Gemcitabine (A Chemotherapy Medication) Vs. Phytochemicals Against Non-Small Cell Lung Cancer – A Computational Approach

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Abstracts: Non-small cell lung cancer is a type of lung cancer, where healthy cells in the lungs grow out of control forming a tumor or a nodule. Although small cell lung cancer and non-small cell lung cancer share few common symptoms and causes, their rate of spread or metastasis differs significantly. Targeted gene therapies involving uses of targeted drugs against specific gene or protein are increasingly being used for the treatment of lung cancer. This study aims at understanding the efficacy of the bioactive compounds over the already existing chemotherapy drug- Gemcitabine. For this study, the target receptor proteins of Non-Small Cell Lung Cancer (NSCLC) were retrieved from Protein Data Bank (PDB) and the ligand compounds were retrieved using PubChem NCBI. Based on various research studies, four target proteins from NSCLC and sixteen bioactive compounds of *Curculigoorchioides* (Black musale) were selected. And their effect of bioactive compounds has been studied by means of *in-silico* approach and further the identified potential active compounds have been compared with control. This comparative in-silico study has predicted that the bioactive principle of *Curculigoorchioides* has better efficacy against cancer receptors and can considered as an effective alternative drug for cancer treatment. Concluding, the present study will be useful in future for designing novel therapeutic plant-based drug with higher efficacy for the treatment of lung cancer.

Keywords: Drug Discovery*,* Gemcitabine, Non-Small Cell Lung Cancer, In-Silico Bioinformatics

1. INTRODUCTION

Cancer is one of a leading cause of death worldwide, accounting for an estimated 19.3 million new cancer cases and almost 10.0 million cancer related deaths in 2020[17]. The number of new cases is expected to exponentially rise over the next few years. Fortunately, cancer therapies have evolved considerably in recent decades, substantially improving the quality of life and survival of cancer patients. In addition to that, recent advancement and development in cutting-edge therapies such as targeted drug therapy along with chemotherapy has promising scope in the diagnosis and treatment of cancer

Prostate, lung, colorectal, and stomach cancer are the most common types of cancer in men, while colorectal, breast, lung, thyroid and cervical cancer are the most common among women. However, some forms of the cancer remain medically intractable. There are some modern treatments that can significantly improve the quality of life and may extend survival [1,2].

Types of Lung Cancer

Lung cancer also known as lung carcinoma, is characterized by abnormal cell growth in the lung. Overtime if untreated on time, this lung cancer cells can proliferate and spread to surrounding healthy tissues, including the lining of the lungs making it difficult to treat with chemotherapy.

Based on the microbial appearances of the cancer, lung cancer is classified into two types namely Small-Cell Lung Carcinoma (SCLC) and Non-Small-Cell Lung Carcinoma (NSCLC). Small-Cell Lung Carcinoma is often aggressive and spreads more quickly than -Small-Cell Lung Carcinoma [3]. Generally, both the types are treated in different ways, so making a distinction between these two types is important.
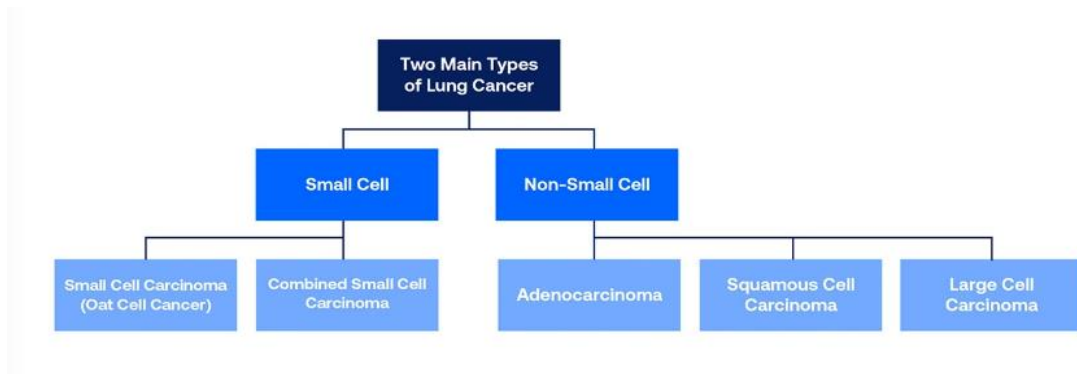
Figure 1 Types of Lung Cancer

Adenocarcinoma: About 50 percentage of non-small cell lung cancers are adenocarcinomas. These types of cancers often arise and spreads from the epithieal cells found the outer area of the lung. In the lung adenocarcinoma tumor eventually proliferates and spreads (metastasize) to surrounding healthy granular cells secreting important substances such as mucus [4].

