Vol. 4 No. 3 (2025)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

Online ISSN 3007-2387

Print ISSN 3007-2379

Identification Of Novel Inhibitors Against Cyclin-Dependent Kinase From Phytocompounds Of *Citrus sinensis* Peel Extract By *In Silico* Approach

Lubna Kanwal*
Department of Zoology, University of Okara, Okara, Pakistan
Phone: +92 (0) 3052999575, Email: lubna.kanwal@uo.edu.pk

Nazia Tabasum
Department of Zoology, University of Okara, Okara, Pakistan

Vishal Haer
Department of Zoology, University of Okara, Okara, Pakistan

Maqsood Ahmad
Department of Statistics, University of Okara, Okara, Pakistan

Author Details

Keywords: *Citrus sinensis*; Cyclin-dependent kinase; Apoptosis; Molecular docking; Pharmacokinetics.

Received on 25 Aug 2025
Accepted on 20 Sep 2025
Published on 30 Sep 2025

Corresponding E-mail & Author*:

Lubna Kanwal*
Department of Zoology, University of Okara, Okara, Pakistan
Phone: +92 (0) 3052999575, Email: lubna.kanwal@uo.edu.pk

Abstract

Sweet orange, biologically known as *Citrus sinensis*, is a citrus fruit with a significant medicinal value. In this study, the bioactive compounds of *C. sinensis* in ethanolic peel extract were identified using gas chromatography-mass spectrometry (GC-MS) and their inhibitory effect on the activity of Cyclin-dependent kinase (CDK), which controls apoptosis, was tested. 12 phytocompounds were isolated from *C. sinensis'* ethanolic peel extract using gas chromatography-mass spectrometry (GC-MS). The SwissADME web program was used to determine the pharmacokinetics and drug-likeness of every compound. The findings were in agreement with Lipinski Rule of Five, the data reflected desirable oral bioavailability and drug-like properties. Additionally, PyRx virtual screening tool was used to dock molecules to measure CDK binding affinity. Discovery Studio Visualiser was used to carry out interaction analysis. Numerous phytocompounds showed significant affinity for the CDK active site in docking tests. The compounds with the highest docking scores were phthalic acid (-6.3 kcal/mol), 2-methoxy-4-vinyl phenol (-5.3), and 4-vinyl phenol (-6). The findings demonstrate that *Citrus sinensis* peel bioactive components suppress CDK, suggeststing their possible therapeutic implications in various diseases particularly in cancer. Nevertheless, additional *in vitro* and *in vivo* research is required to validate the therapeutic effectiveness of the compounds.

Introduction

With the increasing global cancer burden, natural therapeutic drugs should be researched. There is growing body of evidence indicating that phytochemicals prevent and treat cancer. This confirms the use of the plant based drugs in traditional medicine. Herbal medicine involves the application of plant remedies in and out of the body.

Arthritis, allergies, tiredness, migraines, skin infections, wounds, gastrointestinal disorders, burns, and cancer can now be treated with plant-based medicines which proves that food can be medicine (Sam *et al*., 2019). Fruits, vegetables, and whole grains contain chemicals that detoxify free radicals, reducing oxidative damage to biological cells (Saini *et al*., 2022). Anti-inflammatory, antibacterial, antioxidant, and anticancer chemicals are present in fruit peels that are typically kept as agricultural waste. (Parmar *et al*., 2008; Panwar *et al*., 2021).

Citrus sinensisis is often referred to as MUSSAMMI or SWEET ORANGE, which is a species of the family Rutaceae and is among the most extensively grown fruit crops in the world (Parle and Chaturvedi, 2012). Although oranges are typically grown from winter to summer, depending on the type, extracting the juice results in large volumes of peel as waste leading to environmental pollution (Yeoh *et al*., 2008; Shravan *et al*., 2018). Nonetheless, the by-product has more useful phytochemicals than consumable pulp. These are phenolics, flavonoids and essential oils phytochemicals (Sok Sian Liew *et al.*, 2018).

Cyclin-dependent kinases (CDKs) become significant therapeutic targets in cancer since they control the cell cycle, as well as apoptosis (Geleta *et al*., 2016). These enzymes are good targets of therapy since deregulation of CDK activity is prevalent in most cancers. Natural product library screening against these protein targets has been made easier using new computational tools and accelerates the discovery of lead molecules. Such techniques are molecular docking and dynamics simulations (Bhandari *et al*., 2024). *In silico* methods have the ability to rapidly and effectively determine binding affinities and drug-likeness before subjecting to experimental treatment (Zufairo *et al.*, 2023).

