Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Molecular docking Analysis of the phytocompounds found in Citrus seeds and their effects on the hallmark gene of HNSCC

Sudeshna Sengupta, Satavisha Ghorui, Malavika Bhattacharya*

Department of Biotechnology, Techno India University, Kolkata - 700091, West Bengal, India.

ARTICLE HISTORY

Received: 14.03.2023

Accepted: 17.04.2023

Available online: 30.09.2023

DOI:

10.5530/ajphs.2023.13.51

Keywords:

Molecular docking, citrus seeds, hallmark protein, ENTPD1, oral cancer

***Corresponding author:**

Email : malavikab@gmail.com
Phone : +91-

ABSTRACT

Valorization of waste products of fruits and vegetables is attractive to researchers. Fruits and vegetables are high in macro-nutrients. Also, non-nutrient molecules, often known as phytochemicals, are secondary plant metabolites that include polyphenols and triterpenoids and are well known for a variety of biological activities and health advantages. Citrus fruits are rich in ascorbic acid, and citric acid, which boosts our immune system by helping in the production of white blood cells. Citrus fruits are also known as acid fruits because of their soluble solids which are composed of organic acids and sugar. Citrus fruits are also significant because of their elements with anti-oxidant and anti-inflammatory potential within them. Seeds of these fruits are capable of producing new trees, whereas seeds are inedible and waste products too. Seeds of *Citrus aurantifoliia, C.limon, C.reticulata , C.limetta, C. maxima* have been taken for the ligand study, and molecular docking with the protein ENTPD1and also ADMET analysis has been done. Depending upon the phytochemical-rich substances citrus seed can be the potential pool for researchers for reuse and valorization, not only that the significant phytocompounds mentioned and discussed in this paper can be used as a good target for the protein, ENTPD1 which is a hallmark gene of oral cancer. Recycling citrus seeds will add some economic value to produce a new drug delivery system. As the findings indicated limonin present in *C. reticulata* and *C. aurantifoliia* constituted a needful source of medication because it shows good drug likeliness, and may hamper the growth of metastatic cells by boosting immunity.

INTRODUCTION

Oral cancer, a part of Head and squamous cell carcinoma, ensues the dysplasia of the mouthparts, sinus, tonsil, hard and soft palate of the mouth, larynx, tongue, up to oropharynx region. NIH, Cancer Stat Facts estimated 54,540 new cases in 2023 and 11,580 death cases due to Oral cavity and pharynx cancer. [1] It is the 6th most common disease globally; in India, it is the most common disease. [2-4] The low-income population of India is highly affected due to some etiological factors, smokeless tobacco, bidi, gutkha, cigarettes, etc. There are several risk factors that trigger many genetic levels that increase or decrease oral dysplasia through the process of carcinogenesis [5] and in this process of carcinogenesis, there are multistep pathways in which various genes are involved and alter their functions as tumor suppressors or tumor enhancer genes. There are many differentially expressed genes for such processes among them ENTPD1 is a hallmark gene for keratinized cells of mouth parts due to heavy smoking which causes cancer [6]. CD39 is a potential marker of any inflammatory diseases, it metabolizes extracellular adenosine triphosphate to adenosine monophosphate and is encoded by ENTPD1 [ecto- nucleotide triphosphate diphosphohydrolase 1] and depends on membrane proteins. The human ENTPD1 gene is on chromosome 10q24.1. When ADP or ATP is used as the substrate, recombinant CD39 inhibits it. Although CD39 activity initially increased with increasing substrate concentration, it was significantly inhibited at high concentrations of ATP or ADP [7]

The cascade of the proliferation of T cells in hematological malignancies begins with extracellular ATP and leads to immunosuppressant adenosine(eADO) synthesis is regulated by

ectonucleoside triphosphate diphosphohydrolase-1 (CD39) and ecto-5'-nucleotidase (CD73), which influence purinergic signaling by modifying ligand availability [8]

Considering previous molecular signaling databases, limiting CD39-CD73, potential 'immune checkpoint mediator' shows significant changes blood cancer environment [9]

In molecular biology, through molecular docking tools, computational drug designing is a new choice. To control or limit any action of hallmark genes by molecular docking against phytocompounds is a promising approach. Therefore CD39-ENTPD1 activity is key to regulating oral malignancy, in this study we will demonstrate the binding affinities, drug likeliness, and anticarcinogenic and antiviral properties of the phytocompounds in comparison to different citrus fruits available in the market as lead molecules to ENTPD1 protein. This study may help the next-generation low-cost drugs that will come from natural sources involved in lowering the propagation of metastatic cells.

Fruits and vegetables are high in macro-nutrients. Also, non-nutrient molecules, often known as phytochemicals, are secondary plant metabolites that include polyphenols and triterpenoids and are well known for various biological activity and health advantages. Valorization of waste products of fruits and vegetables is attractive to researchers. Citrus fruits are rich sources of Vitamin C, which is a potential antioxidant and shows an immune-supportive solid nature. Nonedible parts of citrus fruits are a considerable amount left after getting the juice. [10]

There are more than 70 citrus species available from the Rutaceae family. On human health it has been well documented that Citrus seeds are enriched sources of phytonutrients, fibers, and pectin, terpenoids like limonoids (limonin, nomilinic acid), and phenolic substances such as phenolic acids and flavonoids, tocopherols, and carotenoids [11] shows a wide array of antioxidant activity along with anti-cancer, anti-inflammatory role[12]. This offers up the possibility of using seeds (byproducts of citrus species) as a source of nutraceuticals [13].