This type of lung cancer occurs in both men and women but it is more common in women and younger people are more susceptible to this cancer than other types of lung cancer. Compared with other types of lung cancer, adenocarcinomas are more prevalent in non-smokers.

Lung adenocarcinoma is further classified into acinar, papillary, and mixed subtypes. each having different progenies and treatment.

- **Acinar:** Acinar lung Adenocarcinomas are cancers that develop in the gland cells that line the lungs. Nearly everyone with lung adenocarcinoma cancer has this type.
- **Papillary lung adenocarcinoma:** Although it is a rare type of lung cancer, the occurrence can cause themalignant cells of the tumor form to complex papillary structures exhibiting proliferative, destructive growth affecting normal lung tissue.[7-12]
- **Mixed subtype**: Mixed lung adenocarcinoma subtype is categorized by the occurrence of a mixed array of different patterns (papillary, acinar, bronchioloalveolar, solid with mucin). This type of adenocarcinomais difficult to treat by chemotherapy.

Squamous cell carcinoma: Also known as epidermoid carcinomas, accounts for about 25% to 30% of all lung cancers. These subtype of non-small cell cancers begins in the top-layer squamous cells that line the inside of the airways in the lungs. They are often linked to a history of tobacco or smoking and tend to be localized in the central part of the lungs, near the main airway (bronchus).

Based on their appearance and prognosis, square cell carcinoma is further classified into the following three types. each of which has different metastasis and treatment.

- keratinizing squamous cell carcinoma
- nonkeratinizing squamous cell carcinoma
- basaloid squamous cell carcinoma

Large cell (undifferentiated) carcinoma: Also known as undifferentiated carcinomas, accounts for about 10% to 15% of all lung cancers. It can occur in any part of the lung. Compared with other cancers, large cell carcinoma tends to be more aggressive which can make it harder to treat.

Other Less Common Types of Lung Cancer

- carcinoid tumour
- salivary gland carcinoma
- pleomorphic
- unclassified carcinoma

Treatment

The type of treatment for lung cancer is usually decided based on the location, cancer severity, and several other health factors such as age, sex, or presence of other comorbidities. Generally, people with non-small cell lung cancer are treated with chemotherapy, surgery, radiation therapy, targeteddrug therapy, or a combination of these treatments. And people with small cell lung cancer are usually treated with chemotherapy and radiation therapy.

Combinations of two chemotherapy drugs are often used to treat early-stage lung cancer. Chemotherapy yields response rates of 25–50% in advanced non-small cell lung cancer (NSCLC) and 60–80% in extensive small cell lung cancer (SCLC), but almost all cancer cells that are not intrinsically resistant to drugs rapidly develop acquired broad cross-resistance to other unrelated chemotherapy agents [13].

Chemotherapy effectively treats many types of cancer. But like other medication treatments, it often causes few side effects. In addition to that, the cost of chemotherapy treatment is usually very high and each person may experience different results. As a result, the search to find new natural plant sources that have biologically active substances against cancer receptors has acquired immense attention. Several studies have been carried out on various plants, vegetables, and fruits because they are rich sources of antioxidants and phytochemicals constituents that prevent free radical damage thereby reducing the risk of cancer, chronic diseases viz., cardiovascular diseases, etc. [5]

The beneficial role of herbal plants has led to an increase in the search for newer plant-based sources for the treatment of a large group of diseases such as cancer. One such unexplored plant is *Curculigoorchioides*.

