin was determined by Lipinski's rule of 5. The SMILES of all seven anthocyanidins were obtained from PubChem and was loaded on SwissADME (http://www.swissadme.ch/) and molinspiration (https://www.molinspiration.com/cgi-bin/properties) to record its physicochemical properties. It was then verified for any objection from Lipinski's rule of 5 which enlists certain limitations for a compound to be an effective oral drug [2].

Protein Preparation

The protein structures, including, 1DI8 (CDK-2), 1XO2 (CDK-6) and 2OJ9 (IGF-1R kinase) were taken from Protein Data Bank (https://www.rcsb.org/pages/help/advanced search/pdbIDs). AutoDock tools- Autodock 4 (Morris et al., 2009) was used for the protein preparation and docking process. The hetatm atoms and water atoms of all the proteins were deleted in the first place, and polar hydrogens and kollman charges were added. Finally, proteins were saved in pdbqt format [3].

Receptor Grid Generation

The grid centered on the entire protein so as to get all the active sites and was saved in gpf format. Then the auto grid was launched for pre-calculation of grids [11].

Molecular Docking

After setting the rigid file name for the macromolecule, required ligand i.e., anthocyanin was also selected. The parameters were set to Genetic Algorithm as well as, the output was set to Lamarckian Algorithm as it enables to handle a large number of degrees of freedom, and it can be utilised to dock ligands with numerous rotatable bonds with high efficiency. Auto dock was launched after saving the file in dpf format [11].

Molecular Docking Analysis

The docking file in dlg format and macromolecule were chosen. Then each conformation was played ranked by energy, after which the information of the complex and H-bonds were analyzed.

Selection of the Best-Scored Pose

The conformation having more binding energy and a good number of hydrogen bonds was selected and the file was saved in pdb format. This conformation was analysed in Discovery Studio. The required ligand, atom within four angstroms were selected and the protein was displayed in a solid ribbon chain. After selecting the hierarchy, the H-bonds which are also present in the auto dock results were selected and the interatomic distances were also displayed. The complex was written and visualized using Biovia Discovery studio 2020 Client (BIOVIA). The pictures of the complex in both 2-D and 3-D structures were saved.

III. RESULTS

Prediction of Druglikeness of Anthocyanidins

The ligand structure was studied for Lipinski rule of 5 and tabulated in Table 1.It was reported that the Bioavailability score should be 0.55 for a neutral organic compound that satisfy Lipinski's rule to act as a good oral drug [12]. It was found that all the anthocyanidins have a bioavailability of 0.55. This Bioavailability score (0.55) showed it to be a good enduring compound for absorption through oral ingestion. Therefore, anthocyanidins proved to act as a good oral drug.

Table 1: Physical properties of natural and standard compounds

Compound name	miLogP	No. of atoms	Molecular weight (g/mol)	No. of H-bond acceptors	No. of H-bond donors	No. of rotatable bonds	Volume
Petunidin	-2.78	34	479.41	12	8	5	392.48
Delphinidin	-3.08	33	465.39	12	9	4	374.95
Pelargonidins	-2.3	31	433.39	10	7	4	358.91
Peonidin	-2.49	33	463.42	11	7	5	384.46
Malvidin	-2.04	33	463.42	11	6	5	385.16
Cyanidin	-2.37	30	419.36	10	7	3	342.08
Palbociclib	2.96	33	447.54	9	2	5	410.58
OSI906	3.63	32	421.5	6	3	3	379.7
SU9516	1.39	18	241.25	5	2	2	209.79

