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Competitive inhibition of catechol from *Andrographis paniculata* in the complex of ERK2 in lung metastasis

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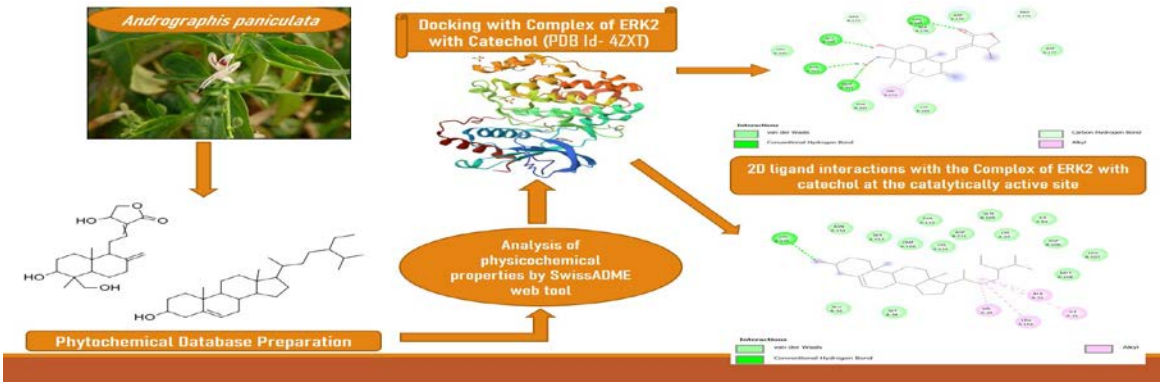
ABSTRACT



Lung cancer is one of the most common cancers worldwide, affecting 18% of cancer patients. There are already 15 drugs available on the market, but their side effects are severe, causing serious damage to other tissue systems, such as white blood cells. To combat these severe problems, alternative therapies are required. Use of medicinal plants is one such option. *Andrographis paniculata* is a medicinal plant used for treating diseases such as cancer, diabetes, high blood pressure, ulcers, leprosy, bronchitis, skin conditions, influenza, dysentery, dyspepsia, and malaria. This study aims to evaluate the role of bioactive compounds of *Andrographis paniculata* against a target ERK2 (Extracellular Signal-related kinase). Two compounds are identified that show more than - 8.00 kcal/mol binding energy, and they are Andrographolide and β-Sitosterol. The molecular analysis and ligand orientation were studied with the help of the computational approach. The binding and intermolecular energy show that the compounds can be explored further in vivo to develop drugs against lung metastasis. *Andrographis paniculata* has the potential to cure lung metastasis by alleviating drug side effects and severe damage to other tissue systems.

Keywords: Lung Cancer, ERK2, Andrographis paniculata, Catechol, Andrographolide, β-Sitosterol

INTRODUCTION

Cancer continues to be one of the primary reasons for mortality across the globe, with nearly 10 million recorded deaths in 2020 alone. Among the most prevalent types of cancer, 2.21 million lung cancer cases were recorded in the same year.[1] In 2020, lung cancer claimed the most lives among cancer-related deaths, with a staggering 1.80 million fatalities. We must continue to promote cancer prevention and early detection measures to reduce the number of deaths caused by this disease.[2,3]

When it comes to lung cancer, there are two main types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC).[4] NSCLC is more common, accounting for 81% of cases, and is further divided into squamous cell carcinoma, adenocarcinoma, and large cell carcinoma.[5] Adenocarcinoma is more prevalent in women and is easier to treat than other subtypes because it is often located in the outer part of the lung and has mutations that can be targeted by treatment. Squamous cell carcinoma is a highly aggressive form of cancer that originates in the cells lining the lungs' airways. In contrast, one of the subtypes of NSCLC is large-cell carcinoma, which can manifest in any part of the lung and is known to be the most aggressive.[6]

Small-cell lung cancer is named after the round, small appearance of the cells under a microscope and accounts for 14% of cases. Small-cell lung cancer is more prevalent in women than men and generally exhibits greater aggressiveness than non-small-cell lung cancer.[7] Unfortunately, Patients with small-cell lung cancer are prone to having a condition that has extended beyond the

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pulmonary system during diagnosis, making treatment more challenging. Even with chemotherapy, it is unfortunate that only a few patients can achieve sustained disease control over a longer period.[8]