Concurrent with the high demand for nutraceuticals-rich innovative functional foods, we have chosen five popular citrus fruits; *Citrus sinensis (sweet orange), Citrus aurantiifolia (patilebu or lemon) Citrus limetta (Musambi), Citrus reticulata (Aroma king lemon), Citrus maxima (pomello)*. Since there is no such IN-Silico study reported for the phytocompounds found in the citrus seeds, molecular docking was performed to identify probable putative bioactive compounds with anti-oral cancer potentiality.

1. MATERIALS AND METHODS

2.1 Protein Preparation

On retrieving the 3D-crystal structure of the protein ENTPD1 (PDB ID: 3ZXO) from the RCSB Protein Data Bank website (https://www.rcsb.org/)[14] the protein structure showed a crystal resolution of 1.90 Å and two chains, A and B, consisting of 129 amino acids. In order to avoid atomic clashes and optimization of hydrogen bonds, the protein crystal structures were prepared before docking by following the standard protein preparation protocol from Discovery Studio Visualizer 21.1. The heteroatoms and water molecules of the protein were eliminated, and polar hydrogen was added. After that, the active site of the prepared protein was predicted.

2.2 Ramachandran Plot

The Phi-Psi Graphical Plot or the Ramachandran Plot gives us the graphical plot of the torsional angles phi (Φ) and psi (Ψ) from amino acids that are present in peptides. For this *in silico* study, the Ramachandran Plot analysis was carried out through the EMBL-EBI PDBsum web server (http://www.ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl) [EBI 2022] Herein the protein's PDB ID was submitted to run the plot analysis with outliers that were labeled based on the type and number of residues and chains. All the labels were displayed.

2.3 Secondary Structure Prediction

In the prediction of secondary structure (Fig. 1) of the mutant-binding domain ENTPD1 (PDB ID: 3ZXO) using the Predict Protein online tool, it was observed that the protein showed a sequence length of 129 amino acids, 46 aligned proteins, and a match of 15 PDB structures. In the predicted secondary structure, the protein helix could be seen colored in blue, the strands in red, and others in yellow.

2.4 Retrieval of Ligands

In order to document the potential inhibitors of the mutant-binding domain ENTPD1, the active phytocompounds of five citrus fruits were retrieved from various literature sources found on the PubChem website (https://pubchem.ncbi.nlm.nih.gov/). The structures of these phytocompounds were downloaded in the 3D Structure Data File (SDF) format [15], the quantification of which has been mentioned in the table (Table 1) below. Next, the ligands were prepared by ligand optimization, minimization of energy, and finally, converting said ligands into a three-dimensional PDB file format via the PyRx software.

2.5 Molecular Docking

Molecular Docking is an essential tool in computer-assisted drug designing, whose primary goal is to predict the predominant binding modes of a protein having a well-defined 3D structure and a ligand. This method helps researchers illustrate how small molecules perform within the binding sites of protein targets, which further gives us an idea of critical biochemical processes within cells by simulating the interaction between small molecules and protein targets at an atomic level [16] In this study, a

Table 1 :

	Citrus limon	*Citrus reticulata*	*Citrus limetta*	*Citrus aurantiifolia*	*Citrus maxima*
Number of Phytocompounds	96	28	5	12	13

virtual screening software tool called PyRx was used for molecular docking[17] All of the active phytocompounds from the five citrus fruits were docked, one fruit at a time, with the mutant-binding domain ENTPD1. When the docking process was completed, we observed a table displaying the binding affinity of each ligand [18] From this data, for further evaluation, the top 3 ligands of each citrus fruit were selected based on the highest binding affinity of the ligand and saved in the PDB file format. The 2D-3D interactions were visualized via Discovery Studio Visualizer 21.1

2.6 . Analysis of Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET)

ADMET is an abbreviation in pharmacokinetics and pharmacology that refers to the characterization of five properties, Absorption, Distribution, Metabolism, Excretion, and Toxicity, that help researchers explore and explain how different biochemical processes take place in order to provide safety considerations for a newly developed drug on which risk-based assessments can be made. For this study, the top 3 compounds of each citrus fruit were chosen for the test of drug-likeness as well as ADMET analysis. The drug-likeness test and ADMET analysis were carried out via the web-based software SWISS-ADME tool (http://www.swissadme.ch/)[19] and ADMETLAB server (https://admetmesh.scbdd.com/service/evaluation/index) [20] respectively. Additionally, a Boiled-Egg Analysis was done through the SWISS-ADME tool. [21] Lipinski's Rule of Five, or simply Rule of 5, is a set of five conditions or guidelines in drug designing and development that an orally active drug has to follow, having no more than one violation. The Rule of 5 was considered for the ADMET analysis.

2. RESULTS

3.1 Ramachandran Plot

The Ramachandran Plot analysis (Fig. 1) was carried out on the mutant-binding domain ENTPD1 (PDB ID: 3ZXO), and its protein geometry has been tabulated below (Table 2):

3.2 Molecular Docking

A molecular docking study was done using the PyRx tool on the five citrus fruits' seeds, and it revealed several active phytocompounds that showed a significant binding affinity with the protein. The top compounds from each of the citrus fruits' seeds that showed the highest binding affinity with the protein have been tabulated below (Table 3). The results of the molecular docking process showed a significant number of compounds from each of the citrus fruits' seeds that had a resulting binding affinity of more than 7 Kcal/mol with the ENTPD1 protein. From this data, the top three compounds of each citrus fruit's seeds were selected for further drug-likeness test and ADME analysis (Table 4).

