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IN SILICO SCREENING OF POTENTIAL DRUG (PHYTOCHEMICALS) AGAINST INTERLEUKIN-6 THE KEY PROTEIN INVOLVED IN METASTASIS

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Abstract:

Cancer is not a single disease. It's a group of diseases characterized by the uncontrolled growth of cells which results in tumors. The cells from the tumor at one point will start spreading to other organs which are termed metastasis. Mostly 90 percent of cancer patients die due to metastasis. A lot of treatments fail because of the complexity of cancer. The cancer cells can keep on mutating to adapt to the new environment. There are many different treatments available for cancer. But the most common ones are radiotherapy and chemotherapy. Both treatments can kill the cancer cells most often they end up killing the normal cells too. Then reduced sensitivity of the cancer cells to these treatments particularly in the advanced stages of cancer leads to ineffective treatment. The release of secretory factors from the cancer cells confers resistance to the cancer cells against therapy. Interleukin 6 which is found to be high in the tumor microenvironment regulates different signaling pathways and confers resistance to the cancer cells against damage. There are a lot of natural phytochemicals which can block interleukin 6 and thereby block metastasis. In this study, we selected 80 phytochemicals and studied their interaction capacity with the Interleukin -6. The phytochemicals were analyzed using molecular docking studies ADME-properties, and drug-likeness using the Schrodinger software. The phytochemicals were then analyzed using different software like protox and toxpro to study the anticarcinogenic, immunogenic, and genotoxic effects as well as the toxicity effects. The phytochemical Secoisolariciresinol has significantly interacted with active residues and it is an Interleukin -6 antagonist according to the protox II app too, which supports our results.

Keywords: *Phytochemicals, Interleukin -6, protox2, ADMET, Molecular docking, Protox-II, Stoptox, Secoisolariciresinol*

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INTRODUCTION:

Cancer is a dreadful disease, and around 10 million people died because of cancer and there are 19.3 million new cases according to Global cancer statistics 2020 (1). Different factors can accelerate the disease and make it worse. It is very difficult to diagnose the disease and this is one of the reasons why cancer is very difficult to cure. Cancer can adapt and develop new strategies to escape our immune system. The cancer mass is also composed of different groups of cells with different characterization and because of this, a single drug is impossible to target the whole mass. Cancer is also a complex disease and it's very difficult to understand its mechanism as it keeps mutating and adapting to new environmental circumstances.

As synthetic drugs often have a lot of side effects. It's always better to have a drug isolated from natural compounds. From the olden days to now, people always used dietary phytochemicals for treating cancer due to their least toxicity, safety, easy availability, and low cost (2). These natural drugs not only help in killing cancer cells but also protect normal cells with no side effects (3). Approximately 50 - 60 percent of American drugs are derived from natural sources such as different plant parts (4). Metastasis is the leading cause of death in cancer. During metastasis, the cells are dislocated from the primary site to other organs. Understanding this process will pave the way to identifying the target for cancer therapy.

Hasini Jayatilaka and her team at Johns Hopkins University observed that when the cells become too densely packed, some would break off and start spreading. At the cellular level, they were able to identify that interleukin 6 (IL-6) and Interleukin 8 (IL-8) are specifically increased with cell density and increase tumor cell migration in a cell density-dependent manner. This effect is specific to metastatic cancer cells. Furthermore, IL-6 and IL-8 do not affect the migration of normal and non-metastatic cancer cells (5). This discovery offers a significant place for cancer treatment

Interleukin – 6 is released by cancerous cells and plays an important role in the spreading, and differentiation of cancer cells. Increased concentration of interleukin- 6 is present in various cancers. Interleukin- 6 can play lots of important roles in the tumorigenesis process such as by regulating proliferation, apoptosis, metabolism survival, angiogenesis, and metastasis (6). In our study, we selected 80 phytochemicals and studied their interaction capacity with the Interleukin -6. The phytochemicals were analyzed using molecular docking studies using the Schrodinger software and drug-likeness using quikpro software.

