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Identification of MAPK1 Inhibitors from *Blumea oxyodonta* and *Cardiospermum halicacabum* for Colorectal Cancer

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Abstract:
Colorectal cancer is significant health concern worldwide. Mitogen-activated protein kinase 1 (MAPK1) is a critical therapeutic target for this malignancy. Natural compounds have potency for different type of cancers due to their diverse structures. Compounds selected for this study were taken from Blumea oxyodonta and Cardiospermum halicacabum. Computational docking analysis revealed promising binding affinities of some of these compounds to MAPK1, suggesting their potential as lead molecules for developing novel therapeutic interventions against colorectal cancer.

Keywords: MAPK1, Colorectal cancer, Blumea oxyodonta, Cardiospermum halicacabum, molecular docking

Introduction:

Colorectal cancer is a prevalent disease, ranking as third most common cancer globally and second in cancer-related mortalities (Bray et al., 2018). Despite lots of research, the origin of colorectal cancer is not clear (Fang et al., 2021). People with fatty diets are prone to colorectal cancer (O'Neill et al., 2016). MAP kinases are vital enzymes that transmit signals within cells (Jonak et al., 1994). Activation of mitogen-activated protein kinases is important for cell proliferation and differentiation and blocking apoptosis (Kyriakis & Avruch, 2012). MAP kinases are activated by phosphorylation in response to extracellular signals, hence also called as extracellular signal-regulated kinases (ERKs) (Jonak et al., 1994). Overactive MAPK signalling leads to excessive growth of the colorectal lining causing tumours (Kundu et al., 2015). Hence, different natural and synthetic inhibitors are used to stop the excessive cell growth. Natural products are a vital source of developing new medicines. Natural products and their synthetically modified forms have been considered as a rich source of potential medicines (Koparde et al., 2019). Lots of natural products have been evaluated for their anticancer property and research is still going on for the locally available and highly effective compounds.

Conventional drug discovery is risky, costly and time-consuming process (Tang et al., 2006 Recent advancements in drug discovery technologies have dramatically transformed the process of identifying potential drugs candidates from nature (Tariq & Siddiqi, 1985). Computer Aided Drug Discovery (CADD) utilizes computational techniques to find potential drug candidates, virtually searching large chemical databases, optimizing drug candidates and predict pharmacological properties (Giri & Bader, 2015). The present study was carried out with an aim to identify the potential of compounds derived from *Cardiospermum halicacabum* and *Blumea oxyodonta* as promising inhibitors for MAPK1 (ERK2) protein.

Materials and methods:

Selection and retrieval of ligands:

Blumea oxyodonta and *Cardiospermum halicacabum*, plants known for their immense medicinal properties, were selected as a source of potential MAPK2 inhibitors. Kulkarni (2023) carried out HRLC-MS for ethanol and acetone extracts of stem of *C. halicacabum* and ethanol extracts of inflorescence of *B. oxyodonta* (Kulkarni, 2023). Compounds reported in the thesis were selected and analysed for their activity against colorectal cancer using PASS analysis (Filimonov et al., 2014). Out of 21 compounds 17 showed antineoplastic activities for colon cancer.

These compounds were then subjected to SwissADME (Daina et al., 2017) analysis to check whether they fulfil the ADME properties. By considering TPSA, Gastrointestinal absoption and Lipinski's rule molecules were sorted for molecular docking. Nine molecules were selected along with the reference ligand trametinib to be docked against MAPK2. PubChem (Kim et al., 2023), a well-known compound database, was used to download 3D SDF files for the selected ligands. These were converted to .pdb format using PyMol (The PyMOL Molecular Graphics System, Version 2.5.5 Schrödinger, LLC; DeLano, 2002).