3.3 Molecular Visualization

The various interactions between receptor and ligand of the top phytocompounds from each citrus fruit's seed were visualized using Discovery Studio Visualizer 21.1. Using the PyRx tool, the docked ligands were first saved in the PDB format and then opened with the prepared protein. Through different 2D and 3D diagrams, numerous receptor-ligand interactions, such as Van der

Table 2

	Number of Residues	Percentage
Residues present in most favored regions: A, B, L	206	94.5%
Residues present in additionally allowed regions: a, b, l, p	10	4.6%
Residues present in generously allowed regions: ~a, ~b, ~l, ~p	2	0.9%
Residues present in disallowed regions: XX	0	0.0%
Number of Non-Glycine and Non-Proline residues	218	100.0%
Number of End-Residues (Excluding Gly and Pro)	4	
Number of Glycine residues	20	
Number of Proline residues	8	
Total number of Residues	250	

Table 3 :

Sl. Number	Name of Citrus Fruit	PubChem Compound ID	Name of Phytocompound	Binding Energy (Kcal/mol)
1	*Citrus limon*	119041	Obacunone	-8.4
2	*Citrus limon*	76312526	Limonin-7-oxime	-8.4
3	*Citrus limon*	76330624	Obacunone-7-oxime acetate	-8.4
4	*Citrus reticullate*	179651	Limonin	-7.8
5	*Citrus reticulata*	439551	Gibberellin A3 (1R,2R,5S,8S,9S,10R,12S)-5,12-dihydroxy-11-methyl-6-methylidene-16-oxo-15-oxapentacyclo[9.3.2.15,8.01,10.02,8]heptadec-13-ene-9-carboxylic acid	-7.8
6	*Citrus reticulate*	102004933	Gibberellin (1R,5S,8S,9S,10R,12S)-5,12-dihydroxy-11-methyl-6-methylidene-16-oxo-15-oxapentacyclo[9.3.2.15,8.01,10.02,8]heptadec-13-ene-9-carboxylic acid	-7.8
7	*Citrus limetta*	446925	Lycopene	-7.7
8	*Citrus aurantiifolia*	179651	Limonin	-7.8
9	*Citrus maxima*	442428	Naringin	-8.6
10	*Citrus maxima*	119041	Obacunone	-8.4
11	*Citrus maxima*	24090	Alpha-Carotene 1,3,3-Trimethyl-2-[3,7,12,16-tetramethyl-18-(2,6,6-trimethylcyclohex-2-en-1-yl)octadeca-1,3,5,7,9,11,13,15,17-nonaenyl]cyclohexene	-8.3

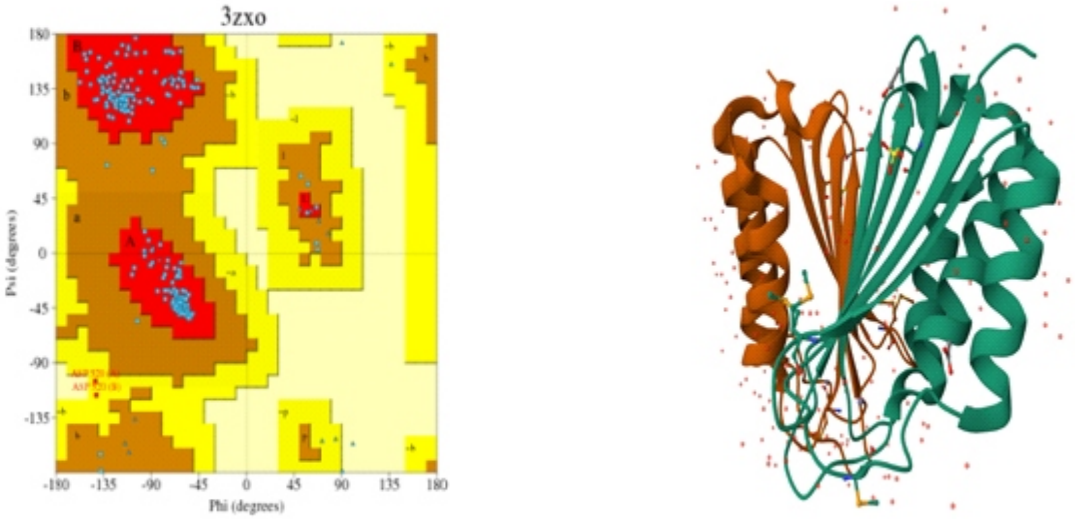


Figure 1 : *Ramachandran Plot Analysis of ENTPD1 and 3D Structure of the mutant-binding domain ENTPD1 (PDB ID: 3ZXO)*

Table 4 :

ADME analysis of top docked phytocompounds based on Lipinski's Rule of Five							
Sl. No.	Citrus Fruit	Name of Ligand	Molecular Weight (g/mol)	Number of H-Bond Donors	Number Of H-Bond Acceptors	Consensus Log^P	Molar Refractivity
1	*Citrus limon*	Obacunone	454.51	0	7	3.17	116.73
2	*Citrus limon*	Limonin-7-oxime	485.53	1	9	2.60	120.39
3	*Citrus limon*	Obacunone-7-oxime acetate	511.56	0	9	3.51	131.11
4	*Citrus reticulata*	Limonin	470.51	0	8	2.55	116.17
5	*Citrus reticulata*	Gibberellin A3	346.37	3	6	1.15	86.87
6	*Citrus reticulata*	Gibberellin	346.37	3	6	1.15	86.87
7	*Citrus lunetta*	Lycopene	536.87	0	0	11.90	188.23
8	*Citrus aurantiifolia*	Limonin	470.51	0	8	2.55	116.17
9	*Citrus maxima*	Naringin	580.53	8	14	-0.79	134.91
10	*Citrus maxima*	Obacunone	454.51	0	7	3.17	116.73
		Alpha-Carotene	536.87	0	0	11.03	184.43

Waals forces, conventional bonds, Pi-Sulphur interactions, Carbon-Hydrogen bonds, Pi-Pi T-shaped interactions, Alkyl and Pi-Alkyl interactions, as well as unfavorable interactions were observed. These interactions have been briefly discussed below:

Obacunone (*Citrus limon*)

Obacunone forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes conventional Hydrogen bonds with residues SER 541, ASP 536, THR 539, and PHE 538, as shown in Figure. 3

Limonin-7-oxime (*Citrus limon*)

Limonin-7-oxime forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes two conventional Hydrogen bonds with residues THR 539 and ASP 536, two Carbon-Hydrogen bonds with residues ASN 503 and GLY 540, along with one unfavorable donor-donor interaction, as shown in Figure. 3.