The docking of anthocyanins including Delphenidin, Malvidin, Cyanidin, Peonidine, Pelargonidin, Petunidin with the cancerous macromoleculesvascular endothelial growth factor receptor 2 (VEGFR-2), B cell lymphoma 2 (Bcl-2), and IGF-1R kinase (insulin-like growth factor 1 receptor),cyclin-dependent protein kinase 6 (CDK-6), and CDK-2, was performed. Additionally, all the protein- ligands interactions are mentioned briefly in Table 2. The CDK2 protein interacted with all the anthocyanins i.e., cyanidin by 3 H-bonds with a binding energy of -3.55, delphenidin by 3 H-bonds with a binding energy of -3.68, malvidin by 1 H-bond with a binding energy of -4.86, pelargonidin by 2 H- bonds with a binding energy of -1.89, peonidine by 3 H- bonds with a binding energy of -2.24, petunidin by 3 H- bonds with a binding energy of -1.13. Similarly, IGF-1R kinase protein has also interacted with all the anthocyanins i.e., cyanidin by 1 H-bond with a binding energy of -4.44, delphenidin by 2 H-bonds with a binding energy of -1.52, malvidin by 3 H-bonds with a binding energy of -2.15, pelargonidin by 2 H- bonds with a binding energy of -3.04, peonidine by 3 H- bonds with a binding energy of -1.68, petunidin by 4 H- bonds with a binding energy of -6.49; VEGFR-2 protein has also interacted with all the anthocyanins i.e., cyanidin by 3 H-bonds with a binding energy of -2.39, delphenidin by 1 H-bond with a binding energy of -1.32, malvidin by 2 H-bonds with a binding energy of -2.68, pelargonidin by H- bond with a binding energy of -3.54, peonidine by 1 H- bond with a binding energy of -3.14, petunidin by 1 H- bond with a binding energy of -5.41; CDK-6 protein has interacted with all the anthocyanins i.e., cyanidin by 2 H-bonds with a binding energy of -4.31, delphenidin by 4 H-bonds with a binding energy of -7.95, malvidin by 3 H-bonds with a binding energy of -4.03, pelargonidin by 3 H- bonds with a binding energy of -4.06, peonidine by 4 H- bonds with a binding energy of -3.00, petunidin by 3 H- bonds with a binding energy of -7.42.

Table 2: Molecular docking results of anthocyanidins with cancer proteins

S. No	Receptor	Ligand	Binding energy	No of H bond formed	AA of receptor	Atom of receptor	Atom of ligand	Distance
1	**1XO2**	Cyanidin	-4.31	4	ALA23	O	H	2.18
					GLY53	O	H	2.33
					GLU52	HN	O	2.35
					GLY53	O	H	1.92
		Delphinidin	-7.95	5	THR106	O	H	2.16
					THR106	OG1	H	2.86
					THR106	OG1	H	2.21
					ARG215	O	H	2.27
					LEU109	O	H	2.14
		Malvidin	-4.03	5	LEU237	O	H	2.38
					ASP275	OD2	H	2.41
					ASP275	OD2	H	2
					ILE262	HN	O	2.83
					ILE262	HN	O	1.92
		Palbociclib	-5.77	4	GLU52	OE1	H	3.5
					ARG168	HE	O	4.7
					ARG168	HH21	O	2.58
					ILE169	H	O	4.92
		Pelargonidins	-4.06	4	ALA253	O	H	1.94
					ASP226	OD1	H	1.81
					HIS255	H	O	2.51
					ASP233	OD1	H	2.27
		Petunidin	-7.42	4	ARG288	O	H	2.03
					HIS137	O	H	3

					HIS137	HD1	O	2.09
					ASP134	O	H	2.32
		Peonidin	-3	3	GLU52	O	H	2
					MET54	O	H	1.97
					ARG60	HE	O	2.03
2	**1DI8**	Cyanidin	-3.55	6	GLU138	OE2	H	2.24
					GLU28	OE1	H	2.07
					VAL29	O	H	5
					GLU28	OE1	H	4.62
					PHE82	O	H	5.05
					HIS84	HE2	O	2.21
		Delphinidin	-3.68	5	TYR15	O	H	2.09
					THR14	O	H	2.23
					ILE35	O	H	3.74
					LEU76	O	H	4.89
					ASN74	O	H	1.97
		Malvidin	-4.86	5	GLU12	O	H	1.97
					HIS84	O	H	5.9
					GLU81	O	H	5.36
					ASP86	O	H	4.46
					ASP86	O	H	3.61
		Pelargonidins	-1.89	4	THR198	O	H	1.97
					ARG199	HE	O	2.11
					VAL197	O	H	4.83
					ARG199	O	H	6.03
		Peonidin	-2.24	4	ARG214	HH12	O	1.88
					VAL251	O	H	1.73
					LEU202	O	H	5.34
					THR198	O	H	4.02
		Petunidin	-1.13	4	GLY229	O	H	2.12
					SER232	O	H	2.24
					LYS178	HN	O	2.14
					CYS177	O	H	5.05
		SU9516	-9.31	2	GLU81	O	H	2
					LEU83	HN	O	1.72
3	**2OJ9**	Cyanidin	-4.44	4	GLY1122	O	H	2.06
					PHE1124	O	H	4.55
					ARG1104	O	H	5.18
					ARG1104	O	H	6.23
		Delphinidin	-1.52	2	GLU963	O	H	1.92
					GLU961	OE1	H	2.25
		Malvidin	-2.15	3	PHE1159	O	H	1.9
					VAL1102	HN	O	4.92