LIGAND SELECTION:

Totally 80 phytochemicals were selected and their structures were retrieved in SDF format from the PubChem database and subsequently converted into PDB format. Hydrogen atoms were added and chargers were assigned to the retrieved structure. Canonical smiles of the ligand molecule were obtained from PubChem and used for the study.

The pharmacokinetic properties of a ligand like absorption, distribution, and excretion (ADME) were tested using Quikpro, a Schodringer module. The pharmacological significance of the Lipinski rule of five was generated and its drug-likeness was evaluated. Those compounds which obey the Lipinski rule of five were selected for further studies.

MOLECULAR DOCKING:

Among the 80 compounds, the 10 compounds which exhibited drug-likeness were selected and taken into account for docking studies using the Glide module. The interaction of Interleukin -6 with the phytochemicals was observed with the Pymol software.

TOXICITY ASSAYS:

Stoptox determines the chemical toxicity of the compounds including acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, skin irritation and corrosion, eye irritation and corrosion, and skin sensitization. We determined the toxicity class of the compounds using this software.

The prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways, and toxicity targets was done by protox.

INTERLEUKIN 6 ANTAGONIST PROPERTY:

The interleukin-6 antagonist's property was predicted to bypass online way2 drug software.

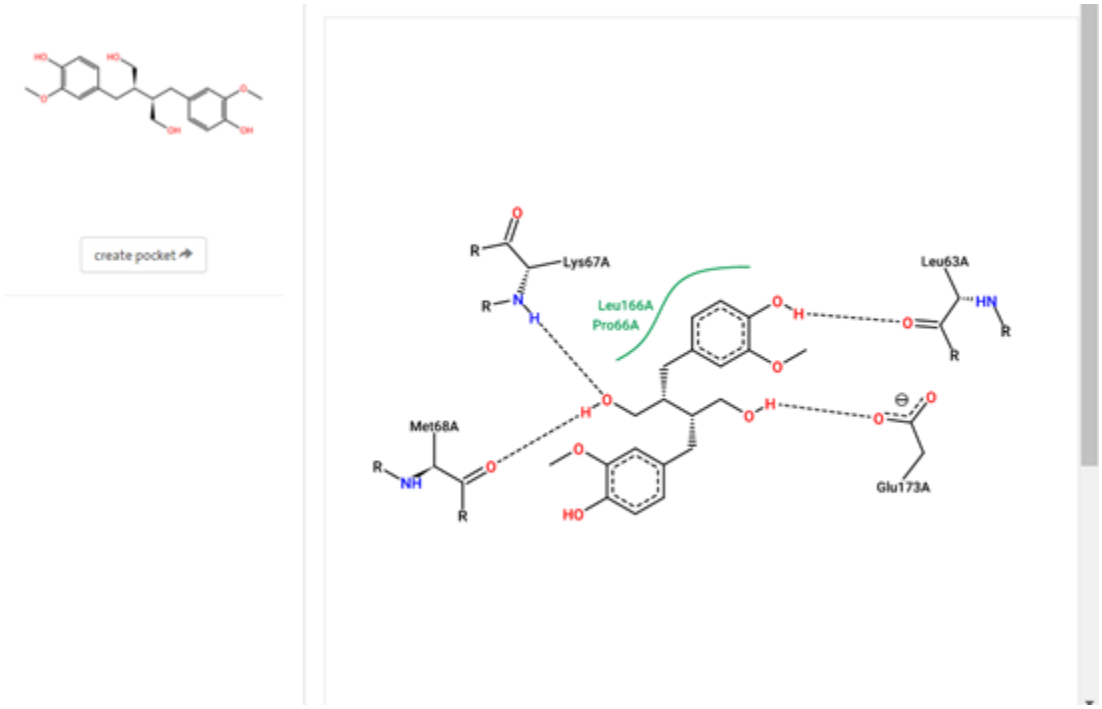

Figure.1 2D Representation of Secoisolariciresinol and protein Interleukin-6 Interactions

RESULTS AND DISCUSSION:

IL-6 represents an important factor of intercellular communication in the cancer cell niche. It also participates in cancer progression, including the formation of the premetastatic niche and the process of metastatic dissemination itself. IL-6 has a remarkable systemic effect, culminating, in the failure of metabolism, severe psychological and mental problems, and finally leading to the death of the cancer patient. Several studies indicate that the high concentrations of IL-6, IL-10, and TNF-α in the serum also help to predict the mortality of patients in advanced stages of cancer (7).