Obacunone-7-oxime acetate (*Citrus limon*)

Obacunone-7-oxime acetate forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes three conventional Hydrogen bonds with residues SER 541, SER 502, and AS_N 503 and a Pi-Donor Hydrogen bond with residue ASP 536, as shown in Figure. 3.

Limonin (*Citrus reticulata*)

Limonin forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes three conventional Hydrogen bonds with residues SER 541, THR 539, and PHE 538, along with one Carbon-Hydrogen bond having residue ASP 536, as shown in Figure. 3.

Gibberellin A3 (*Citrus reticulata*)

This type of gibberellin forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes three conventional Hydrogen bonds having residues ASP 536, GLY 542, and SER 541, as shown in Figure. 3.

Gibberellin (*Citrus reticulata*)

This gibberellin type forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes five

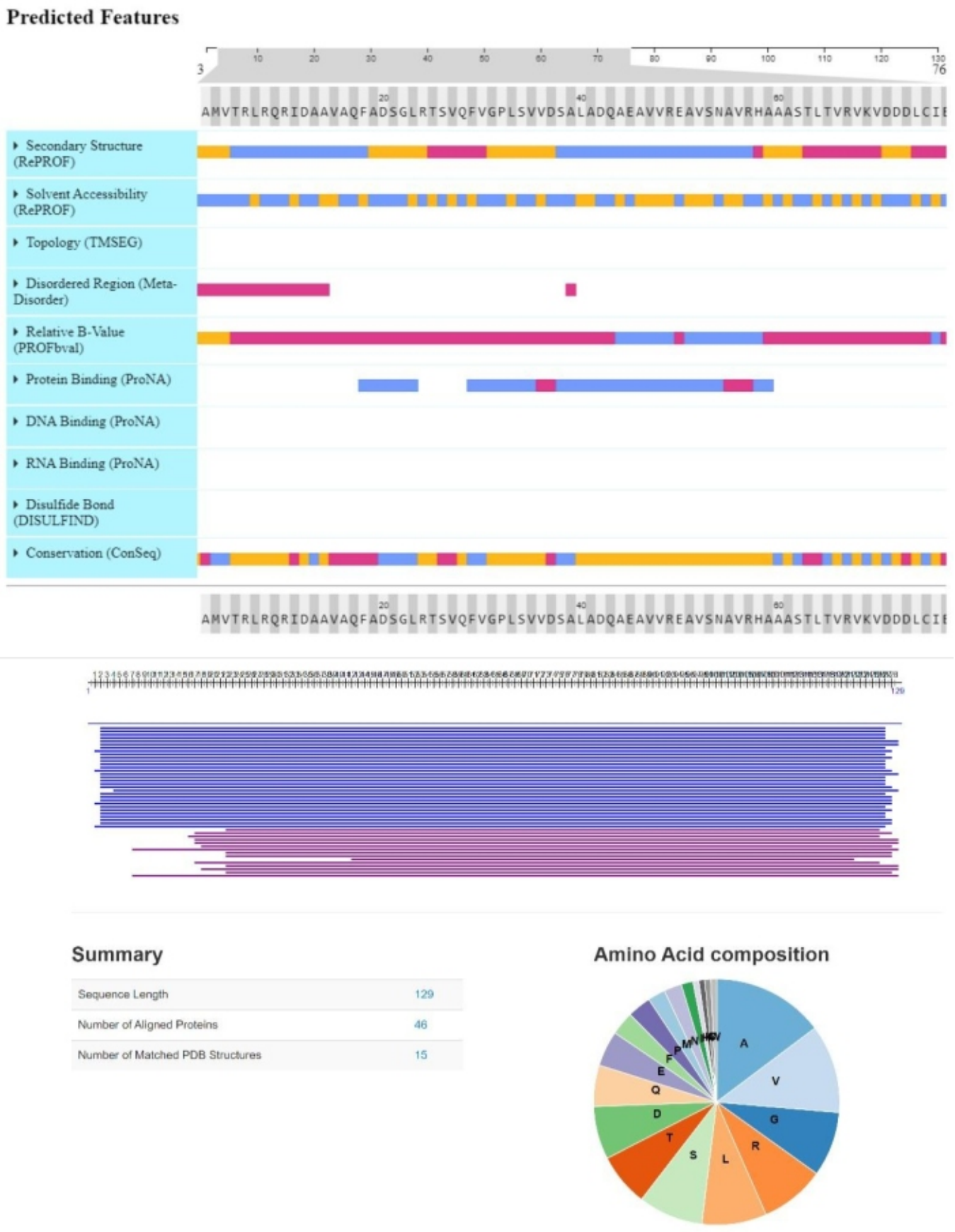


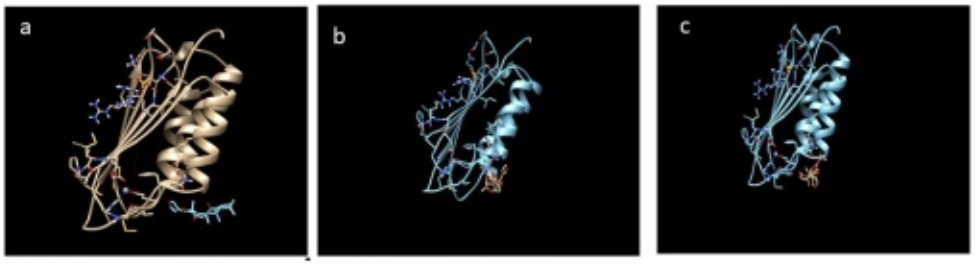
Figure 2 : Prediction of Secondary Structure of the mutant-binding domain ENTPD1 (PDB ID: 3ZXO)