					GLY1157	O	H	2.24
		OSI906	-10.97	2	ASP1202	O	H	2.16
					ASP1205	HN	N	4.27
		Pelargonidins	-3.04	4	THR1161	OG1	H	2.25
					LYS1224	O	H	2.25
					PHE1229	O	H	4.38
					ALA1094	O	H	2.56
		Peonidin	-1.68	3	PHE1159	O	H	4
					LEU1143	O	H	5.2
					ARG1104	HE	O	4.52
		Petunidin	-6.49	4	ASN1097	HD22	O	2.21
					ALA1094	O	H	2.26
					PHE1229	HN	O	2.15
					ARG1226	O	H	2.21

Finally, Bcl-2 protein has interacted with all the anthocyanins i.e., cyanidin by 2 H-bonds with a binding energy of -3.44, delphenidin by 2 H-bonds with a binding energy of -6.9, malvidin by 2 H-bonds with a binding energy of -3.49, pelargonidin by 3 H- bonds with a binding energy of -3.52, peonidine by 3 H- bonds with a binding energy of -3.67, petunidin by 3 H- bonds with a binding energy of -6.08.

The other interactions such as *van der waals*, Alkyl, Pi-Sigma and Pi-Alkyl interactions were also formed between the receptors and ligand as shown in Fig.'s 1, 2 and 3.

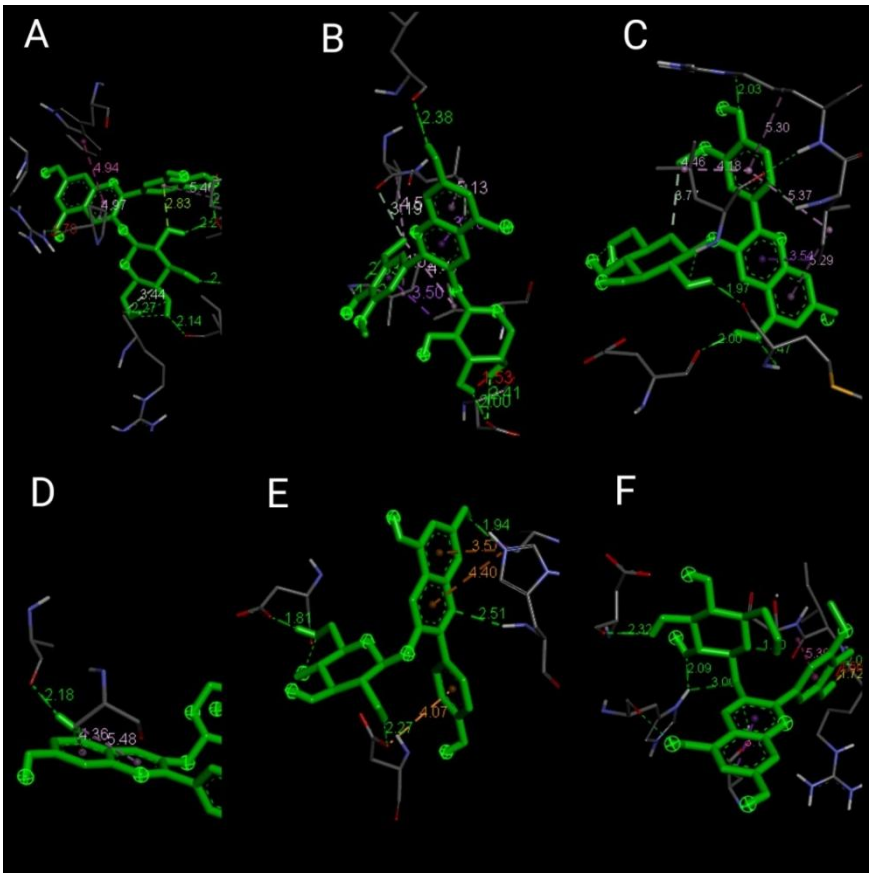

Fig 1: Molecular docking visualisation of CDK-6 protein with anthocyanidins
A- delphinidin; B- Malvidin; C- Peonidin; D- Cyanidin; E- Pelargonidin; F- Petunidin.