Selection and retrieval of protein target:

A pivotal kinase, Mitogen activated protein kinase-1 (MAPK1), has emerged as promising target

in cancer due to its role in regulating cellular proliferation and differentiation. Hence, was selected as target for this study. Its structure was download from AlphaFold database (Jumper et al, 2021; Varadi et al., 2024) with ID: AF-P28482-F1 (https://alphafold.ebi.ac.uk/search/text/P28482).
Structure was downloaded in PDB format (Alfafold: AF-P28482-F1). AlphaFold predicts structures by using protein sequence. It is now widely used for proteins whose experimental structures are not available. Active site of molecule must be predicted to avoid unusual results while docking. Therefore, ProteinsPlus server (Fährrolfes et al., 2017; Schöning-Stierand et al., 2020; Schöning-Stierand et al., 2022) was used to predict the binding pocket for trametinib. Amino acids in binding pocket were predicted which were used for adjusting grid box in later step.

Molecular docking:

PyRx (Dallakyan & Olson, 2015), a virtual screening software was using to dock selected ligands with the target. Ligands were energy minimized and converted to .pdbqt using OpenBabel (O'Boyle et al., 2011) in PyRx. Then protein was loaded and converted to .pdbqt format. Grid box was generated and adjusted according to the amino acids predicted from ProteinPlus server. Binding affinity and binding pose were generated using AutoDock Vina (Trot & Olson, 2009) in PyRx.

2D interactions of protein ligand complex:

Protein-ligand complex files were prepared using PyMol and 2D interactions were viewed using LigPlot+ (Laskowski & Swindells, 2011) software. It enables to predict hydrogen bonds and hydrophobic interactions within the protein-ligand complex.

Result and Discussion:

Selection and retrieval of ligands:

Selected ligands were searched for antineoplastic activity related to colorectal cancer using PASS analysis. Table 1 shows selected compounds with those (highlighted) having activity against colorectal cancer.

Table 1. Compounds selected for study (Kulkarni, 2023).

Sr. No.	Compounds	SMILES
1	**(-)-Bisdechlorogeodin**	CC1=CC(=C2C(=C1)OC3(C2=O)C(=CC(=O)C=C3OC)C(=O)OC)O
2	**(+)-Sophorol**	C1C(C(=O)C2=C(O1)C=C(C=C2)O)C3=CC4=C(C=C3O)OCO4
3	Chlorogenic acid	C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O
4	**Dactylin**	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)O)OC5C(C(C(C(O5)CO)O)O)O
5	Dihydrocapsaicin	CC(C)CCCCCCC(=O)NCC1=CC(=C(C=C1)O)OC
6	**Diosmetin 7-O-beta-Dglucuronopyranoside**	COC1=C(C=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)OC4C(C(C(C(O4)C(=O)O)O)O)O)O)O
7	**Formononetin**	COC1=CC=C(C=C1)C2=COC3=C(C2=O)C=CC(=C3)O
8	**Genistein 8-c-glucoside**	C1=CC(=CC=C1C2=COC3=C(C2=O)C(=CC(=C3C4C(C(C(C(O4)CO)O)O)O)O)O)O
9	**Hypaconitine**	CC(=O)OC12C3C(CC(C3OC(=O)C4=CC=CC=C4)(C(C1O)OC)O)C56C(CCC7(C5C(C2C6N(C7)C)OC)COC)OC
10	**Isorhamnetin**	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O
11	**Jaceidin**	COC1=C(C=CC(=C1)C2=C(C(=O)C3=C(O2)C=C(C(=C3O)OC)O)OC)O
12	**Luteolin**	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)O
13	**Luteolin 4'-o-glucoside**	C1=CC(=C(C=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O)OC4C(C(C(C(O4)CO)O)O)O
14	**Morindon**	CC1=C(C2=C(C=C1)C(=O)C3=C(C2=O)C=CC(=C3O)O)O
15	**Myricitrin**	CC1C(C(C(C(O1)OC2=C(OC3=CC(=CC(=C3C2=O)O)O)O)C4=CC(=C(C(=C4)O)O)O)O)O)O
16	Pheophorbide a	CCC1=C(C2=NC1=CC3=C(C4=C(