Research is being carried out throughout the world to identify the natural inhibitors from natural compounds. Chun J Y et al., 2010 (8) found that andrographolide, a diterpenoid lactone from *Andrographis paniculata*, could inhibit IL-6 expression and suppress IL-6–mediated signals. Based on this evidence we decided to screen various phytochemicals that could inhibit interleukin 6.

The phytochemicals selected were first analyzed for the ADMET properties. Based on Lipinski's rule the drug-likeness analysis was done using the quid pro software. Table 1 shows the properties such as octanol-water partition coefficient, blood-brain partition coefficient, absorption, distribution, metabolism, and excretion ability of the phytochemicals, which can be predicted using this software, and out of 80 compounds, only 10 compounds adhere to Lipinski's rule. All the phytochemical compounds under study were found to be absorbed in the gastrointestinal tract and could cross the blood-brain barrier.

Molecular docking studies of 10 phytochemicals were carried out with Interleukin -6 protein. The interaction of the phytochemicals with interleukin 6 protein was tabulated with G-Score, number of hydrogen bonds, bond length, number of bonds, and the interacting residues (Table 2). The LIGSITE tool is used to determine the amino acid residues.

The results of docking studies were recorded (Table 2) and found the compound secoisolariciresinol scored a significant G-score of -11.71 Kcal/mol among the other compounds tested. The interactions were observed with amino acid residue Methionine at two different positions 68, Lysine at position 67, Leucine at 63, and Glu at 173 positions with each of bond lengths of 2.5Å,2.6Å,2.1Å, 2.1 Å and 1.8 Å respectively. The compound secoisolariciresinol had significant interaction with the target protein which is presented in Figure 1 and the pymol structure of the same in Figure - 2.

Table 1 Analysis of ADME Properties for the Phytochemicals using QikProp

Molecule Name	**Rotatable bonds**	**Donor Hydrogen Bonds**	**Acceptor Hydrogen bonds**	**Octanol / Water partition coefficient**	**The surface area in square**	**Brain /Blood partition coefficient**	**Percent Human Oral Absorption**	**Rule Of Five**
	0-15	**0-6**	**2-20**	**2.0 – 6.5**	**300 – 1000**	**-3.0 - 1.2**	**>80% is high <25% is poor**	**Max- 4**
Secoisolariciresinol	13	4	6.4	2.467	629.502	-1.744	85.333	0
Enterolactone	6	2	4.5	2.555	550.644	-1.236	86.458	0
6 shogaol	10	1	3.5	4.036	641.09	-1.056	100	0
Hesperetin	4	2	4.75	1.803	542.569	-1.532	75.462	0
Apigenin	3	2	3.75	1.642	492.826	-1.446	73.538	0
Hispidulin	4	2	4.5	1.895	523.83	-1.349	78.349	0
Xanthohumol	10	2	4	4.082	694.288	-1.776	95.065	0
Lipochalcone	9	2	4.25	3.871	648.4	-1.284	100	0
Baicalein	3	2	3.75	1.757	489.252	-1.276	77.146	0
Curcumin	12	2	7	2.9	701.596	-2.079	85.501	0