Table 5 :

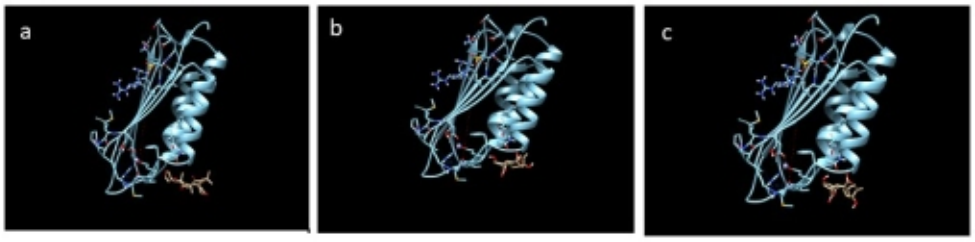
Prediction of Toxicity using ProTox-2 Web Server						
Sl. No.	Citrus Fruit	Name of Ligand	Predicted LD50 (mg/kg)	Predicted Toxicity Class	Average Similarity	Prediction Accuracy
1	*Citrus limon*	Obacunone	555	4	66.92%	68.07%
2	*Citrus limon*	Limonin-7-oxime	244	3	60.09%	68.07%
3	*Citrus limon*	Obacunone-7-oxime acetate	555	4	55.33%	67.38%
4	*Citrus reticulata*	Limonin	244	3	70.49%	69.26%
5	*Citrus reticulata*	Gibberellin A3	6300	6	100%	100%
6	*Citrus reticulata*	Gibberellin	6300	6	100%	100%
7	*Citrus limetta*	Lycopene	5700	6	79.06%	69.26%
8	*Citrus aurantiifolia*	Limonin	244	3	70.49%	69.26%
9	*Citrus maxima*	Naringin	2300	5	80.21%	70.97%
10	*Citrus maxima*	Obacunone	555	4	66.92%	68.07%
11	*Citrus maxima*	Alpha-Carotene	1510	4	71.45%	69.26%

Table 5 shows the prediction of toxicity of the phytocompounds as mentioned as name of the ligands

3D illustration of the docked structure of the mutant-binding domain ENTPD1 and the phytocompounds



(a) Obacunone, (b) Limonin-7-oxime, and (c) Obacunone-7-oxime acetate, respectively from *Citrus limon.*



(a) Limonin, (b) Gibberellin A3, and (c) Gibberellin respectively from *Citrus reticulata.*

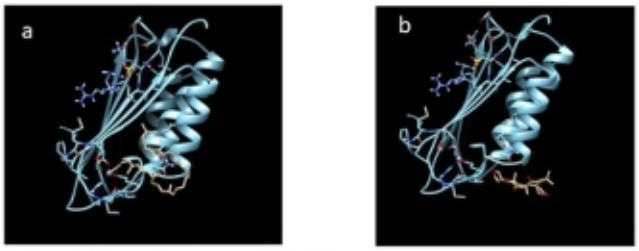
Figure 3 : 3D illustration of the docked structure of the mutant-binding domain ENTPD1 and phytocompounds from *C.limon , C.reticulata.*

Table 6 :

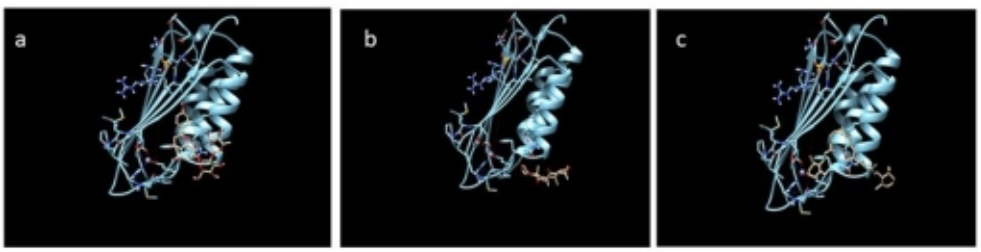
ADMET analysis using ADMET Lab 2.0								
Sl. No.	Citrus Fruit	Name of Ligand	Log S	HIA	Pgp-sub	BBB	Carcinogenicity	Lipinski's Rule of Five
1	*Citrus limon*	Obacunone	-4.355	0.022	0	0.989	0.777	Accepted
2	*Citrus limon*	Limonin-7-oxime	-4.61	0.057	0.001	0.921	0.833	Accepted
3	*Citrus limon*	Obacunone-7-oxime acetate	-4.404	0.171	0.001	0.922	0.673	Accepted
4	*Citrus reticulata*	Limonin	-4.694	0.018	0	0.988	0.739	Accepted
5	*Citrus reticulata*	Gibberellin A3	-2.973	0.629	0.005	0.535	0.391	Accepted
6	*Citrus reticulata*	Gibberellin	-2.973	0.629	0.005	0.535	0.391	Accepted
7	*Citrus limetta*	Lycopene	-7.642	0.02	0.758	0.001	0.026	Rejected
8	*Citrus aurantiifolia*	Limonin	-4.694	0.018	0	0.988	0.739	Accepted
9	*Citrus maxima*	Naringin	-3.43	0.927	0.974	0.347	0.795	Rejected
10	*Citrus maxima*	Obacunone	-4.355	0.022	0	0.989	0.777	Accepted
11	*Citrus Maxima*	Alpha-Carotene	-7.973	0.038	0.353	0	0.043	Rejected

Table 6 shows the carcinogenicity analysis using ADMET lab 2.0 of the phytocompounds found in citrus seeds.