Numerous receptors and proteins, such as CXCL12/CXCR4, Bombesin receptors, CD24, Fibroblast Growth Factor Receptors(FGFR), Vasopressin and Oxytocin, Transcription Factors, Neural Cell Adhesion Molecule(NCAM)/CD56, Insulin-Like Growth Factor-1R, Activated Leucocyte Adhesion Molecule(ALAM)/CD166, PETA-3/CD151, Bradykinin Receptor, *Mer* or *Axl* as Receptor Tyrosine Kinase(RTK), Epidermal Growth Factor Receptor(EGFR), and Interleukin-22 Receptor have been identified as being overexpressed in lung cancer.[9]

Andrographis paniculata has been utilised in ancient Ayurvedic and Oriental medicine. The Acanthaceae family includes about 40 species within the genus Andrographis, with only a few renowned for their medicinal properties. The most notable species, i.e., *Andrographis paniculata*, is also known as Kalmegh, the king of bitters. This annual plant is erect, well-branched, and grows up to 1 meter tall. *Andrographis paniculata (AP)* is indigenous to peninsular Sri Lanka and India, and it is also found in various regions of the Southeast part of Asia, the West Indies, America, China, and Christmas Island. *Andrographis paniculata* has numerous medicinal uses. The whole plant treats respiratory infections, insect stings, dysentery, malaria, dyspepsia, influenza, and snakebites. The leaf is effective against gastrointestinal disorders, loss of appetite, fever, irregular stools, colic pain, diarrhoea, tuberculosis, common cold, cough, fever, hepatitis, mouth ulcers, bronchitis, and sores. The shoot treats diabetes, common cold, hypertension, cancer, urinary tract infections, malaria, and snakebites. The root is known for its febrifuge, tonic, stomachic, and anthelmintic properties.[10]

ERK2 is part of the MAP kinase family. It is crucial in numerous biological processes, including differentiation, transcription regulation, and cell growth, and cytokines, UV, and growth factors such as EGF and insulin-like growth factors trigger this process. Additionally, ERK2 remains activated in numerous human cancers, including lung, breast, colon, and skin cancers.[11] The development of lung cancer can be attributed to various carcinogens that activate the EGFR/RAS/MAPK signalling pathway. Studies have shown that many lung cancer patients have constitutively active extracellular signal-regulated kinase 2 (ERK2). Targeting ERK2 through the development of compounds could be beneficial in fighting lung carcinogenesis.[12] In this study, different compounds of *Andrographis paniculata* have been evaluated for their ADMET properties and then used against the ERK2 receptor.

EXPERIMENTAL

MATERIAL AND METHOD

Through an evaluation of the physicochemical properties, an insight into the effectiveness of the drug candidate in traversing cell membranes to reach its target receptor can ultimately be assessed. To conduct a more thorough investigation into the drug's pharmacokinetics, swissADME software was utilised. This software provides information on ligand absorption, distribution, metabolism, excretion and toxicity based on our structure's molecular weight and lipid solubility index.

Selection of the target

The 3-D structure of the Complex of ERK2 with catechol (PDB: 4ZXT) was selected from the RCSB Protein Data Bank database.[13]

Selection of Ligand

The chemical structure of phytochemicals from *Andrographis paniculata* was prepared using the reliable PubChem compound database. To design the structures, the ChemBioDraw tool is used and effectively transforms the MOL SDF format of the ligand into a PDBQT file with the assistance of the PyRx tool.[14]

Figure 1 shows all the compounds used for this study, i.e., 5-Hydroxy-7,2',6'-trimethoxyflavone **(1)**, 5-hydroxy-7,8,2',3'-tetramethoxyflavone **(2)**, 5-hydroxy-7,8,2',5'-tetramethoxyflavone **(3)**, 7-O-Methylwogonin **(4)**, 14-Deoxy-11,12-didehydroandrographolide **(5)**, Andrographolide **(6)**, Andrographoside **(7)**, β-Sitosterol **(8)**, Cinnamic Acid **(9)**, Dihydroskullcapflavone **(10)**, Neoandrographolide **(11)**.