Curculigoorchioides belong to the Amaryllidaceae family, are an endangered Rasayana herb with leaves resembling that of palm leaves. The plant is native to India and holds a space place for its potent aphrodisiac and adaptogen in the Ayurvedic system of medicine. It is being traditionally used as an important ingredient of many Ayurvedic preparations owing to its immunostimulant, aphrodisiac, antioxidant, hepatoprotective, anticancer, and antidiabetic activities. Various chemical constituents like glycosides, phenolics, mucilage, saponins, and aliphatic compounds from the plant have been reported to pose beneficial health properties [14].

Gemcitabine a class of nucleosidic chemotherapy (anticancer or antineoplastic) drug, is commonly is used in combination with other drugs to treat the advanced or metastatic types of lung cancer (such as non-small cell lung cancer (NSCLC) or small cell lung cancer (SCLC)) that has spread to other parts of the body and cannot be treated with conventional therapy or surgery. Usually, the Gemcitabine work by damaging the RNA or DNA that guides the cell how to multiply itself in the division. If the cells are unable to divide further, they are digested by the immune system. The faster the cells are dividing, the more likely it is that chemotherapy will disrupt the cells, causing the tumor to shrink. They also induce cell suicide (apoptosis or self-death) [15]. This study will be useful in the future for designing plant-based novel drugs for the treatment of lung carcinoma.

2. MATERIALS AND METHODS
A. STRUCTURE

The 3D structure of five target proteins viz., Rab GTPase, Human KAP1 PHD finger bromodomain, Human alpha-catenin, Human heat shock protein HSP70, Human peroxiredoxin from NSCLC were retrieved from the protein data bank (PDB) [15], with PDB ID of IX3S, 2RO1, 4IGG, and 4PO2 respectively. The attached water molecules and ligand molecules were removed to avoid interference during binding site prediction.

Based on various review literature articles, sixteen bioactive phytochemical compounds from *Curculigoorchioides*are selected. The 3D structure of the active compounds is obtained from PubChem [16,17] in the SDF file format (*.sdf). Apart from these bioactive compounds mentioned above, the 3D structure of Gemcitabine with PubChem CID: 60750 was selected for comparison. Two bioactive compounds were selected for drug-like molecules by Lipinski's rule of five. Only these molecules were taken into consideration for docking.

B. DOCKING

LIGANDFIT which performs docking based on cavity detection algorithm was the docking method used for our study (Accelyr's Discovery Studio 4.0) and allow us to virtually screen compounds and predict the strongest binders based on various scoring functions. For our study, the molecular docking analysis of 1X3S, 2RO1, 4IGG, 4PO2 with ligands was carried using the Dreading parameter in which the partial charges of target proteins and ligands in which the Gasteiger charging method was employed to calculate. The energy grid extension was set to $5.0 A^o$ and '0' was set as the conformation search number of the Monte Carlo trial. The number of poses for ligands in the receptor cavity was limited to 10 and other input parameters for docking were set as default options and docking was performed. Broyden-Flecher Gold Farshanno(BFGS) methods are employed on LIGANDFIT for the final energy refinement of the ligand pose (or) pose optimization.

3. RESULTS AND DISCUSSION

Two drug-like molecules filtered by Lipinski's rule of five are Syringic acid and 2-Bromo-4-(trifluoromethoxy) benzoic acid. Possible binding sites were predicted in Discovery Studio. Among the 2 drug-like molecules, Syringic acid showed significant interaction with target proteins which are listed in Table 1. Dockscore is used to screen the top 10 poses which possess less ligand/protein interaction energy and the internal energy of the ligand which is used in grading the energy. From the top 10 poses, only the highest dock score poses were used for post docking scoring. Gemcitabine a chemotherapeutic agent is used as a control and was docked with 5 target proteins. The Dockscores were recorded and the strength of the bond formed is studied in Discovery Studio. The Dockscores of Syringic acid and Gemcitabine are compared for all the targeted proteins and listed in Graph1.The compounds of Syringic acid with highest dock score is compared with Gemcitabine.

Table 1 Various scores of the docking of the drug-like molecule for the pose which has the highest Dock score.