Table 2: Docking of Phytochemicals with Interleukin -6 protein

Name of the ligand	G-Score	Residues	Bond	No of Bonds
PubChem ID	**(Kcal/mol)**	**Interaction**	**length (Å)**	
Secoisolariciresinol/ 65373	-11.71	LEU -63A LYS-67A MET-68A ARG-169A	2.2 2.1 2.6 2.8	4
Enterolactone/ 10685477	-10.21	LEU-65A LEU-65A LYS-67A MET-68A GLU-173A	2.5 3.1 2.1 3.2 2.5	5
6 shogaol/ 5281794	9.95	LEU- 63A LYS-67A MET-68A	1.6 2.1 2.8	3
Hesperitin/72281	9.35	ASN-62A LEU-65A SER-177A	2.8 2.4 2.8	3
Apigenin/5280443	-9.33	ASN-62A LEU-65A LEU-65A GLU-173A SER-177A	3.0 2.2 3.5 2.9 2.8	4
Hispidulin /5281628	-9.28	LEU-65A SER-177A	2.4 2.8	2
Xanthohumol/639665	-9.01	LYS- 67A MET-68A LYS-87A SER-170A	2.3 2.3 3.4 3.0	4
Lipochalcone/5318998	-8.73	LEU-65A LEU-65A ARG-169A	2.2 3.1 2.3	3
Baicalein/5281605	-8.57	LEU-63A LEU-65A	1.9 2.8	2
Curcumin/969516	-8.46	LUE-63A SER-177A	1.9 2.9	2

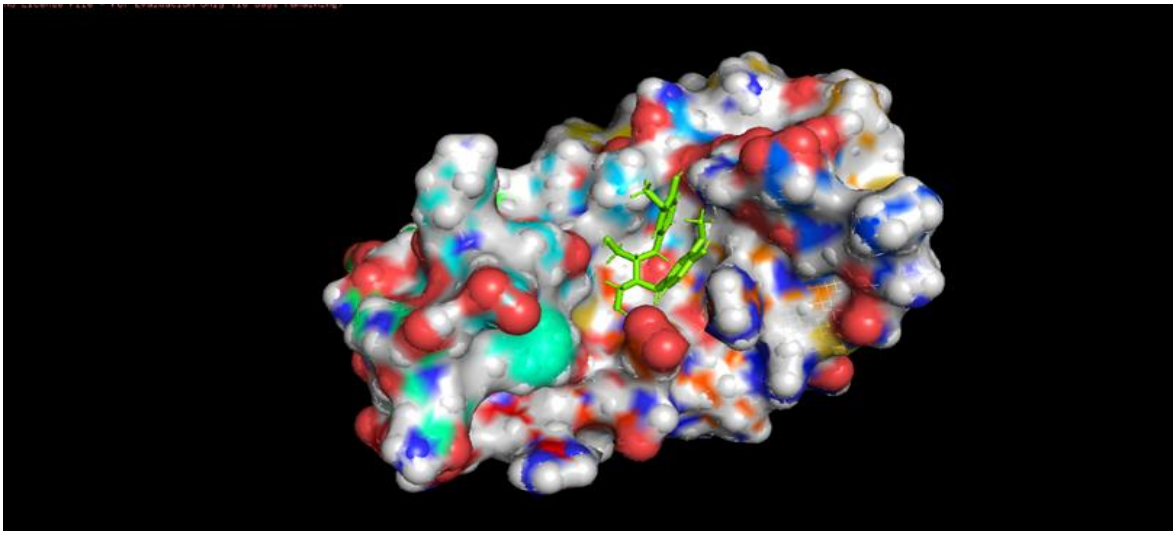

Figure 2: Representation of the docking of ligand (Secoisolariciresinol) with Interleukin using Pymol software The green color structure is the ligand and the multicolour structure is the protein.

Stoptox is an in-silico tool, which helps to identify both putative toxicants and non-toxicants in chemical libraries of their interest (9). The toxicity data of the phytochemicals are presented in Table 3. Secoisolariciresinol, Enterolactone, 6-Shagol, Curcumin, and Gingerol are all non-toxic to inhalation, oral, dermal, and eyes. But these are sensitive to the skin. Hesperidin, Apigenin, Hispidulin, Licochalcone A, and Baicalein can cause acute dermal toxicity. Apigenin, Xanthohumol, and Baicalein could cause eye irritation and corrosion. Hesperidin, Xanthohumol, and Licochalcone A are non-sensitizers to the skin and other compounds are sensitizers.