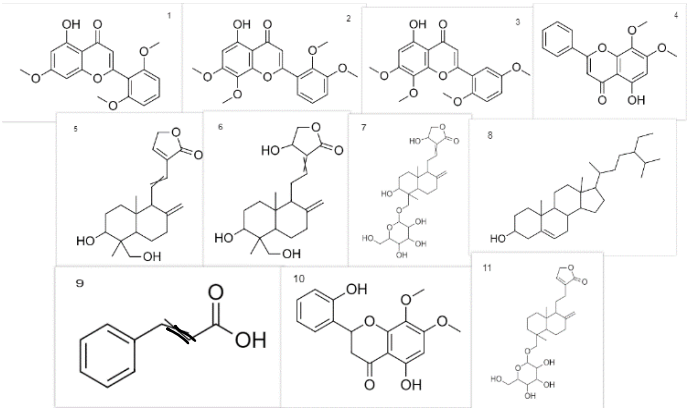


Figure 1. Selected Phytocompounds from *Andrographis paniculata.*

Target and ligand optimisation

The PDB coordinates of the phytochemicals and target protein were optimised for docking analysis using the AutoDock Vina and Drug Discovery Studio software. The resulting coordinates were in a state of stable conformation and minimum energy.[15]

Molecular docking studies

In drug discovery, molecular docking is a reliable, time-saving, and cost-effective technique. Autodock Vina is an open-source software that performs docking studies. The empirical scoring function is utilised to calculate the binding affinity of the protein-ligand complex. To better understand the interactions between ligand and target, the most effective active compounds were successfully inserted into the active site pockets of extracellular signal-related kinase 2 (ERK2), a crucial target for lung cancer drugs. The PDB ID for this protein target is 4ZXT. Water molecules were eliminated, and introduced polar hydrogens into a macromolecule. Using Chemsketch, sketches of the ligands were created and saved in MDL file format. The PyRx tool was used to load both the target and ligand molecules. The ligands' energies were minimised and converted to PDBQT file format. The selected protein was a macromolecule, and the active site of the target molecules was identified with the help of the online server known

as CASTp. A 3D grid box was set up that covered the active site of the target molecule and performed molecular docking. Conformations were based on their binding energy and it determined that the conformation with the lowest binding energy had the highest docking score.[16]

Results and Discussion

Molecular Docking was utilised to evaluate the molecular interactions and determine the binding strength of existing drugs with the anti-tumour active site. The complex of ERK2 and catechol (PDB: 4ZXT) was used as a target for this work. This receptor has been docked with eleven compounds from *Andrographis paniculata*. It was found that andrographolide showed maximum binging energy of -8.24 kcal/mol with the selected receptor (Figure 2).

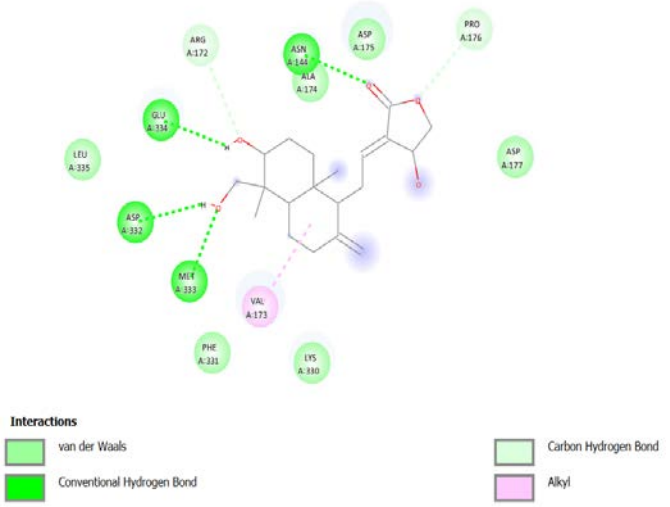


Figure 2. Docking of Andrographolide with complex of ERK2 and catechol

This research has produced valuable data on eleven phyto-compounds obtained from AP. PubChem software is used to obtain 2D structures and Molinspiration online software to obtain 3D structures, presented in Table 1. Table 2 showcases the computed physicochemical properties of the screened compounds. Out of the eleven compounds tested, nine displayed no violations, while one had one violation and another had two. As for the bioavailability score, nine compounds scored 0.55, one scored 0.17, and one scored 0.85, all obtained through SwissADME. Assessing the drug-likeness of a compound involves evaluating its physical and chemical properties concerning its pharmacological or biological activity and determining whether it has the potential as an orally active drug in humans relies on Lipinski's rule of five.

