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Molecular docking studies of phytocompounds from *Artemisia monosperma* against ERK2 kinase in lung cancer

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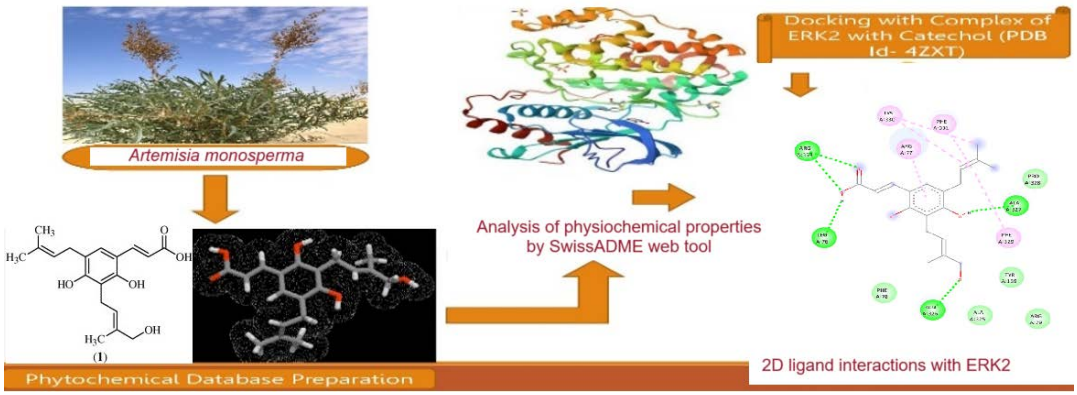
ABSTRACT



We looked into Artemisia monosperma's possible therapeutic benefits against lung cancer in this study. We used in-silico research to look at the molecular pathways that lead to the development of lung cancer. According to our findings, the EGFR/RAS/MAPK signaling pathway is crucial for the advancement of lung cancer. Since ERK2 activation is frequently seen in lung cancer patients, drugs that target ERK2 in lung carcinogenesis are advantageous. Lung cancer cells treated with catechol experienced G1 phase arrest and decreased expression of proteins associated with G1-S progression. Many medicinal uses for artemisia species exist in traditional medicine around the globe. They have qualities that are anti-inflammatory, antioxidant, anticancer, antispasmodic, antibacterial, insecticidal, and antifungal. We found that 4, 6-Dihydroxy-3-(3-Methyl-2-Butenyl)-5-(4-Hydroxy-3-Methyl-2-Butenyl) Cinnamic Acid was the top hit, with a MolDock score of −6.96 kcal/mol, using molecular docking-based virtual screening. Based on Lipinski's rule of five, this molecule was determined to be drug-like, and its ADMET characteristics showed average pharmacokinetic profiles. This implies that the discovered substance could have therapeutic uses in the management of lung cancer.

Keywords: Artemisia monosperma, Antitumor, Phytochemicals, ERK2, Catechol

INTRODUCTION

India is the third-biggest producer and user of tobacco products worldwide. There are over 267 million tobacco users in the nation, or 28.6% of the total population.. As per the National Cancer Registry Programme, tobacco-related cancers account for 27% of all cancers in India. Lung cancer is the leading cause of cancer-related deaths worldwide.[1] In India, it is responsible for 8.1% of cancer-related fatalities and 5.9% of all cancer cases. Almost eighty percent of lung cancer patients smoke.2. among India, lung cancer is one of the leading causes of disease and mortality, particularly among men. The illness has changed throughout time, now primarily affecting women, nonsmokers, and younger age groups. Standard platinum doublet chemotherapy using generic medications is administered to the majority of patients with non-oncogene-addicted NSCLC and SCLC.[3]

Fruits and vegetables naturally contain a substance called catechol (pyrocatechol).[4] By directly attaching to signaling molecules crucial to the development of cancer, several phytochemicals with the catechol moiety have demonstrated anti-