The analysis through gas chromatography-mass spectrometry (GC-MS) is capable of revealing complex plant extract phytochemical composition (Aboul et al., 2020). Computational guiding enhances the discovery and optimization of hits and thus such techniques are up to date in the studies of natural products The incorporation of *in silico* strategies, such as ADMET prediction and molecular docking, is still limited, despite recent works demonstrating its usefulness for aiding the mechanistic interpretation of in vitro results and better understanding the drug-like properties of biologically active compounds.

(Hmamou *et al*., 2026).

In this study the phytocomponents of the *C. sinensis* ethanolic peel extract were analyzed through the GC-MS. The drug-like properties of the compounds were also determined using Lipinski Rule of five (RO5). This study aims to locate potential natural sources of CDK inhibitors to use in future anticancer drugs development and provide contribution to the growing body of research that constitute the usefulness of citrus processing wastes as a source of pharmaceutical solutions.

Materials and Methods

Collection of plant material

Citrus sinensis peel was purchased from different local markets and their peels were removed using a peeler. Following that, the peels were cleaned and dried in the shade.

Preparation of ethanolic extract of *Citrus sinensis peel*

Citrus sinensis peels were dried in the shade at room temperature. After drying, the peels were ground into a coarse powder with an electric grinder. 50 grams of peel powder and 75% ethanol were mixed in 1 gram to 10 milliliters on an orbital shaker. The mixture was stirred for 72 hours at 24°C. Following this, the mixture was filtered by passing it through a Whatman filter paper No. 1 and then evaporated using rotay evaporator . After drying the extract was refrigerated at 4 °C until use (Nauroze *et al.*, 2023).

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Bioactive chemicals were examined using GC-MS in an ethanolic *citrus sinensis* extract. Agilent's GC 7890B and MS 5977A gas chromatography-mass spectrometry (GC-MS) system used a capillary standard and DB 5MS nonpolar column. It measured 30 millimeters long, 0.25 millimeters wide, and 0.25 micrometers thick. Helium was the carrier gas, and the mobile phase flowed one milliliter per minute. The oven was heated to 280°C at 10°C per minute. Each injection was given a volume of 1 μL. For GC-MS, a 70 eV electron ionization energy system was used. Duration was 65 minutes. The identification of the specific compounds was done by comparing the m/z ratios with the samples that were proven to be valid by Sigma-Aldrich using the data of the mass spectrometry of the NIST Mass Spectral Library (Saravanan *et al.*, 2022).

Pharmacokinetics profile

The phytochemicals were put via Swiss ADME pharmacokinetic profile after *Citrus sinensis* GCMS analysis. Individual active component SMILES formulas were used to achieve this.

Study of Ligand-protein docking

This study used molecular docking to analyze *C. sinensis* phytocompounds and their effects on CDK. Molecular docking studies using PyRx 0.8 revealed ligand-protein binding mechanisms. PDB provided the receptor protein's 3D structure in PDB format. The PubChem database included SDF chemical structures of active phytoconstituents. After integrating polar hydrogen atoms, eliminating water molecules, and releasing protein ligands, the desired protein structure was created. Then, the BIOVIA Discovery Studio Visualizer (version 21.1.0.20298) was used to generate and visualize phyto compound-target protein interactions and maps (Arif *et al*., 2024).

Results

Identification of bioactive compounds of *C. sinensis* peel extract by GCMS Analysis

Gas chromatography-mass spectrometry analysis reveals 12 peaks that show the presence of 12 bioactive compounds and their identification was based on their retention time (Rt) and peak area. Retention duration and mass spectra are compared to databases or standards to locate peaks. This method allows for a more precise identification of the compounds that are present in the ethanolic extract of plants. A higher peak area shows more concentration of that compound in the extract. These compounds with their class name, peak area, retention time, and structure are given in Table (1). As shown in fig. (1), 2-methoxy-4-vinylphenol shows the highest peak of 15.03% with RT of 7.092 minutes while pathelic acid shows the lowest peak of 1.59% with RT of 18.341 minutes.