Compound	Target	Dockscore
Syringic acid	**1X3S**	**279.52**
Syringic acid	2RO1	99.156
Syringic acid	4IGG	93.313
Syringic acid	4PO2	99.546
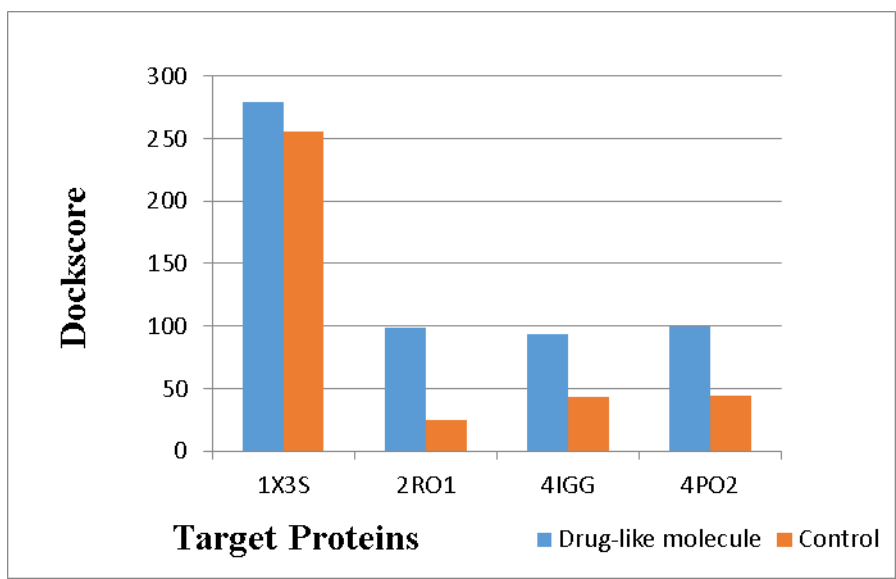
Graph 1 Dockscoresof Control and Drug-like molecule to targeted proteins of NSCLC

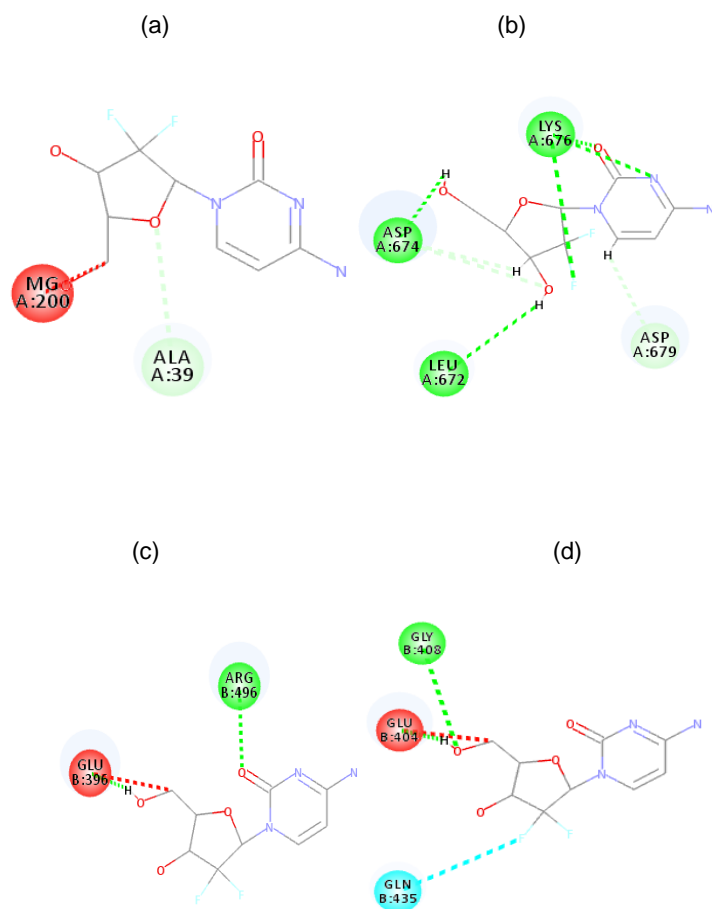**Figure 2** 2D structure of control (Gemcitabine) having dock score less than the drug like molecules. (a) 1X3S (b) 2RO1 (c) 4IGG (d) 4PO2