3D illustration of the docked structure of the mutant-binding domain ENTPD1 and the phytocompounds



(a) Lycopene from *Citrus limetta* and (b) Limonin from *Citrus aurantiifolia*, respectively.



(a) Naringin (b) Obacunone (c) Alpha carotene from *Citrus maxima* , respectively.

Figure 4 : 33D illustration of the docked structure of the mutant-binding domain ENTPD1 and phytocompounds from *C.aurantiifolia* and *C.maxima*

Table 7 :

Prediction of Biological and Pharmacological activities using PASS Server					
Sl. No.	Name of Citrus Fruit	Name of Ligand	Shows Anticarcinogenic Properties	Shows Antiviral Properties	Name of Viruses (Antiviral Properties)
1	*Citrus limon*	Obacunone	Yes	Yes	Herpes
2	*Citrus limon*	Limonin-7-oxime	Yes	Yes	HIV
3	*Citrus limon*	Obacunone-7-oxime acetate	Yes	Yes	Rhinovirus
4	*Citrus reticulata*	Limonin	Yes	Yes	Herpes
5	*Citrus reticulata*	Gibberellin A3	Yes	Yes	Rhinovirus
6	*Citrus reticulata*	Gibberellin	Yes	Yes	Rhinovirus
7	*Citrus limetta*	Lycopene	Yes	Yes	Rhinovirus
8	*Citrus aurantiifolia*	Limonin	Yes	Yes	Herpes
9	*Citrus maxima*	Naringin	Yes	Yes	Herpes, Hepatitis B, Rhinovirus
10	*Citrus maxima*	Obacunone	Yes	Yes	Herpes
11	*Citrus maxima*	Alpha-Carotene	Yes	Yes	Herpes

Table 7 Predicts the role of of Biological and Pharmacological activities using PASS Server.

conventional Hydrogen bonds having residues GLY 542, SER 541, THR 539, PHE 538, and ASP 536, as shown in Figure. 3.

Lycopene (*Citrus limetta*)

Lycopene forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes two Alkyl interactions with residues LEU 559 and LEU 543, as shown in Figure. 4.

Limonin (*Citrus aurantiifolia*)

Limonin forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes three conventional Hydrogen bonds having residues SER 541, THR 539, and PHE 538, as well as a single Carbon-Hydrogen bond with residue ASP 536, as shown in Figure 4.

Naringin (*Citrus maxima*)

Limonin forms different 3D interactions with the mutant-binding domain ENTPD1. It includes three conventional Hydrogen bonds having residues ASP 536, THR 539, and THR 544, two Carbon-Hydrogen bonds with residues THR 558 and GLY 540, as well as two Pi-Alkyl interactions having residues LEU 559 and LEU 543, as shown in Figure. 4.

Obacunone (*Citrus maxima*)

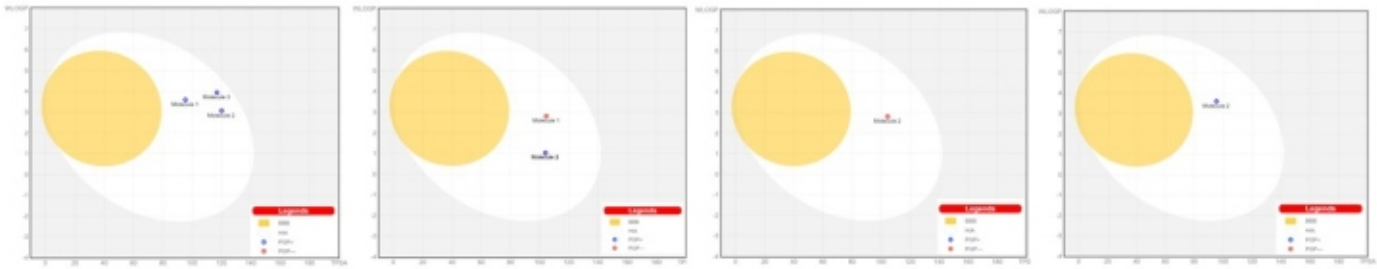
Obacunone forms different 3D interactions with the mutant-binding domain ENTPD1. It includes four conventional Hydrogen bonds having residues SER 541, THR 539, ASP 536, and PHE 538, as shown in Figure 4.

Alpha-Carotene (*Citrus maxima*)

Alpha-Carotene forms different 2D and 3D interactions with the mutant-binding domain ENTPD1. It includes only one Alkyl interaction having residue LEU 543. Figure 4.

Drug-likeness Prediction, ADMET analysis, and Toxicity Prediction

Lipinski's Rule of Five or Rule of 5 helps researchers differentiate between compounds that show drug-like characteristics and others that are simple non-drug-like biomolecules. For this study, the drug-likeness test of the topmost well-docked compounds was carried out following the Rule of Five or Lipinski's Rule of Five. The ADMET analysis of the same was done, and a Boiled-Egg illustration was generated using the Swiss-ADME web-based tool and ADMETLAB 2.0, as shown in Table 4. The Boiled-Egg analysis also helped us predict and



(a) Obacunone, (b) Limonin-7-oxime, and (c) Obacunone-7-oxime acetate, respectively, from ***Citrus limon.***

(a) Limonin, (b) Gibberellin A3, and (c) Gibberellin, respectively, from ***Citrus reticulata.***

(a) Lycopene and (b) Limonin from ***Citrus limetta*** **and** ***Citrus aurantiifolia***

(a) Naringin, (b) Obacunone, and (c) Alpha-Carotene, respectively, from ***Citrus maxima***

Boiled-Egg analysis of the phytocompounds

Figure 5

analyse the Brain-access Blood Barrier (BBB) and gastrointestinal or Human Intestinal Absorption (HIA) characteristics of the best-docked compounds. Additionally, the toxicity of the phytocompounds (Table 5) was predicted using ProTox 2 prediction server. In the end, the top phytocompounds from each of the citrus fruits' seeds were analysed within the standard scale of water solubility (log_s), permeability glycoprotein substrate, BBB, HIA, carcinogenic characteristics, as well as Lipinski's Rule of Five validation (Table 6).