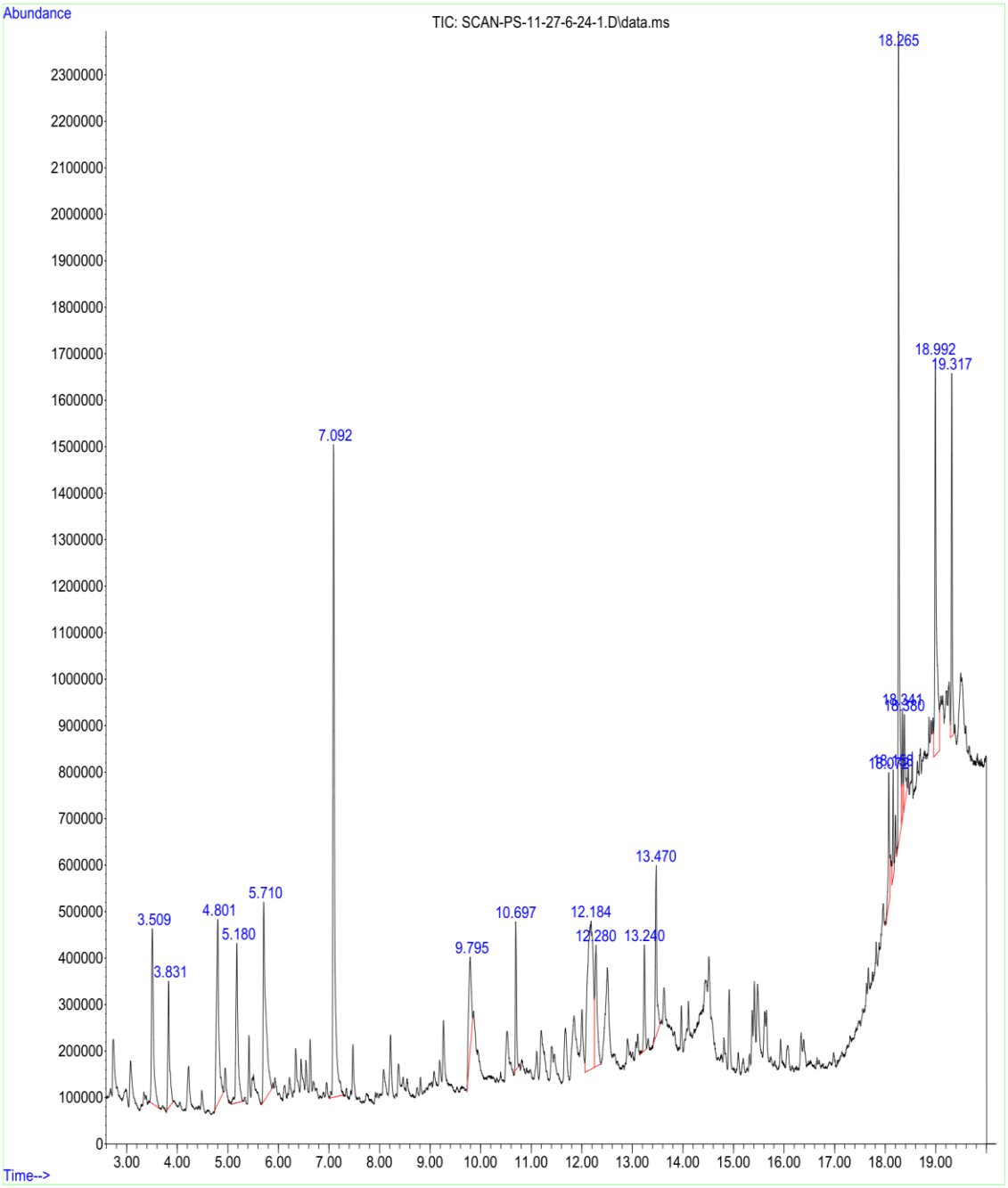

Figure 1: GCMS chromatogram of an ethanolic extract of *Citrus sinensis*

Table1: Bioactive compounds derived from ethanolic peel extract of *Citrus sinensis* using GC-MS

Sr No.	Compound name	Class name	Peak area (%)	RT (Min)
1	3,5-Octanedione	Diketone	5.00	3.509
2	4,5-Diamino-2-hydroxypyrimidine	Pyrimidines	**2.99**	**3.831**

3	4H-pyran-4-one	Pyranones	**6.16**	**4.801**
4	1,3,5-Trioxane	cyclic ethers	**4.69**	**5.180**
5	4-vinyl phenol	Phenol derivatives and vinyl group	**7.41**	**5.710**
6	2-methoxy-4-vinyl phenol	Phenols	**15.03**	**7.092**
7	2-pentanone	Pentanota	**3.45**	**9.795**
8	Phenols	Dihydroxyphenols	**2.32**	**10.697**
9	Propanoic acid	Non-Steroidal Anti-inflammatory Drugs	**11.61**	**12.184**
10	Phthalic acid	Aromatic dicarboxylic acid	**1.59**	**18.341**
11	Valeric acid	Straight-chain fatty acids	**1.70**	**18.380**
12	13-docosenamide	Fatty amides	**4.96**	**19.317**

Pharmacokinetic profile

Pharmacokinetic analyses have been done through SwissADME by entering their SMILES formula to investigate the effects of these drugs or compounds within a biological system over time, the results are shown in Table (2).