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cancer action. Nevertheless, no studies have been conducted to date to clarify catechol's direct molecular targets or anti-carcinogenic actions. A member of the MAP kinase family, sometimes referred to as extracellular signal-regulated kinases (ERKs), which function as an integration point for various biological signals, is encoded by the MAPK1 (mitogen-activated protein kinase 1) gene. Numerous cellular functions, including development, differentiation, transcription control, and proliferation, are influenced by them. This kinase has to be phosphorylated by upstream kinases in order to activate. This kinase translocates to the nucleus of the stimulated cells upon activation, where it phosphorylates targets that are nuclear. In addition, this protein functions independently of its kinase activity as a transcriptional repressor. The protein in question has been classified as a moonlighting protein due to its capacity to carry out diverse molecular tasks. By directly binding to ERK2, catechol inhibits its function. In human lung cancer cell lines and murine KP2 cell lines, this inhibition results in reduced downstream signaling, which includes phosphorylation and stability of the oncogene c-Myc. H460.[5]

Classification of *Artemisia monosperma*

Scientific classification	
Kingdom:	Plantae
Family:	Asteraceae
Genus:	*Artemisia*
Species:	***A. monosperma***

Figure 1. *Artemisia monosperma* plant

In the Middle East's desert sand dunes and sandy Mediterranean, Artemisia monosperma is a common plant. This shrub can tolerate being covered and uncovered by sand, making it a good fit for the dynamic sand dune habitat. It is found in the phytogeographic zones of the Sahara, Arabia, and the Mediterranean. In Israel and the northern Sinai, it predominates in the mid-seral stage of plant succession, which is the process of stabilizing sand. This plant flowers from September to December. When moist, the mature mucilaginous achenes of A. monosperma stick to sand surfaces and are dispersed by wind between November and January. Achenes may float on runoff water and spread into depressions and along runnels in regions where cyanobacterial sand crusts are prevalent. Because A. monosperma achenes are extremely light-sensitive and need a thin layer of sand—roughly 2 to 20 mm—to germinate, the germination process helps to keep the environment wet. However, these achenes' rates of germination decrease with increasing depth in the sand.[6]

EXPERIMENTAL

MATERIAL AND METHOD

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.[6]

Selection of Ligand

The reliable PubChem compound database was used to prepare the chemical structure of phytochemicals from Artemisia monosperma. The ChemBioDraw tool was utilized to design the structures and effectively transform the ligand's MOL SDF format into a PDBQT file with the assistance of the PyRx tool.[8]

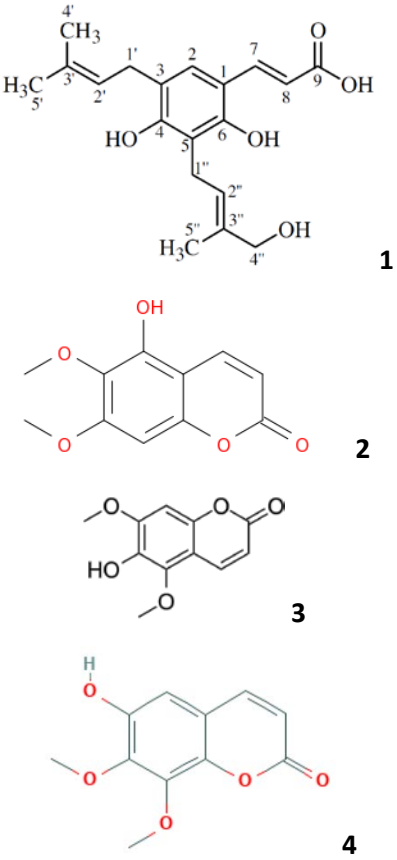

Figure 2. Chemical Structure of Compounds used in this study

Target and ligand optimisation

The coordinates of the phytochemicals and target protein were optimized for stable conformation and minimum energy during docking analysis.[9]