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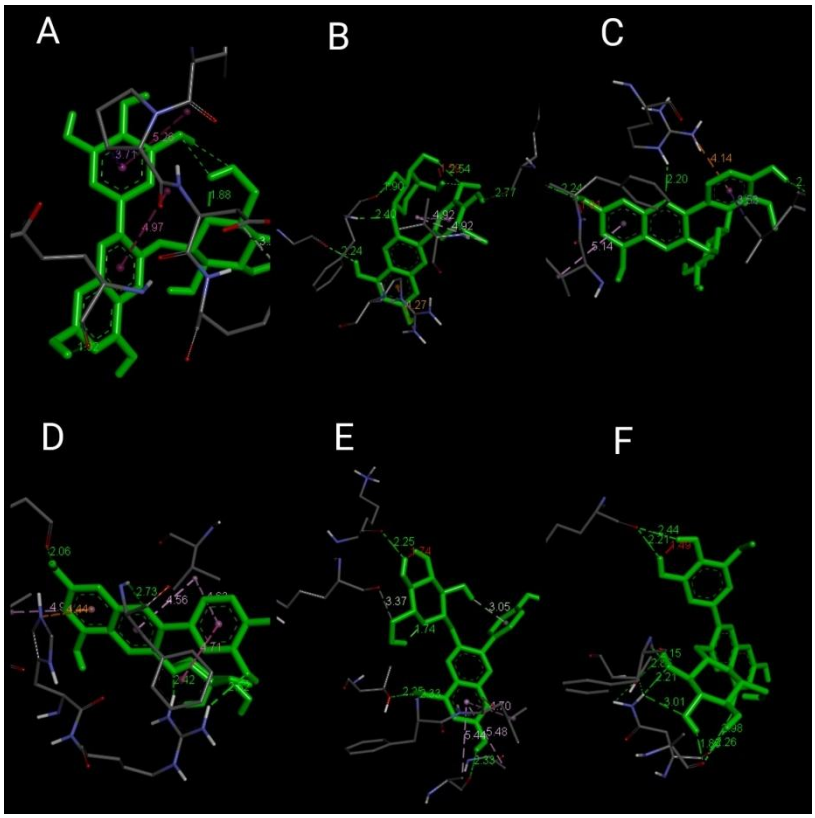

Fig 2: Molecular docking visualisation of IGFR protein with anthocyanidins
A- delphinidin; B- Malvidin; C- Peonidin; D- Cyanidin; E- Pelargonidin; F- Petunidin

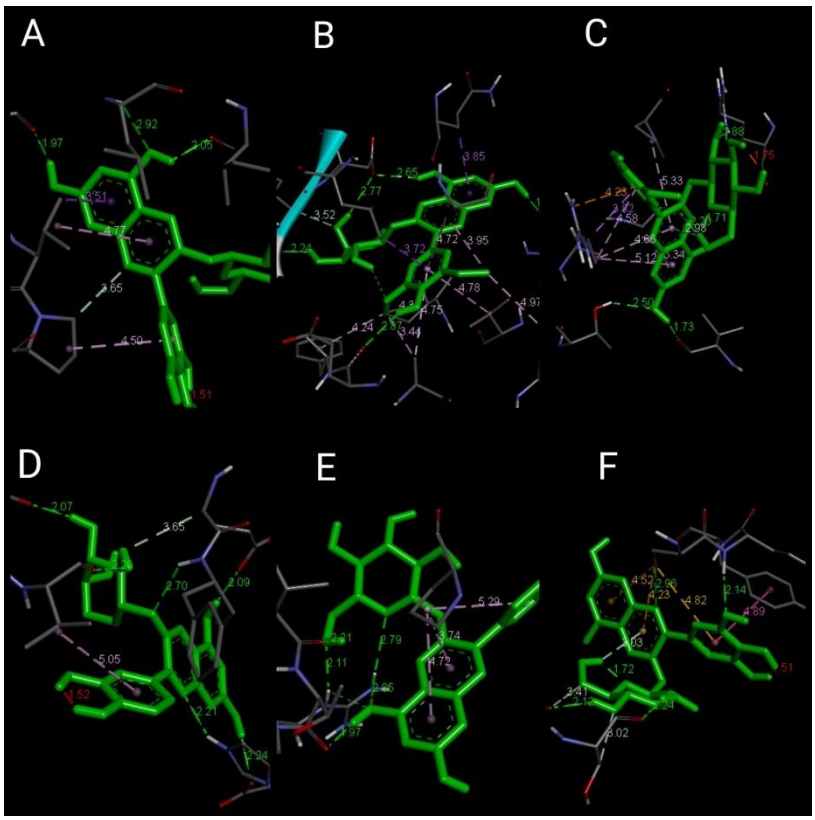

Fig 3: Molecular docking visualisation of CDK-2 protein with anthocyanidins.
A- Delphinidin; B- Malvidin; C- Peonidin; D- Cyanidin; E- Pelargonidin; F- Petunidin