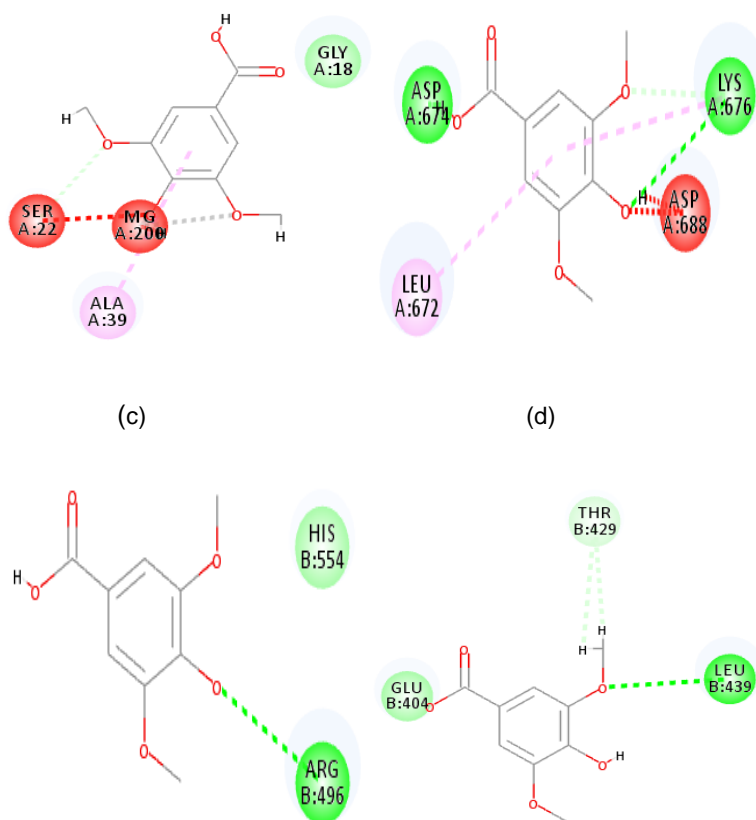(a)   (b)

(c)   (d)
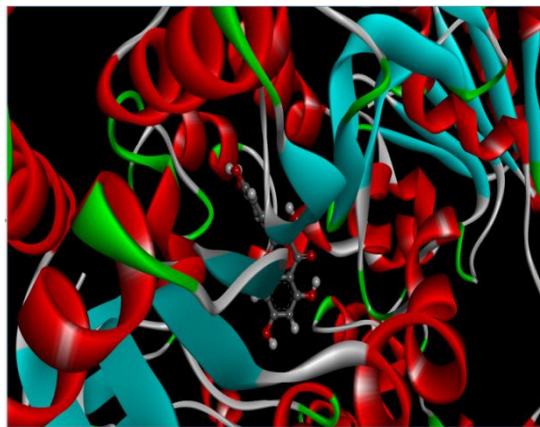
Figure 3 2D structure of Syringic acid having dock score more than the control.