Table 1: Pharmacokinetic analysis of propolis constituents.

Sr. no	Compound	Molecular mass (g/mol)	Acceptor H	Donor H	Log P	Lipinski
1	3,5-Octanedione	142.20	2	0	1.68	yes
2	4,5-Diamino-2-hydroxypyrimidine	126.12	2	3	-0.09	yes

3	4H-pyran-4-one	96.08	2	0	1.31	yes
4	1,3,5-Trioxane	90.08	3	0	1.26	yes
5	4-vinyl phenol	120.15	1	1	1.64	yes
6	2-methoxy-4-vinyl phenol	150.17	2	1	2.14	yes
7	2-pentanone	86.13	1	0	1.61	yes
8	Phenols	197.45	1	1	1.97	yes
9	Propanoic acid	74.01	2	1	0.89	yes
10	Phthalic acid	166.3	4	2	0.60	yes
11	Valeric acid	102.13	2	1	1.32	yes
12	13-docosenamide	337.58	1	1	5.10	yes

In Silico-based screening of *Citrus sinensis* to identify cyclin-dependent kinase (CDK)

Molecular docking of bioactive compounds found in the ethanolic extract of *C. sinensis* has been performed against CDK using the Pyryx docking tool and the results are shown in table (3).

Table 2:Bioactive compounds with docking score and biological activities.

Serial no.	Compound	Class	Biological activity	CID	Docking
1.	3,5-Octanedione	Diketone	It has antibacterial and antifungal effects (Sevcikova *et al*., 2014)	80602	-4.7
2.	4,5-Diamino-2-hydroxypyrimidine	Pyrimidines	It has anti-microbial activity (Singh *et al.,* 2024)	248637	-4.9
3.	4H-pyran-4-one	pyranones	It has anti-microbial,anti-fungal, anti-oxidant, and anti-inflammatory activity (El-Bana *et al*., 2024)	7968	-4.3
4.	1,3,5-Trioxane	cyclic ethers	It has anti-malarial, antiviral, anti-bacterial and anti-tumor activities	8081	-3.6
5.	4-vinyl phenol	Phenol derivatives and vinyl group	It has anti-microbial and anti-oxidant properties (Girawale *et al.,* 2023)	62453	-6
6.	2-methoxy-4-vinyl phenol	Phenols	It has anti-oxidant, anti-microbial, Anti-inflammatory, Neuroprotective, and Potential Cancer Chemopreventive Effects (Jeong *et al.*, 2011)	332	-5.3
7.	2-pentanone	Ketone	It has anti-fungal, anti-bacterial, and anti-tubercular activity	7895	-4.1

			(Germinara *et al*., 2012).		
8.	Phenols	Dihydroxyphenols	It has anti-oxidant, anti-genotoxic, and cytostatic activities (Stasiuk *et al*., 2010).	7271	-4.9
9.	Propanoic acid	Non-Steroidal Anti-inflammatory Drugs	It has antibacterial, anticancerous, Anti-Convulsant, and anti-inflammatory activities (Mokale *et al*., 2010).	1032	-3.8
10	Phthalic acid;	Aromatic dicarboxylic acid	allelopathic, anti-microbial, and insecticidal (Huang *et al*., 2021).	1017	-6.3
11	Valeric acid	Straight-chain fatty acids	Suppressing Oxidative Stress, Neuroinflammation, and Modulating Autophagy Pathways (Chen *et al*., 2018).	7991	-4.2

Molecular Docking of Phytocompounds with CDK

A total of 12 bioactive compounds are found in the ethanolic peel extract of *C. sinensis*. The compounds identified with good binding energy and their attraction with the target protein are shown in figures (2-4) below using the Discovery Studio tool. These are 2-methoxy-4-vinylphenol with a binding score of -5.3 in fig.(2), Phthalic acid with a binding score of -6.3 in fig.(3), 4-vinylphenol with a binding score of -6 in fig.(4).