		C(C(=C5C(C(C(=CC6=NC(=C2)C(=C6C)C=C)N5)C)CCC(=O)O)C4=N3)C(=O)OC)O)C)C
17	**physalin G**	CC12CC3C4(C56C1C(=O)C(O5)(C7CC(C8=CC=CC(=O)C8(C7CCC6(C(=O)O4)O)C)O)OCC2C(=O)O3)C
18	**Quercetin**	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O)O
19	**quercetin 3,7-dirhamnoside**	CC1C(C(C(C(O1)OC2=CC(=C3C(=C2)OC(=C(C3=O)OC4C(C(C(C(O4)C)O)O)O)C5=CC(=C(C=C5)O)O)O)O)O)O
20	**Quinacrine**	CCN(CC)CCCC(C)NC1=C2C=C(C=CC2=NC3=C1C=CC(=C3)Cl)OC
21	Quinic acid	C1C(C(C(CC1(C(=O)O)O)O)O)O)O

ADME property of these compounds was predicted using SwissADME. Compounds having following properties were selected for docking.
Topological Polar Surface Area (TPSA) < 138 Å²
Lipinski's rule with 0 violations
High Gastrointestinal aborption
On this basis, 9 compounds were selected for docking: **(-)-Bisdechlorogeodin, (+)-Sophorol, Formononetin, Isorhamnetin, Jaceidin, Luteolin, Morindon, Quercetin and Quinacrine.**

Selection and retrieval of protein target
Protein: Mitogen-activated protein kinase 1
Source organism: Homo sapiens (Human)
UniProt: P28482
Experimental structures: 145 structures in PDB for P28482
Biological function: Acts as a transcriptional repressor. Binds to a [GC] AAA [GC] consensus

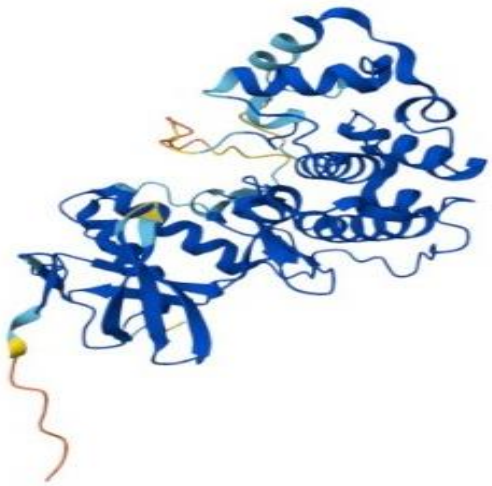

Figure 1: Mitogen activated protein kinase-1 (AlphaFold: AF-P28482-F1)

Molecular docking

Binding pocket was predicted for MAPK-1 for ligand Tametinib. Amino acids in binding pocket are as follows:
GLY_34_A ALA_35_A TYR_36_A LYS_54_A GLU_71_A ASN_82_A ILE_83_A ILE_133_A LEU_134_A GLY_136_A LEU_137_A LYS_138_A ILE_140_A HIS_141_A VAL_145_A LEU_146_A HIS_147_A ARG_148_A ASP_149_A LEU_150_A LYS_151_A PRO_152_A SER_153_A ASN_154_A LEU_155_A LEU_156_A LYS_164_A ILE_165_A CYS_166_A ASP_167_A PHE_168_A GLY_169_A LEU_170_A ALA_171_A ARG_172_A THR_206_A LYS_207_A ILE_209_A ASP_210_A ILE_211_A SER_213_A VAL_214_A ILE_217_A VAL_304_A

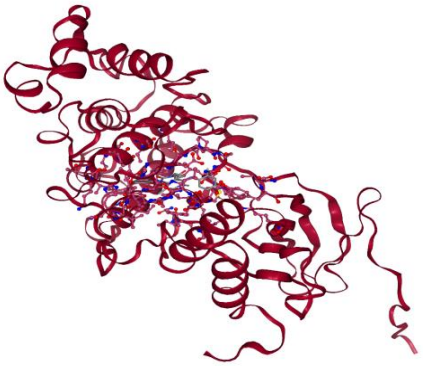

Figure 2. Binding pocket for MAPK-1 for Trametinib

Docking was performed using PyRx for above highlighted compounds with MAPK-1 protein. Binding affinity for ligands is displayed in Table 3. It shows that 7 compounds were having higher binding affinity than selected reference compound, Trametinib (-6.8 kcal/mol).