(a) 1X3S (b) 2RO1 (c) 4IGG (d) 4PO2
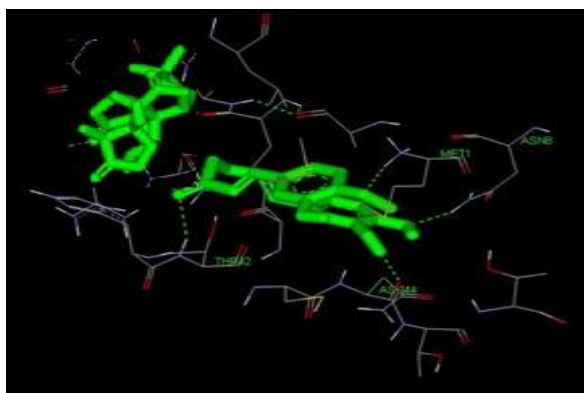

Figure 4 Interaction of Syringic acid   with 1X3S

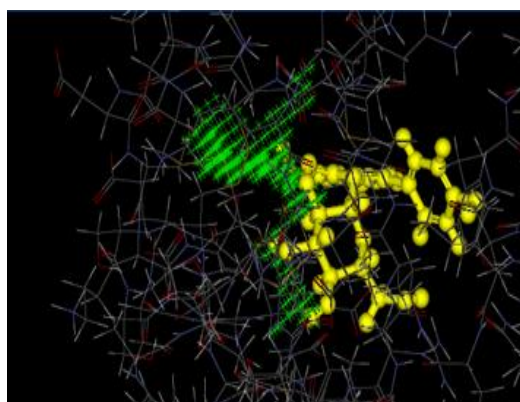**Figure 5** Interaction of Syringicacid with 2RO1
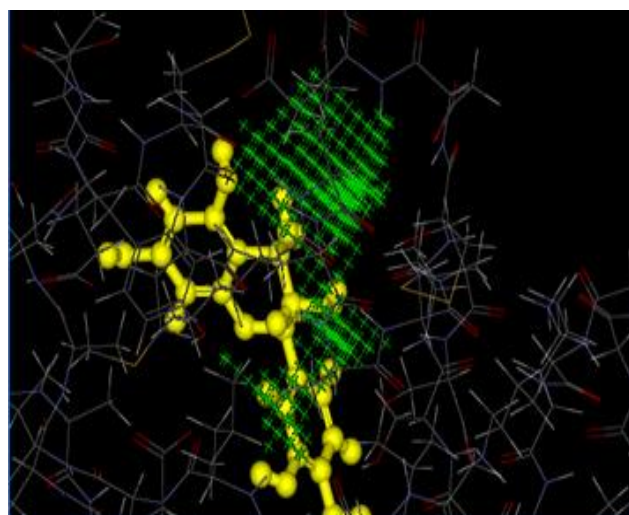

Figure 6 Interactionof Syringicacid with 4IGG

Figure 7 Interaction of Syringicacid with 4PO2

Pharmacodynamic Study

Figure 8 Pharmacodynamic results of Syringic acid

0,887	0,003	Antiseptic
0,888	0,014	Membrane integrity agonist
0,875	0,004	UGT1A6 substrate
0,872	0,003	Reductant
0,877	0,010	Ubiquinol-cytochrome-c reductase inhibitor
0,870	0,004	Membrane permeability inhibitor
0,869	0,003	Alkane 1-monooxygenase inhibitor
0,864	0,003	UGT1A9 substrate
0,860	0,003	Antimutagenic
0,861	0,008	HIF1A expression inhibitor
0,856	0,008	Mucomembranous protector
0,847	0,015	Testosterone 17beta-dehydrogenase (NADP+) inhibitor

Side Effects

Click this place to view possible adverse & toxic effects
(prediction is based on clinical manifestations, which are sometimes observed in a few or even in a single patient)

All   Pa>Pi   Pa>0,3   Pa>0,7   ok

Pa	Pi	Activity
0,905	0,004	Urine discoloration
0,901	0,004	Hematemesis
0,899	0,006	Shivering
0,857	0,005	Panic
0,853	0,008	Ulcer, aphthous
0,837	0,009	Fibrillation, atrial
0,822	0,005	Gastrointestinal hemorrhage
0,813	0,004	Hypercholesterolemic
0,831	0,026	Twitching
0,787	0,016	Postural (orthostatic) hypotension

Figure 9 Side effects of Syringic acid

Click this place to view possible adverse & toxic effects
(prediction is based on clinical manifestations, which are sometimes observed in a few or even in
a single patient)

Pa	Pi	Activity
0,992	0,002	Anemia
0,975	0,003	Diarrhea
0,963	0,001	Ulcer, aphthous
0,964	0,003	Respiratory failure
0,964	0,003	Stomatitis
0,963	0,003	Hepatitis
0,956	0,004	Inflammation
0,956	0,004	Hypotension
0,950	0,005	Acidosis
0,947	0,003	Hyperthermic
0,947	0,005	Headache