Table 3 - Prediction of toxicity of the phytochemicals using Stoptox Software

S.No	Phytochemicals	Acute Inhalation Toxicity	Acute Oral Toxicity	Acute Dermal Toxicity	Eye Irritation and corrosion	Skin Sensitisation	Skin Irritation and corrosion
1.	Secoisolariciresinol	Non-toxic	Non-toxic	Non-toxic	Non-toxic	sensitizer	Negative
2.	Enterolactone	Non-toxic	Non-toxic	Non-toxic	Non-toxic	sensitizer	Negative
3.	6- Shagol	Non-toxic	Non-toxic	Non-toxic	Non-toxic	sensitizer	Negative
4.	Hesperidin	Non-toxic	Non-toxic	Toxic	Non-toxic	Non-sensitizer	Negative
5.	Apigenin	Non-toxic	Non-toxic	Toxic	toxic	sensitizer	Negative
6.	Hispidulin	Non-toxic	Non-toxic	Toxic	Non-toxic	sensitizer	Negative
7.	Xanthohumol	Non-toxic	toxic	Non-toxic	toxic	Non-sensitizer	Negative
8.	Licochalcone A	Toxic	Non-toxic	Toxic	Non-Toxic	Non-sensitizer	Negative
9.	Baicalein	Non-toxic	Non-toxic	toxic	toxic	sensitizer	Negative
10.	Curcumin	Non-toxic	Non-toxic	Non-toxic	Non-toxic	sensitizer	Negative

Table 4 Assessment of the medicinal property by protox and Interleukin 6 antagonist property by pass online way2 drug software.

S.No	Phytochemical	Medicinal Property	Interleukin -6 antagonist
1.	Secoisolariciresinol	Cancer-related disease treatment, Apoptosis, Free radical scavenger, Antileukemia, Anthelmintic	Positive
2.	Enterolactone	Cytoprotectant, Cholesterol antagonist, anticarcinogenic, Antimutagenic, Antileukemia, Anti-inflammatory	Positive
3.	6 shogaol	Anti-mutagenic, free radical scavenger, Anti-inflammatory, Lipid peroxide inhibitor, Anti-carcinogenic, Anti-viral (Rhinovirus), Anti-leukemic, Interleukin -4 antagonist	Positive
4.	Hesperidin	Anti-carcinogenic, Apoptosis, Agonist, Hepatoprotective, Antineoplastic, Interleukin -12 agonist	Negative
5.	Apigenin	Anti-mutagenic, Antioxidant, free radical scavenger, Anti-leukemic, Anti-helminthic	Positive
6.	Hispidulin	Antioxidant, Hepatoprotective, Anti-carcinogenic, Anti-mutagenic, Anti-bacterial, Anti-neoplastic	Positive
7.	Xanthohumol	Anti-ulcerative, Chemoprevention, Free Radical scavenger, Interleukin antagonist, Interleukin -5 and 1 antagonist	Positive
8.	Lipochalcone	Free radical Scavenger, Anti-inflammatory, Anti-leukaemia, Anti-metastatic, Angiogenesis inhibitor, Interleukin -1 antagonist	Negative
9.	Baicalein	Anti-mutagenic, Apoptosis agonist, Antioxidant, Free radical scavenger, Anti-inflammatory, Anti-allergic, Interleukin- 1 antagonist	Negative
10.	Curcumin	Free radical scavenger, Anti-inflammatory, Cytoprotectant, Interleukin agonist, Interleukin-1 antagonist	Negative

Enterolactone-6, shogaol, Hesperidin, and Hispidulin have anticarcinogenic properties. Xanthohumol, Lipochalcone, Baicalein and Curcumin are Interleukin-1 antagonists. Secoisolariciresinol, Enterolactone, 6 shogaol, Apigenin, Hispidulin and Xanthohumol are Interleukin -6 antagonists and it was determined by passonline way2 drug software (Table - 4). This software gives the medicinal properties of the compounds and also clearly states whether they are Interleukin-6 antagonists or not.