Comparisons between Swiss ADMET Lab and ProTox-2 along with Pass Server

It was observed that in the ADMET Lab 2.0 (Table 6), all the top phytocompounds followed Lipinski's Rule of Five, with the exception of Lycopene (from *Citrus limetta*), Naringin and Alpha-Carotene (from *Citrus maxima*). During the prediction of toxicity using Pro-Tox-2 Server (Table 5), it was detected that that phytocompounds Gibberellin A3 and Gibberellin (from *Citrus reticulata*) showed the highest LD50 (1100 mg/kg) value, that is, 6300 mg/kg. In the PASS Server, the antiviral and anticarcinogenic properties of the top phytocompounds were noted, with the compound Naringin (from *Citrus maxima*) showing antiviral properties against many viral diseases (Table 7).

3. DISCUSSION

The review "The Second Life of Citrus Fruit Waste: A Valuable Source of Bioactive Compounds" published by MDPI journal probed into the chemical composition of discarded seeds of Hamlin, Natal, Perario, and Valencia orange varieties account for the high content of carotenoids, phenolic compounds, tocopherols, and phytosterols, which play an important role in the free radical scavenging capacity of this by-product [22] Another study named "The Potential of Tree Fruit Stone and Seed Wastes in Greece as Sources of Bioactive Ingredients" from the journal Food Waste Strategies to Reuse and Prevention stated that citrus seeds are the only naturally occurring source of limonoid aglycones.The total limonoid content and composition can vary greatly depending on the cultivar and the method of analysis. Lemon and orange seeds, according to Bonaccorsi and colleagues, contain 375 and 114 mg/kg of limonoids, respectively. These values were significantly lower than those reported by other authors for lemon and orange seeds (18.93 and 22.33 mg/g dry seed, respectively). In lemon, grapefruit, tangerine, and orange seeds, the average concentration of total limonoid glucosides and aglycones has been found to be 6.1 and 13.5 mg/g, respectively. Limonin is the most abundant constituent and one of six limonoid aglycones that have been identified as inherently bitter (limonin, nomilin, obacunonic acid, changing, deoxylimonoic acid, and nomilinic acid). Limonin and some of its derivatives and analogs (limonin 17- d-glucopyranoside, limonin carboxymethoxime, and deoxylimonin) are regarded as potent antineoplastic agents. On the other hand, two nomilin derivatives, deacetylnomilin and nomilin glucoside, have been reported to be the most effective inhibitors of estrogen receptor-positive breast cancer cells. The furan group, which is a structural feature shared by all limonoids, appears to be the site of several physiological activities. Changes in the A ring of the limonoid nucleus, for example, can result in a loss of anti-cancer activity[23,24]

Among the five lemon samples, we have 11 phytocompounds depending upon good binding energy with the target protein. From *Citrus limon* three phytocompounds i.e Obacunone, Limonin -7 Oxime, and Obacunone 7 oxime shows drug likeliness, prediction accuracy was 68.07%,68.07 %, and 67.38% respectively, from *Citrus reticulata* Limonin, Gibberellin A3, and Gibberellin, among them two shows drug likeliness, PGP+, And PGP- with 100% accuracy. Whereas from *C. limetta* and *C.aurantiifolia,* two phytocompounds lycopene don't show drug likeliness and Limone shows drug likeliness (PGP -) respectively, with both accuracy levels (69.26%). From C.maxima among three phytocompounds only one; Obacunone has shown drug likeliness (PGP+), Naringin, and Alpha-carotene don't show drug likeliness with an accuracy level of 70.97%, 68.07 %, 69.26% resp. Obacunone, Limonin-7-oxime, Obacunone-7-oxime acetate from *C.limon,* Limonin, Gibberellin A3, Gibberellin from *C.reticulata*, Lycopene from *C.limetta*, Limonin from *C. aurantiifolia*, Naringin, Obacunone, Alpha-Carotene from *C. maxima* has shown anticancer activity. These

ligands from the citrus fruits show potential binding to the hallmark gene ENTPD1, and the results extracted from the PASS server justify this statement that all of these ligands have anticancer activity

The above-mentioned ligands show antiviral properties too, Obacunone, Limonin-7-oxime, and Obacunone-7-oxime acetate from *C.limon show* antiviral properties against Herpes, HIV, and Rhinovirus. Limonin, Gibberellin A3, and Gibberellin from *C.reticulata* show antiviral properties against Herpes, Rhinovirus, and Rhinovirus respectively. Lycopene from *C.limetta* has antiviral properties against Rhinovirus, and Limonin from *C. aurantiifolia* shows antiviral properties against Herpes. Naringin from *C. maxima* has shown antiviral properties against Herpes, Hepatitis B, and Rhinovirus. Obacunone, Alpha-Carotene from *C.maxima* has shown antiviral properties against Herpes.

4. CONCLUSION

Depending upon the phytochemical-rich substances citrus seed can be the potential pool for researchers for reuse and valorization, not only that the significant phytocompounds mentioned and discussed in this paper can be used as a good target for the protein which is a hallmark gene of oral cancer. Recycling citrus seeds will add some economic value to produce a new drug delivery system. The findings indicated limonin present in *C. reticulata and C. aurantiifolia* constituted a needful source of medication because it shows good drug likeliness, which may hamper the growth of metastatic cells by boosting immunity. Molecular docking analysis of different citrus seeds shows that Obacunone from *C.limon and C. maxima* can be a new choice for anti-Herpes medication.