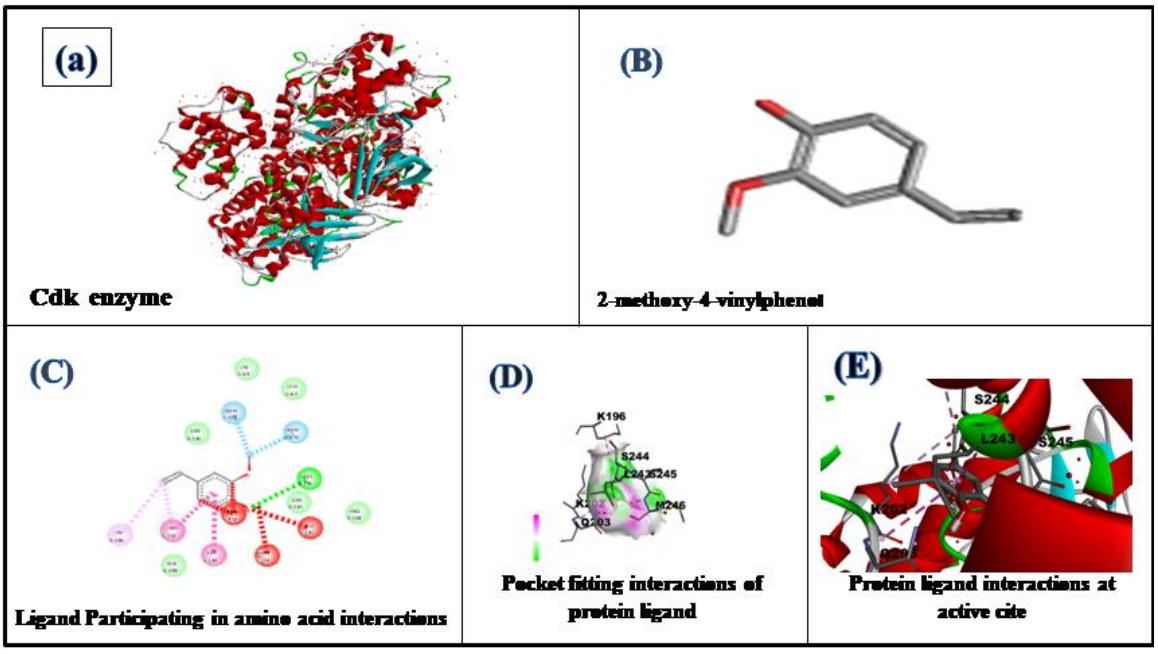

Figure 2: Molecular interaction of 2-methoxy-4-vinyl phenol with cyclin-dependent kinase