Molecular docking studies

Molecular docking is a computer process used in drug development that forecasts how a ligand will orient to create a stable compound with its macromolecular target. An empirical scoring function is used by the open-source program Autodock Vina to compute the binding affinity of the protein-ligand complex when conducting docking investigations. To learn more about the interactions between ligand and target, our research introduced active molecules into the active site pockets of extracellular signal-related kinase 2 (ERK2), an important target for lung cancer therapies. This protein target's PDB ID is 4ZXT. Polar hydrogens were added to the macromolecule after water molecules were eliminated. Chemsketch was used to construct the ligand sketches, which were then saved in MDL file format. PyRx was used to reduce the energies of the ligands and convert them to PDBQT format. The target molecules' active site was found. A 3D grid box was set up to cover the target molecule's active site for molecular docking. The conformation with the lowest binding energy was determined to have the highest docking score.[10,11]

RESULTS AND DISCUSSION

Molecular Docking was used to evaluate drug binding strength with the anti-tumour active site of ERK2 and catechol (PDB: 4ZXT) complex. Past research on the ERK2-catechol complex aimed to comprehend how the protein and small molecule bind together. Nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography were used to precisely identify the complex's atom arrangement and examine the binding site's structural details. Four *Artemisia monosperma* compounds were docked with this receptor. In this recent research, four *Artemisia monosperma* compounds were docked with the complex of ERK2 with catechol (PDB: 4ZXT). 4,6-dihydroxy-3-(3'-methyl-2'-butenyl)-5-(4"-hydroxy-3"-methyl-2"-butenyl) Cinnamic Acid **1** (Figure 2) showed maximum binding affinity (Figure 3)

According to a recent study, Artemisia monosperma was used to make silver nanoparticles (AM-AgNPs), which were then examined for their capacity to combat cancer in MCF-7 cells. Various methods, including UV-vis spectroscopy, FT-IR, TEM, SEM, EDX, and XRD, were employed to study the AM-AgNPs.To test their anticancer potential, MTT and NRU assays were performed. The investigation also looked at cellular damage, ROS generation, and mitochondrial membrane malfunction to determine how the AM-AgNPs led to the death of cancer cells. The findings demonstrated that the AM-AgNPs had an average size of 24 nm and were cubic and crystalline. AM-AgNPs generated by the green synthesis approach dramatically decreased cell viability by 13–86% in the MTT assay and 9–79% in the NRU assay at 10-100 µg/mL. The IC_{50} value for both assays was around 32 µg/mL, indicating strong anticancer properties.[12]

Another study found that extracts from *A. monosperma* had a significant impact on human colorectal carcinoma cells. The extracts caused a decrease in cell viability in HCT-116 cells and changed their morphology in a dose-dependent manner. AM-C extract exhibited particularly high cytotoxic activity, with an

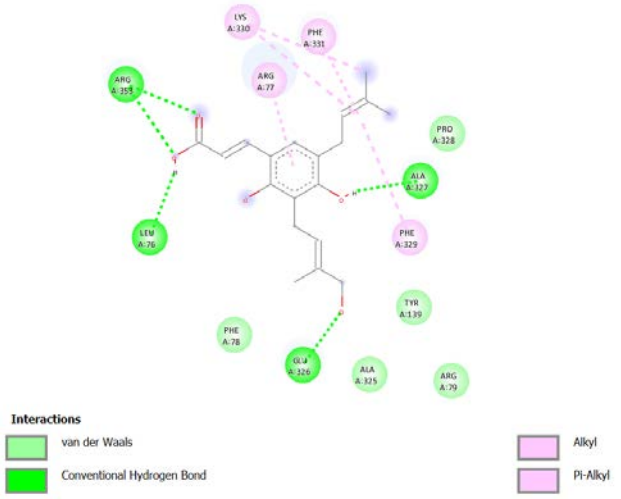

Figure 3 Docking of 4,6-dihydroxy-3-(3'-methyl-2'-butenyl)-5-(4"-hydroxy-3"-methyl-2"-butenyl)Cinnamic Acid **1** with receptor