ACKNOWLEDGEMENTS

The authors are thankful to Chancellor, Techno India University, Kolkata, West Bengal for providing the necessary infrastructural facilities.

FUNDING

Not applicable.

CONFLICT OF INTEREST

There is no conflict of interest.

AUTHOR CONTRIBUTION

MB and SS contributed to the concept and design. SG contributed to the data acquisition and analysis. SS contributed to the writing and review of the paper. MB contributed overall supervision of the study. All authors read and approved the manuscript.

REFERENCE

1. National Cancer Institute, Surveillance, Epidemiology, and end result program. [cited 2023 March] [https://seer.cancer.gov/]
2. Chen Y-F, Wei Y-Y, Yang C-C, Liu C-J, Yeh L-Y, Chou C-H*et al*. miR-125b suppresses oral oncogenicity by targeting the anti-oxidative gene PRXL2A. Redox Biol. 2019 , 22:101140.
3. Kao SY and Lim E. An overview of detection and screening of oral cancer in Taiwan, Chin. Chin J Dent Res.2015. 18(1):7-12.
4. Krishna A, Singh S, Kumar V, *et al.* Molecular concept in human oral cancer. *National Journal of Maxillofacial Surgery*,2015 *6*(1), 9-15.
5. Sengupta S and Bhattacharya M. Different Types of Gene Expression Profiles and Pathway Enrichment Analysis of Oral Dysplastic Keratinocyte Cells Caused by Heavy Smoking. Asian J Biol Life Sci. 2022. 11(3):690-7.
6. Vadlamani VMK, Gunasinghe KKJ, Chee XW, Rahman T *et al.* Human soluble CD39 displays substrate inhibition in a substrate-specific manner. Sci Rep. 2023 2;13(1):8958.
7. Vaisitti T, Arruga F, Guerra G, Deaglio S. Ectonucleotidases in blood malignancies: A tale of surface markers and therapeutic targets. Front Immunol 2019. 2301(10). 1664-3224
8. Jiang X, Wu X, Xiao Y, Wang P *et al.* The ectonucleotidases CD39 and CD73 on T cells: The new pillar of hematological malignancy. Front. Immunol. 2023 14:1110325.
9. Zayed A, Badawy M. T, & Farag M. A. Valorization and extraction optimization of Citrus seeds for food and functional food applications. Food Chemistry,2021; 355, 129609.
10. Moulehi I , Bourgou S , Ourghemmi I and Tounsi MS. Variety and ripening impact on phenolic composition and antioxidant activity of mandarin (*Citrus reticulate* Blanco) and bitter orange (*Citrus aurantium* L.) seeds extracts. *Ind. Crops Prod.* 2012 *39*, 7480.
11. Sir Elkhatim KA., Elagib R. AA., & Hassan AB. Content of phenolic compounds and vitamin C and antioxidant activity in wasted parts of Sudanese citrus fruits. Food Science & Nutrition, 2018. 6(5), 12141219.
12. Falcinelli B, Famiani F, Paoletti A, D'Egidio S *et al.* Phenolic Compounds and Antioxidant Activity of Sprouts from Seeds of *Citrus* Species. *Agriculture*.; 2020 10(2):33.
13. Berman HM, Westbrook J, Feng Z, Gilliland G, *et al*. 2000 The protein data bank. Nucleic Acids Res; 28 235-42.
14. European Bioinformatics Institute. PDBsum Home Page. Cambridge: European Bioinformatics Institute. [Last accessed on 2022 Sep 09]. [https://www.ebi.ac.uk/]
15. Kim S, Chen J, Cheng T, Gindulyte A, *et al.* 2021: New data content and improved web interfaces. Nucleic Acids Res PubChem in 2021 49:D1 388-95. [https://www.ebi.ac.uk/pdbsum]
16. McConkey BJ, Sobolev V, Edelman M. The performance of current methods in ligand-protein docking. Curr Sci 2002. 83:845-56.
17. Trott O, Olson AJ. Auto Dock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem; 2010. 31: 455-461.
18. O'Boyle NM, Banck M, James CA, Morley, *et al*. Open Babel: An open chemical toolbox. J Cheminform 2011 3:33.
19. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep; 2017. 7:42717.

20. Xiong G, Wu Z, Yi J, Fu L, *et al.* ADMETlab 2.0: An integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res; 2021. 49:W5-14.

21. Daina A, Zoete V. ABOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. ChemMedChem 2016.11 1117-11121.

22. Russo, C.; Maugeri, A.; Lombardo, G.E.; Musumeci, L.; Barreca, D.; Rapisarda, A.; Cirmi, S.; Navarra, M. The Second Life of Citrus Fruit Waste: A Valuable Source of BioactiveCompounds.Molecules 2021,26 (19): 5991.

23. Ordoudi, S.A.; Bakirtzi, C.; Tsimidou, M.Z. The Potential of Tree Fruit Stone and Seed Wastes in Greece as Sources of Bioactive Ingredients. Recycling 2018, 3 (1) : 9.

24. Anthony C. Cemaluk, Egbuonu & Osuji, Chigozirim. Proximate Compositions and Antibacterial Activity of Citrus sinensis (Sweet Orange) Peel and Seed Extracts. European Journal of Medicinal Plants. 2016. 12 (1-7). 10.9734

Cite this article : Sudeshna Sengupta, Satavisha Ghorui, Malavika Bhattacharya
Molecular docking Analysis of the phytocompounds found in Citrus seeds and their effects on the hallmark gene of HNSCC
Asian J. Pharm. Hea. Sci.. 2023;13(3):2878-2899. DOI : 10.5530/ajphs.2023.13.51