IV. DISCUSSION

In this work, we have used three tools, specifically Auto Dock, Open Babel, and Discovery Studio. Auto Dock is a suite of automated docking tools and is used to perform computational molecular docking of small molecules to proteins, DNA, RNA and other important macromolecules, by treating the ligand and selected parts of the target as conformationally flexible. Auto Dock 4 actually consists of two main programs, namely, auto dock, auto grid. Auto dock performs the docking of the ligand to a set of grids describing the target protein and auto grid on the other hand pre-calculates these grids. Additionally, the atomic affinity grids can be visualized in this. Auto dock's scoring function is based on the AMBER force field, and also estimates the free energy of binding of a ligand to its target. Open Babel is used to interconvert chemical file formats. Discovery studio, on the other hand is a suite of software for simulating small molecule and macromolecule systems however, we have used this to analyze the docking results in this project.

It was reported that the Bioavailability score should be 0.55 for a neutral organic compound that satisfy Lipinski's rule to act as a good oral drug [3]. It was found that all the anthocyanidins have a bioavailability of 0.55 and thus are a good enduring compound for absorption through oral ingestion. Therefore, anthocyanidins proved to act as a good oral drug.

The different types of anthocyanins namely, Delphenidin, Malvidin, Cyanidin, Peonidin, Pelargonidin, Petunidin have showed good binding interaction with the cancer proteins namely, vascular endothelial growth factor receptor 2 (VEGFR-2), CDK-2, IGF-1R kinase (insulin-like growth factor 1 receptor), cyclin-dependent protein kinase 6 (CDK-6), and B cell lymphoma 2 (Bcl-2). The above mentioned anthocyanins were proved to be a potent anti-cancerous agent and furtherpoly herbal formulation based on its individual efficiency will be formulated since it already showed good results in vitro. Further, dosing will be decided based on the in vitro anticancer analysis to carry the work on experimental animals in vivo to know its efficiency.

Anti-Carcinogenic activity against various cell types *in vitro* as well as tumor types *in vivo* of cancer was shown by anthocyanins. The expression and activation of numerous genes correlated with the cellular functions together with genes involved in JNK, PI3K/Akt, MAPK pathways [1]. Radical scavenging activity, reduced cell proliferation, induction of apoptosis and differentiation, stimulation of phase II detoxifying enzymes. Inflammation, as well as angiogenesis and invasiveness are the prospective cancer chemo-preventive processes of the anthocyanins found from *in vitro* studies.

By acting on the NF- KB and PI3K/Akt pathways in the initial stages, anthocyanin inhibits inflammation to decrease the COX- 2 and iNOS expression which stops normal cells from transformation by regulating phase II antioxidant enzymes expression in order to attain anti-oxidation through Nrf2/ARE signal system. During the formation phase, anthocyanins target the MAPK pathway and AP-1 as well as inhibits RTK activity, and its signal cascade pathway regulating the expression of cancer-associated genes to block carcinogenesis leading to cell cycle arrest and DNA repair. Anthocyanins activates the capase mediated by ROS and JNK/p38- MAPK, which leads to the induction of cancer cells apoptosis during the development stage. Furthermore, anthocyanins target the VEGF signal pathway and extracellular matrix degradation to stop metastasis of cancer, as well as reverses the resistance of multidrug resistance of cancer cells to enhance the sensitivity of chemotherapy. [11]

Cyanidin was reported to be an antioxidant, anti-Carcinogenic, anti-Mutagenic, and anti-Proliferative and anti-Inflammatory agent. Delphenidin was reported to inhibits proteolytic activity and act as an antitumor agent. Malvidin was reported to be an Antioxidant, anti-Proliferative and anti-inflammatory agent. Pelargonidin was reported to be an antioxidant, antitumor and anti-inflammatory agent. Peonidin was reported to be an antioxidant and anti-proliferative action. Petunidin has an antioxidant, and anti-proliferative agent [11].

V. CONCLUSION

The anthocyanidins have proved to be good oral drug for treating cancer based on the bio availability score. They have also shown good binding interaction with the cancer proteins. The anthocyanidins modulate the expression and activation of multiple genes associated with the cellular functions. These can also reverse the multidrug resistance of cancer cells to improve their chemotherapy sensitivity. Hence, Anthocyanidins could be studied further more deeply and can be utilised as a good oral drug.

Consent for Publication

Not applicable

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise

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