IC50 of 250.5 µg/mL. Additionally, the extract caused an increase in ROS production and a decrease in MMP levels in HCT-116 cells. Additionally, it caused cell death by downregulating the antiapoptotic gene Bcl-2 and upregulating genes linked to apoptosis, such as p53, Bax, caspase-3, and caspase-9. These results imply that AM-C extract may be useful as a therapeutic agent in the treatment of colorectal cancer. In particular, it could accelerate apoptosis in HCT-116 cells by producing reactive oxygen species and disrupting the mitochondria.[13]

Another research shows that consuming *Artemisia monosperma* A. (wormwood) oil extract can have powerful effects on improving the quality of life. Not only can it help with chronic complex diseases like colon cancer, but it can also benefit overall health. The oil extract has anti-tumour and anti-inflammatory properties, which are reflected in reduced levels of various markers in the blood. Additionally, the extract has an antioxidant effect, which helps to reduce oxidative stress. In tests on malignant rats, the oil extract improved both nutritional and hematological parameters. Surprisingly, as compared to the other treatment groups (G4 and G5), group G6 shown the greatest protection against CRC. The colon's histological analysis supported these findings. Wormwood oil has been shown to significantly reduce the growth of tumors and have anti-inflammatory and antioxidant properties.[14]

The combined effects of cisplatin and Artemisia absinthium extract on Calu-6 human lung cancer cells were investigated in this workImportant ingredients like artemisinin, thujone, and costunolide were discovered to be present in the extract. MTT test results showed that the extract and cisplatin both suppressed Calu-6 cell growth in a dose-dependent way. When combined, they considerably decreased cell viability in comparison to the control group ($p < 0.001$). Apoptotic body formation, nuclear chromatin condensation, and cell membrane shrinkage were among the alterations in nuclear morphology that were shown by DAPI labeling. Appendix V/PI An increase in apoptosis in Calu-

6 cells was further validated by assays and changes in the expression of genes involved in apoptosis. The active ingredients in the extract of A. absinthium improved the cisplatin's cell sensitivity. By lowering the dosage and making use of the plant's ingredients, the combination of A. absinthium extract with cisplatin may lessen the drug's adverse effects in lung cancer cells. For the treatment of lung cancer, artemisia can be regarded as an adjuvant substance with fewer negative effects.[15]

According to a separate research, comparable soil and climatic circumstances in three distinct locales led to minor chemical variations in the essential oils (EOs) recovered from the A. monosperma species. However, because of fluctuating weather circumstances and soil moisture levels, the EO production and composition varied significantly across seasons. The EOs contained 72 chemical compounds, mainly sesquiterpenes. The EOs of *A. monosperma* exhibited significant allelopathic and antioxidant activity, which varied depending on the season and chemical composition of the samples. .. While EOs collected in the summer and fall showed greater allelopathic and antioxidant activity, there was no discernible seasonal trend in the concentration of the main bioactive components. These results emphasize the significance of time and season for collecting aromatic plants, since these factors can greatly affect the biological activities of their Eos. [16]

One of the studies reported that the *Artemisia monosperma* is a fragrant perennial plant that grows widely in the Arabian deserts. Some women in Jordan use its leaves as a folk remedy to induce abortions. The purpose of this study was to assess how the ethanolic leaf extract of the plant affected the rat pregnancy result. In comparison to the control, the administration of 150 mg/kg or 300 mg/kg of the extract on days 3-5 of gestation led to a decrease in the implantation of viable fetuses and an increase in the number of absorbing sites. In the meanwhile, mid-term abortion was triggered by the extract's administration at doses of 50 mg or 300 mg per kilogram between days 10 and 12 of pregnancy. Furthermore, the extract at doses of 150 mg/kg or 300 mg/kg on days 19–21 of pregnancy resulted in a delayed start of labor, an unsuccessful spontaneous birth, and a significant rise in the level of the hormone oxytocin. Because Artemisia monosperma can negatively impact rat pregnancy results, pregnant women should avoid using the plant's leaves. [17]