Table 2. Predicted Binding affinities using PyRx virtual screening software

Sr. No.	Compounds	Binding affinity (kcal/mol)
Reference ligand	Trametinib	-6.8
1	(-)-Bisdechlorogeodin	-5.9
2	**(+)-Sophorol**	**-8.5**
3	**Formononetin**	**-7.6**
4	**Isorhamnetin**	**-8.6**
5	**Jaceidin**	**-8**
6	**Luteolin**	**-8.4**
7	**Morindon**	**-8.2**
8	**Quercetin**	**-8.4**
9	Quinacrine	-6.2

LigPlot+ was used for evaluating 2D interactions of protein ligand complex. Like Trametinib, Jaceidein and Quercitin also showed hydrogen forming with Ser153. Other hydrophobic interactions also play role stabilizing in protein-ligand interaction.

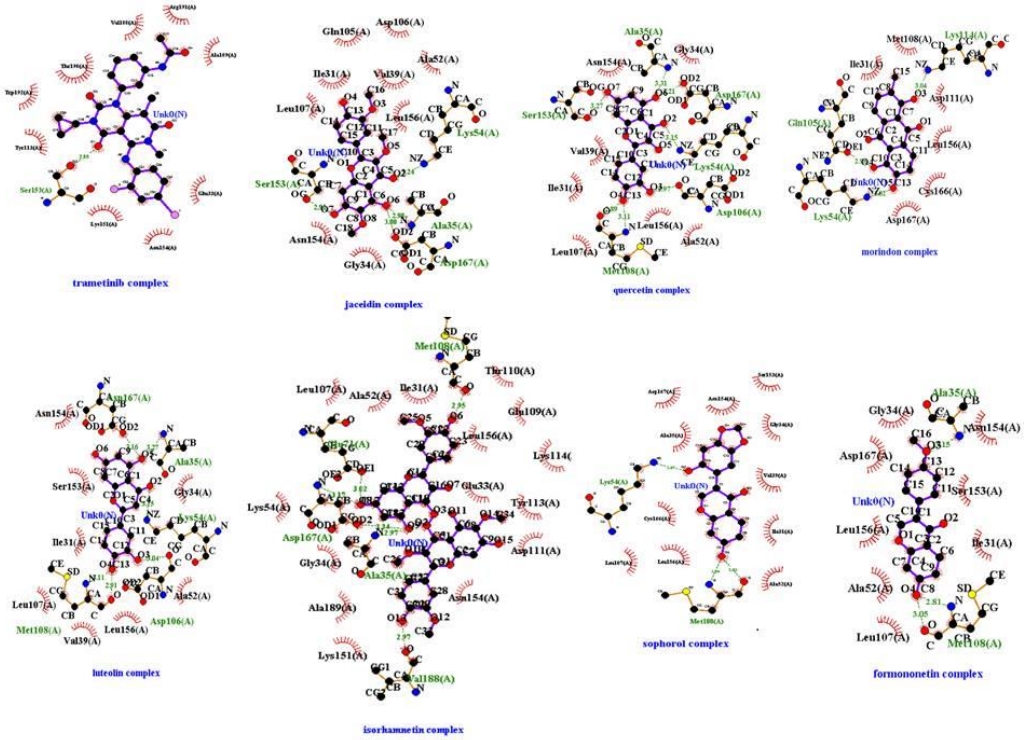

Figure 3. : 2D interaction of molecules using LigPlot+

Conclusion:

Advancement in computational approaches is enhancing the drug discovery process for novel candidates. Our findings demonstrate that the selected compounds were potent enough to inhibit MAPK1 and are important drug candidates related to cancer. They can also be evaluated for other proteins involved signalling pathways leading to cancer.

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