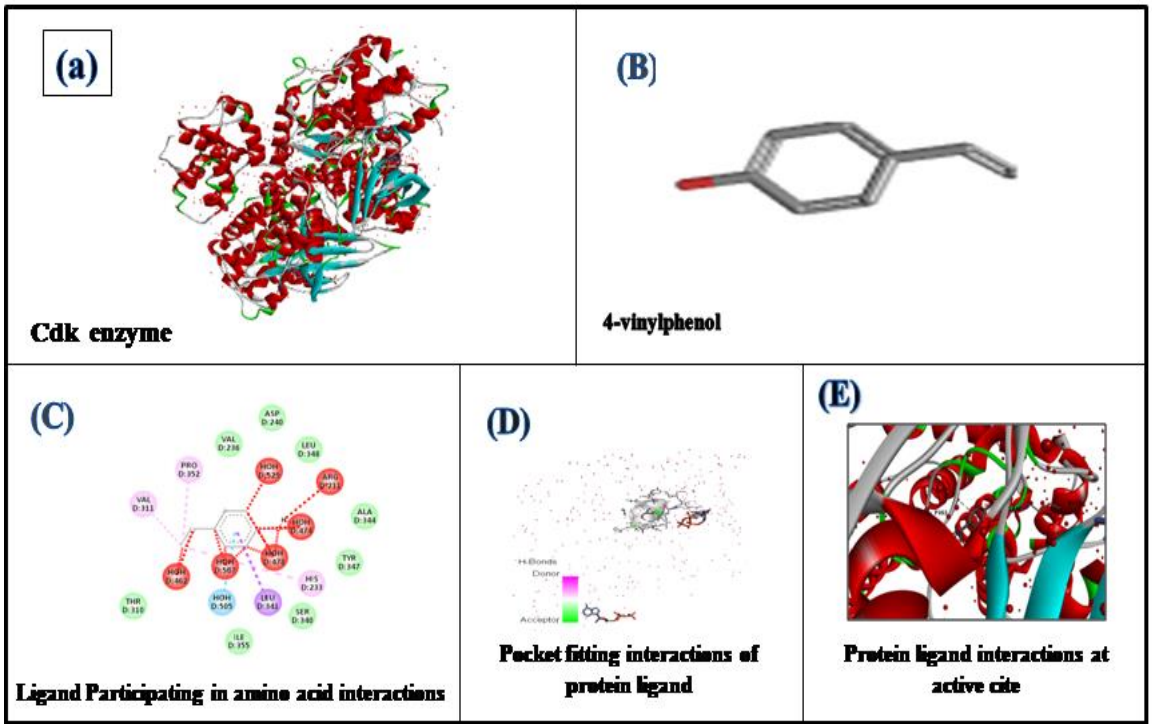

Figure 3: Molecular interaction of 4-vinyl phenol with cyclin-dependent kinase

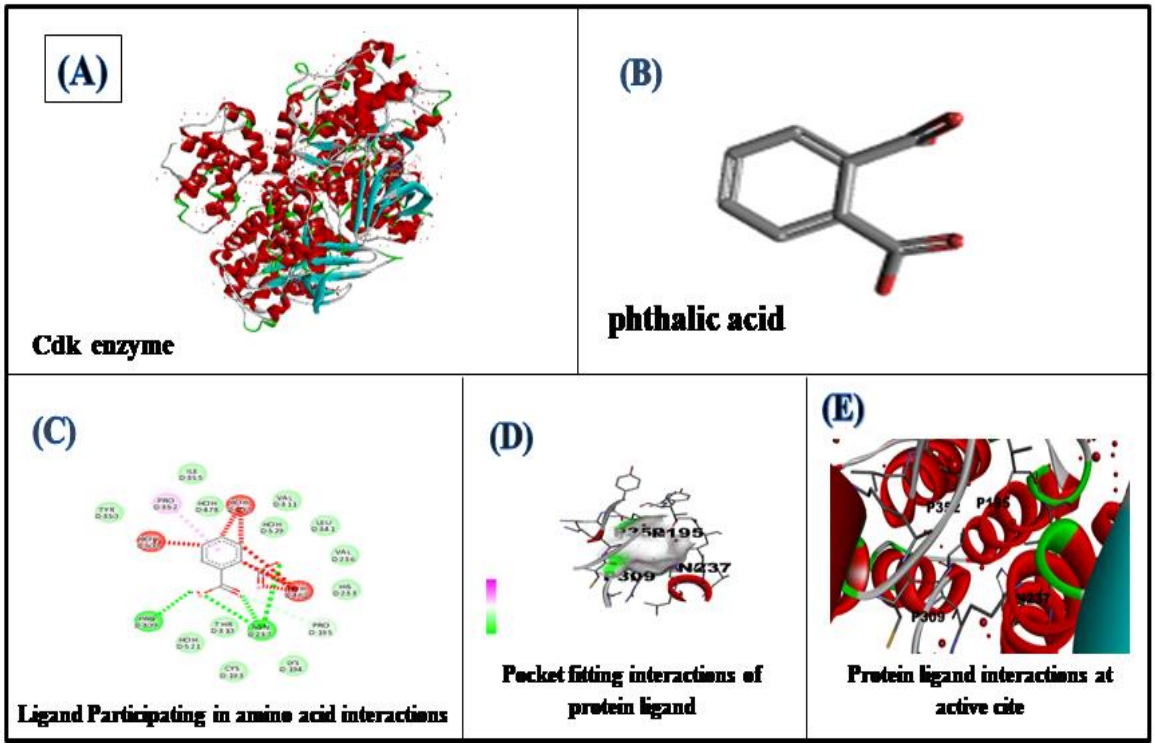

Figure 4: Molecular interaction of Phthalic acid with cyclin-dependent kinase

Discussion

Medicinal plants have different types of phytochemicals that may treat many diseases. Many people use medicinal plants to cure different diseases. Plant-based natural remedies are widely used in developing nations and are given special attention because of their multiple protective benefits and beneficial effects on human health (Alam *et al*., 2020). The goal of the current research was to identify the chemical components and assess their biological and pharmacological potential to understand the significance of the ethanolic extract of *C. sinensis.*

The GC–MS analysis was carried out to identify the various volatile matter components found in the ethanolic extract of *C. sinensis* peel. This study identified 12 phytocompounds from the ethanolic extract of peel of *C. sinens.* While the maximum peak was acquired for 2-methoxy-4-vinyl phenol which is approximately 15.03%, indicating the presence of a large quantity of this compound in this extract. 2-methoxy-4-vinyl phenol has been reported for potential antioxidant activity as well as anticancer potential. The amount of 2-methoxy-4-vinyl phenol in *C. sinensis* peel extract increases the possibility that it has natural anticancerous, antibacterial, and antifungal properties. Citrus plants contain flavonols, anthocyanins, favas, and flavones. *Citrus sinensis* contains several chemicals. Coumarins, flavonoids, hydroxy amides, steroids, alkanes, fatty acids, carbohydrates, peptides, carotenoids, volatile molecules, and minerals including potassium, magnesium, calcium, and salt. It has also been used to treat obesity, menstrual irregularities, high blood pressure, anxiety, depression, and stress.