Table 3 shows the docking score of compounds derived from *Andrographis paniculata* with 4ZXT. According to Table S4, several 2D ligand interactions from the compounds found in *Andrographis paniculata* when interacting with the Complex of ERK2 with catechol at the catalytically active site. Table S5 showcases the bioavailability radar analysis of screened compounds using SwissADME, which initially assesses a molecule's drug-like qualities. The pink section on the radar

Table 1: 2D & 3D Structures of compounds from *Andrographis paniculate*

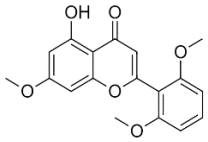
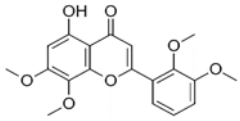
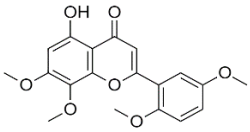
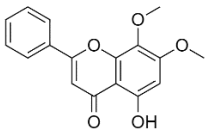
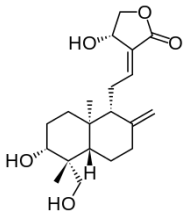
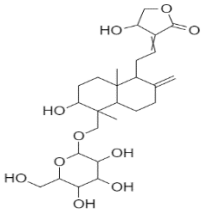
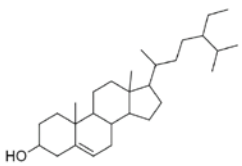
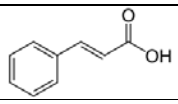
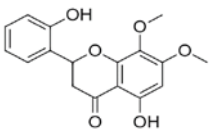
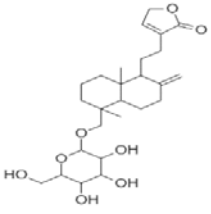
Compounds	2-D Structure
5-Hydroxy- 7,2',6'-trimethoxyflavone	
5-hydroxy- 7,8,2',3'-tetramethoxyflavone	
5-hydroxy- 7,8,2',5'-tetramethoxyflavone	
7- O -Methylwogonin	
Andrographolide	
Andrographoside	
β-Sitosterol	
Cinnamic Acid	
Dihydroskullcapflavone	
Neoandrographolide	

Table 2: Computed physicochemical properties of screened compounds

Compounds	PubChem Id	MW (g/mol)	H Bond Donor	H Bond Acceptor	TPSA (Å²)	Log *P* (iLOGP)	Violations	Bioavailability Score
5-Hydroxy- 7,2',6'-trimethoxyflavone	5318369	328.32	1	6	78.13	3.38	0	0.55
5-hydroxy- 7,8,2',3'-tetramethoxyflavone	5319378	358.34	1	7	87.36	3.4	0	0.55
5-hydroxy- 7,8,2',5'-tetramethoxyflavone	10948318	358.34	1	7	87.36	3.6	0	0.55
7- O -Methylwogonin	188316	298.29	1	5	68.9	2.99	0	0.55
14-Deoxy- 11,12-didehydroandrographolide	5708351	332.43	2	4	66.76	2.85	0	0.55
Andrographolide	5318517	350.45	3	5	86.99	2.45	0	0.55
Andrographoside	6439612	512.59	6	10	166.14	2.68	2	0.17
β-Sitosterol	222284	414.71	1	1	20.23	4.79	1	0.55
Cinnamic Acid	444539	148.16	1	2	37.3	1.55	0	0.85
Dihydroskullcapflavone	12098358	316.31	2	6	85.22	2.46	0	0.55
Neoandrographolide	9848024	480.59	4	8	125.68	3.27	0	0.55

Table 3: Docking score of compounds derived from *Andrographis paniculata* with Complex of ERK2 with catechol (PDB: 4ZXT)

Ligands	Binding Energy (ΔG)(kcal/mol)	Ligand Efficiency	Inhibition Constant (μM)	Intermolecular Energy (kcal/mol)	Vdw H-bond desolvation (kcal/mol)
5-Hydroxy- 7,2',6'-trimethoxyflavone	-6.27	-0.26	25.37	-7.76	-7.58
5-hydroxy- 7,8,2',3'-tetramethoxyflavone	-7.45	-0.25	3.44	-9.84	-9.1
5-hydroxy- 7,8,2',5'-tetramethoxyflavone	-6.32	-0.24	23.47	-8.11	-7.98
7- O -Methylwogonin	-7	-0.32	7.38	-8.19	-8.18
14-Deoxy- 11,12-didehydroandrographolide	-6.52	-0.33	16.73	7.71	-7.69
Andrographolide	-8.24	-0.33	916.9	-10.03	-9.22
Andrographoside	-6.32	-0.18	23.27	-9.9	-9.16
β-Sitosterol	-8.22	-0.27	949.5	-10.3	-10.21
Cinnamic Acid	-6.34	-0.58	22.68	-7.23	-5.21
Dihydroskullcapflavone	-6.45	-0.28	18.6	-7.95	-7.64
5-Hydroxy-7,2',6'-trimethoxyflavone	-6.78	-0.2	10.71	-10.06	-9.68