A. ADME Analysis Test

The study of pharmacokinetics (PK) involves understanding how a drug moves throughout the body, which provides valuable information about how the drug affects the body over time. On the other hand, pharmacodynamics (PD) refers to what the drug does to the body and the resulting pharmacologic response when it reaches the site of action. PK and PD data can then be used to establish dose-exposure-response relationships, which enable us to describe the safety and efficacy of the drug. It's important to note that different patients may experience varying effects from the same drug due to biological differences. The processes of absorption, distribution, metabolism, and excretion, collectively known as ADME, describe how a drug moves through and is

Table 1: 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
4,6-Dihydroxy-3-(3-Methyl-2-Butenyl)-5-(4-Hydroxy-3-Methyl-2-Butenyl)Cinnamic Acid **1**	11267570	332.39	4	5	97.99	2.82	0	0.55
5-Hydroxy-6,7-Dimethoxycoumarin (Tomentin) **2**	14059525	222.19	1	5	68.9	2.17	0	0.55
6-Hydroxy-5,7-Dimethoxychromen-2-One (Fraxinol) **3**	3047739	222.19	1	5	68.9	2.12	0	0.55
6-Hydroxy-7,8-Dimethoxycoumarin **4**	11345068	222.19	1	5	68.9	2.1	0	0.55

Table 2: Docking score of compounds derived from ***Artemisia monosperma*** with Complex of ERK2 with catechol (PDB: 4ZXT)

Compounds	PubChem Id	Binding Energy (ΔG)(kcal/mol)	Ligand Efficiency	Inhibition Constant (μM)	Intermolecular Energy (kcal/mol)	Vdw H-bond desolvation (kcal/mol)
4,6-Dihydroxy-3-(3-Methyl-2-Butenyl)-5-(4-Hydroxy-3-Methyl-2-Butenyl)Cinnamic Acid	11267570	-6.96	-0.29	7.97	-10.24	-8.94
5-Hydroxy-6,7-Dimethoxycoumarin (Tomentin)	14059525	-1.94	-0.12	37.87	-2.83	-2.72
6-Hydroxy-5,7-Dimethoxychromen-2-One (Fraxinol)	3047739	-1.93	-0.12	38.32	-2.83	-2.71
6-Hydroxy-7,8-Dimethoxycoumarin	11345068	-5.73	-0.36	62.66	-6.63	-6.25

processed by the body. These processes are evaluated through data collected in clinical pharmacology studies and provide insight into the PK processes of a drug. It is crucial to understand the ADME properties of a drug for safe and effective pharmacotherapy development.[18] The team collected 2D structures from PubChem and 3D structures from Molinspiration online software, which are provided in Table S1. The computed physicochemical properties of the screened compounds are showcased in Table 1. All four compounds had zero violations and a bioavailability score of 0.55 obtained through SwissADME.

B. Bioavailability Radar

The bioavailability radar, which offers a rapid evaluation of a molecule's drug-like qualities, is shown in Table S2. Six physicochemical parameters are considered by this radar: size, polarity, lipophilicity, solubility, flexibility, and saturation. The pink area on the radar represents the optimal physicochemical space in which the molecule must fall to be classified as drug-like i.e., 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 hybridization not less than 0.25).[19] The molecule 4,6-dihydroxy-3-(3'-methyl-2'-butenyl)-5-(4"-hydroxy-3"-methyl-2"-butenyl) Cinnamic Acid **1** was said to be orally bioavailable as it falls in six physicochemical properties but on the other hand, 5-Hydroxy-6,7-Dimethoxycoumarin (Tomentin) **2**, 6-Hydroxy-5,7-Dimethoxychromen-2-One (Fraxinol) **3** and 6-Hydroxy-7,8-Dimethoxycoumarin **4** showed high unsaturation due to its low fraction of carbon in the sp3 hybridization and, therefore, is not orally bioavailable (Table S2).[20-22]

SUPPLEMENTARY INFORMATION

The data of the phytoconstituents used for the in-silico docking investigations are included in the supplemental information file. Every table is contained in the supplemental data.

CONFLICT OF INTEREST STATEMENT

The author states that there is no financial or academic conflict of interest with the publishing of this work.

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