Molecular docking is an exigent part of in silico approaches and also an important tool in structural molecular biology and computer-assisted-drug design. The pharmacokinetics parameter analysis by SwissADME is also a part of the in silico approaches (Shaik *et al.,* 2025). In this current study molecular docking was also performed against the 12 bioactive compounds with CDK enzyme by using PyRx screening tool and Discovery studio. 3 out of 12 displayed a very good binding activity to control the overexpression of CDK. These are 2-methoxy-4-vinyl phenol with a binding score of -5.3, Phthalic acid with a binding score of -6.3, and 4-vinyl phenol with a binding score of -6. The pharmacokinetics and the drug-likeness properties of these phytochemicals were then analyzed using the SwissADME web tool. All the 12 compounds of *C. sinensis*, showed drug-like properties following Lipinski's Rule of Five (RO5).

The sweet orange, also known as *C. sinensis,* can lower cholesterol, and blood pressure, and boost the immune system. It also prevents cancer, kidney stones, stomach ulcers, and arteriosclerosis (Etebu and Nwauzoma 2014). CDK dysregulation, which is connected to cancer genetic pathology, increases anti-apoptotic protein production and decreases cell-cycle checkpoint function (Geleta *et al*., 2016). In this study, we use the CDK enzyme which is a multifunctional enzyme that can modify various protein substrates involved in cell cycle progression. Cancer is distinguished from other diseases by uncontrolled cell growth that leads to tumors. The results open up avenues for further investigations of the long-term use of such bioactive chemicals in cancer management.

Conclusion

Using GC-MS analysis, the current study effectively showed that the ethanolic peel extract of *Citrus sinensis* includes a variety of bioactive phytochemicals, with all 12 discovered compounds demonstrating drug-likeness qualities in accordance with Lipinski's Rule of Five. The molecular docking study showed that these compounds exhibit favourable binding interactions with cyclin-dependent kinase (CDK), a cancer treatment target. The traditional use of *C. sinensis* peel in cancer prevention and the potential therapeutic compounds in citrus industry waste are supported by computational analysis. However, additional cytotoxicity testing,both *in vitro,* and *in vivo,* is required before using these bioactive chemicals as anticancer therapy.

References

Aboul Naser, A., Younis, E., El-Feky, A., Elbatanony, M., & Hamed, M. (2020). Management of Citrus sinensis peels for protection and treatment against gastric ulcer induced by ethanol in rats. *Biomarkers*, *25*(4), 349-359.

Alam, S., Emon, N. U., Rashid, M. A., Arman, M., & Haque, M. R. (2020). Investigation of biological activities of Colocasia gigantea Hook. f. leaves and PASS prediction, in silico molecular docking with ADME/T analysis of its isolated bioactive compounds. *BioRxiv*, 2020-05.

Arif, R., Bukhari, S. A., Mustafa, G., Ahmed, S., &Albeshr, M. F. (2024). Network Pharmacology and Experimental Validation to Explore the Potential Mechanism of Nigella sativa for the Treatment of Breast Cancer. Pharmaceuticals, 17(5), 617.

Bhandari, D. P., Chaudhary, P., Upadhyaya, S. R., Ranjitkar, R., Satyal, R., Adhikari, A., & Parajuli, N. (2024). Chemical variability, antioxidant and larvicidal efficacy of EOs from Citrus sinensis (L.) Osbeck peel, leaf, and flower. *Horticulturae*, *10*(6), 566.

Chen, X., Wang, H., Zhou, M., Li, X., Fang, Z., Gao, H., & Hu, W. (2018). Valproic acid attenuates traumatic brain injury-induced inflammation in vivo: involvement of autophagy and the Nrf2/ARE signaling pathway. *Frontiers in Molecular Neuroscience*, *11*, 117.

El-Bana, G. G., Salem, M. A., Helal, M. H., Alharbi, O., & Gouda, M. A. (2024). A review on the recent multicomponent synthesis of 4H-pyran derivatives. *Mini-Reviews in Organic Chemistry*, *21*(1), 73-91.