indicates the ideal range for various properties, including polarity (TPSA between 20 and 130 Å2), size (MW between 150 and 500 g/mol), lipophilicity (XLOGP3 between -0.7 and +5.0), solubility (log S not exceeding six), flexibility (no more than nine rotatable bonds), and saturation (fraction of carbons in sp3 hybridisation not less than 0.25). Similarly, a study reported that andrographolide could effectively combat cancer cells by stopping the cell cycle at the G0/G1 phase. This can be achieved by activating the cell-cycle inhibitory protein p27 and decreasing cyclin-dependent kinase 4 (CDK4) expression levels. Additionally, andrographolide has been shown to increase lymphocyte proliferation and interleukin-2 production, indicating its immune-stimulatory activity. Moreover, andrographolide has been found to boost the production of tumour necrosis factor-α and CD marker expression. This leads to an improvement in the cytotoxic activity of lymphocytes against cancer cells, ultimately contributing to its indirect anticancer properties.[17]

A study reported anti-tumour properties of WA against six different types of cancer receptors, i.e., 3ERT, 4ZXT, 4J96, 4UYA, 7SAP and 6XXP. 4ZXT showed a high binding energy of -8.03 kcal/mol with Withaferin-A, a bioactive compound in *Withania somnifera*. It was observed to form direct hydrogen bonds with 167-ASP, 153-SER, 71-GLU, and 54-LYS. It also demonstrated pi-pi stacking and hydrophobic interactions with 156-LEU, 113-TYR, 84-ILE, and 35-ALA.[18]

A study reported that active components of AP can be a potential agent against COVID-19. They used network pharmacology and identified 11 potential targets and 24 ligands. The study revealed

that andrographidine C made a stable complex with ACE2, making it a potential candidate for treating the virus.[19]

Another study reported that the components of *Andrographis paniculata* have anti-migratory effects and can inhibit metastasis-related factors such as MMP2, HER2, MMP9, CXCR4, and TM4SF3 in esophageal cancer cells. In addition, it was reported that the water extract of *Andrographis paniculata* (at a dosage of 1600mg/kg) possesses anti-tumour and anti-metastatic properties, as evidenced by its effectiveness in treating mice with metastatic esophageal xenograft. Notably, the water extract of *Andrographis paniculata* demonstrated a synergistic impact when used with cisplatin and 5-fluorouracil, effectively impeding tumour nodule growth.[20]

CONCLUSIONS

Continuous research into complementary and alternative medicine for cancer treatment remains ongoing. Recently, there has been a lot of attention on *Andrographis paniculata.* This study has identified two compounds, Andrographolide and β-Sitosterol, with a binding energy of more than -8.00 kcal/mol. Demonstration of molecular binding interaction from *in-silico* analysis elucidated that Andrographolide and β-Sitosterol have more specificity towards the target protein 4ZXT and could be an efficient anti-cancer compound. According to Ayurveda, *Andrographis paniculata* has anti-cancer properties, and the action of andrographolide is believed to be based on similar principles. It has been found that Andrographolide effectively induces G0/G1 cell-cycle block in various cancer cells. Additionally, it induces TRAIL-mediated apoptosis, activates death receptor pathways, and activates p53 by enhancing phosphorylation. It is crucial to remember that several naturally occurring substances have been discontinued from clinical trials because they did not show effectiveness and had undesirable side effects. Performing *in-vitro* experiments on cancer cell lines to screen compounds has resulted in identifying potential candidates for pre-clinical and clinical studies. The potential of Andrographolide and its semi-synthetic derivatives as a source for new anti-cancer drugs is an intriguing prospect. Andrographolide and β-Sitosterol show potential as anti-cancer agents. However, further research is needed to ascertain their anti-cancer properties fully.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUPPLEMENTARY INFORMATION

The supplementary information file contains the data of phytoconstituents selected for the in-silico docking studies. Tables 4 and 5 are included in the supplementary information.

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