Figure 10 Side effects of Gemcitabine
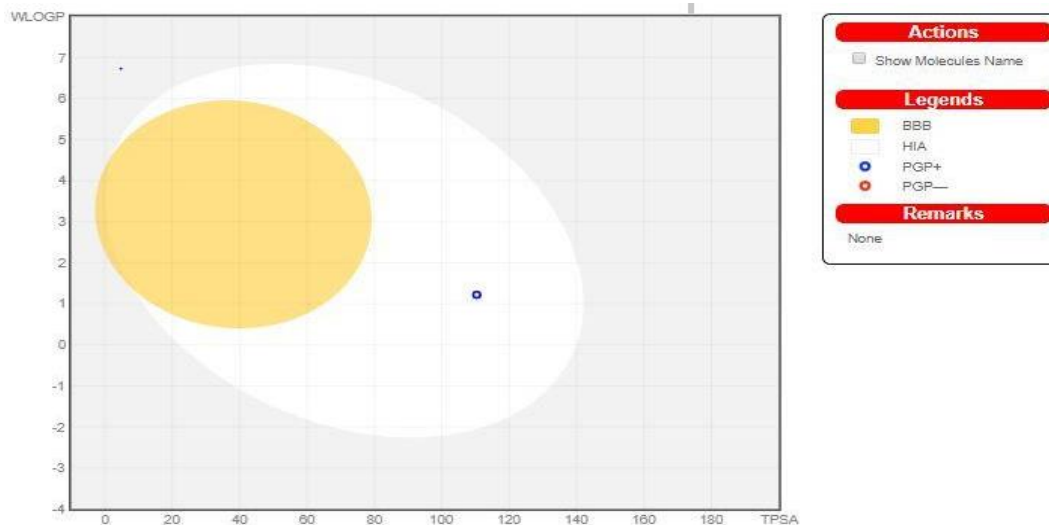
Pharmacokinetic study

Figure 11 Boiled egg predictive model for Intestinal and brain permeation of Syringic acid

The ADME studies carried out using SwissADME revealed the pharmacokinetic properties of Syringic acid. The gastrointestinal absorption is high whereas penetration of the blood-brain barrier is absent in the ligand. The tox tree results are suggestive of no risk of toxicity as there are no hints of toxicity found in the prediction.

CONCLUSION

The fundamental goal of in-silico drug design is to predict whether a given ligand molecule will dock to a targeted protein and to identify potential inhibitors. This computational tool has a vast range of applications ranging from refining the experimental data to predicting the efficacy of the selected drug compound. A comparative in-silico interaction analysis between a therapeutic drug molecule and plant-based bioactive phytochemicals also helps identify potential drugs like bioactive phytochemicals. The use of complementary experimental and informatics techniques increases the chance of success in many stages of the discovery process, from the identification of novel targets, development of lead compounds with desired properties, and elucidation of their functions of the discovery Molecular docking is frequently used to predict the binding orientation of small molecule drug candidates against their protein targets to order predict their affinity and activity of the small molecule.

- The cancer receptors namely Rab GTPase, Human KAP1 PHD finger bromodomain, Human alpha-catenin, Human heat shock protein HSP70, Human peroxiredoxin of NSCLC were downloaded from Protein Data Bank (PDB)
- Energy minimization for cancer receptors was done using SwiSS Viewer.
- The active principles from the herbal plant *Curculigoorchioides*namely 2-Bromo-4-(trifluoromethoxy) benzoic acid Syringic acidandcontrol drug Gemcitabine, were downloaded from the PubChem database.
- Molecular docking was carried out in Accelrys Discovery Studio commercial software.
- Among the potential phytochemicals, the component - Syringic acid showed effective interaction with the targeted cancer receptors.
- The **highest dock score of 279.52** was observed on the molecular interaction of 1X3S with Syringic acid, which is high compared to the standard control drug Gemcitabine.

Indiahas given to the medicinal world, natural remedies such as Ayurveda, Yunani, and Siddha. Based on such systems, we can find not only new remedies; but also new lead molecules may be obtained. Though data from clinical trials are positive, further investigations coupled with extensive clinical trials are required. Based on Ayurveda, Yunani, and Siddha, the current analysis provides an opportunity to treat active compounds of *Curculigoorchioides*as a potential novel drug target for the treatment of cancer in the future.

Acknowledgment:

Conflicts of interest:

We the authors of this paper have no conflicts of interest.

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