Etebu, E., & Nwauzoma, A. B. (2014). A review on sweet orange (Citrus sinensis L Osbeck): health, diseases and management. American Journal of Research Communication, 2(2), 33-70.

Geleta, B., Makonnen, E., & Abay, S. M. (2016). Cyclic dependent kinase (CDK): role in cancer pathogenesis and as drug target in cancer therapeutics. J Cancer Sci Ther, 8(6), 160-167.

Germinara, G. S., De Cristofaro, A., & Rotundo, G. (2012). Bioactivity of short-chain aliphatic ketones against adults of the granary weevil, Sitophilus granarius (L.). *Pest management science*, *68*(3), 371-377.

Girawale, S. D., Meena, S. N., Yadav, A. A., & Kodam, K. M. (2023). Biocatalysis at its finest: Bacillus paralicheniformis produces 4-vinylphenol with potent biological activities. *Biocatalysis and Agricultural Biotechnology*, *52*, 102842.

Hmamou, A., El Khomsi, M., Elrherabi, A., Khibech, O., Bendaoud, A., Bouakline, H., Lahkimi, A. (2026). *Papaver rhoeas* L. fruits: optimized extraction, phytochemical profiling, *in vitro* bioactivities, *in silico* ADMET and molecular docking analysis. *International Journal of Food Properties*, *29*(1). https://doi.org/10.1080/10942912.2026.263584

Huang, L., Zhu, X., Zhou, S., Cheng, Z., Shi, K., Zhang, C., & Shao, H. (2021). Phthalic acid esters: Natural sources and biological activities. *Toxins*, *13*(7), 495.

Mokale, S. N., Shinde, S. S., Elgire, R. D., Sangshetti, J. N., & Shinde, D. B. (2010). Synthesis and anti-inflammatory activity of some 3-(4, 6-disubtituted-2-thioxo-1, 2, 3, 4-tetrahydropyrimidin-5-yl) propanoic acid derivatives. *Bioorganic & medicinal chemistry letters*, *20*(15), 4424-4426.

Jeong, J. B., Hong, S. C., Jeong, H. J., & Koo, J. S. (2011). Anti-inflammatory effect of 2

methoxy-4-vinylphenol via the suppression of NF-κB and MAPK activation, and acetylation of histone H3. *Archives of pharmacal research*, *34*(12), 2109-2116.

Panwar, D., Saini, A., Panesar, P. S., & Chopra, H. K. (2021). Unraveling the scientific perspectives of citrus by-products utilization: Progress towards circular economy. Trends in Food Science & Technology, 111, 549–562.

Parle, M., &Chaturvedi, D. (2012). Orange: Range of benefits. *International research journal of pharmacy*, *3*(7), 59-63.

Parmar, H. S., & Kar, A. (2008). Antiperoxidative, antithyroidal, antihyperglycemic and cardioprotective role of Citrus sinensis peel extract in male mice. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *22*(6), 791-795.

Sam, S. (2019). Importance and effectiveness of herbal medicines. *Journal of pharmacognosy and phytochemistry*, *8*(2), 354-357.

Shaik, M. R., Patan, A., & Yadala, P. C. (2025). A Review of SwissADME in Morden Medicinal Chemistry: Insights on Anti-Cancer, Anti-Tubercular, and Anti-Microbial Agents. *International Journal of Current Trends in Pharmaceutical Research*, *13*(02), 90-96.

Sevcikova, Z., Pour, M., Novak, D., Ulrichova, J., & Vacek, J. (2014). Chemical properties and biological activities of cyclopentenediones: a review. Mini reviews in medicinal chemistry, 14(4), 322-331.

Singh, N., Tyagi, N., Singh, M., Kushwaha, H. R., Sharma, R. K., Shree, P., ... & Garg, M. (2024). Characterization, phytochemical profiling, antioxidant, and cytotoxicity of underutilized medicinal plants and composite flour. *Food Chemistry*, 139985.

Stasiuk, M., & Kozubek, A. (2010). Biological activity of phenolic lipids. *Cellular and Molecular life sciences*, *67*(6), 841-860.

Yeoh, S., Shi, J. T. A. G., &Langrish, T. A. G. (2008). Comparisons between different techniques for water-based extraction of pectin from orange peels. *Desalination*, *218*(1-3), 229-237.

Zufairo, S. K., Rahmawati, D. R., Meiyanto, E., & Susidarti, R. A. (2023). Citrus sinensis peel extract synergistically enhances the cytotoxic effect of chemotherapeutic agents on HepG2 cells. *Indonesian Journal of Cancer Chemoprevention*, *14*(3), 151-159.