Table -5 Toxicity classes of the phytochemicals predicted by Protox

S.no	Phytochemicals	Predicted ld50 mg/kg	Predictedochemicals toxicity class	MolecularWeight (sa)
1.	Secoisolariciresinol	2000	Class 4	130-725
2.	Enterolactone	2000	Class 4	362.422
3.	6- Shagol	687	Class 4	298.338
4.	Hesperitin	2000	Class4	276.375
5.	Apigenin	2500	Class5	302.283
6.	Hispidulin	4000	Class 5	270.241
7.	Xanthohumol	3800	Class 5	300.267
8.	Licochalcone A	1000	Class4	354.402
9.	Baicalein	3919	Class 5	338.402
10.	Curcumin	2000	Class 4	270.241

Protox-II helps to predict the different levels of toxicities of the phytochemical compounds (https://tox-new.charite.de/protox_II/index.php?site=home). Secoisolariciresinol, Enterolactone,6- Shagol, Hesperetin, Licochalcone A, and Curcumin belong to the class 4 toxicity group. Apigenin, Hispidulin, Baicalein, and Xanthohumol belong to class 5. Gingerol belongs to class 3. Class 3 is toxic if swallowed (50 <LD50<300). Class 4 is harmful if swallowed (30<LD50≤2000). Class 5 may be harmful if swallowed (2000<LD50≤5000).

The bioavailability radar plots of the tested Secoisolariciresinol indicated that it was fairly inside the pink area, which indicates their drug-likeness along with a very good bioavailability profile (Figure -3).

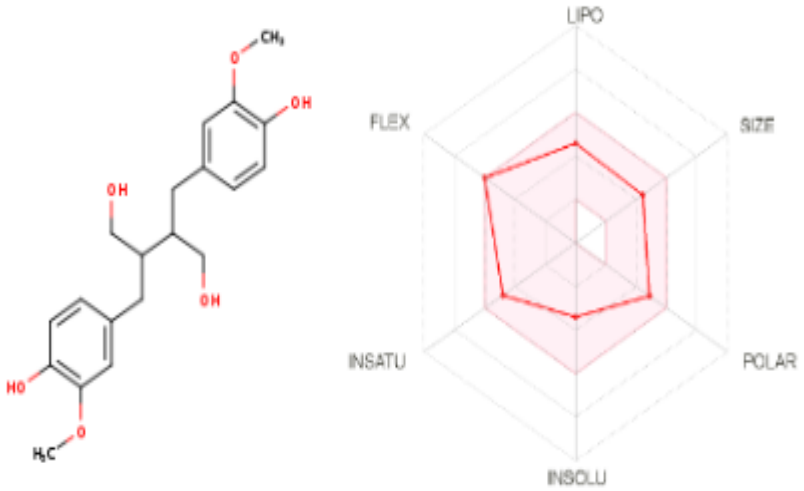

Figure 3- Toxicity radar chart of Secoisolariciresinol

There are several studies reported using the target protein Interleukin -6. The network pharmacology research showed that TCM could decrease IL-6 using several compounds, such as quercetin, ursolic acid, luteolin, and rutin. Molecular docking results showed that the molecular binding affinity with IL-6 of all compounds except γ-aminobutyric acid was < -5.0 kJ/mol, indicating the potential of numerous active compounds in TCM to directly interact with IL-6, leading to an anti-inflammation effect (10).

IL-6 protein showed strong interactions with the isoorientin, lupeol, and andrographolide with the least binding energies (-7.1 to -7.7 kcal/mol). These phytocompounds also have promising drug-likeliness and ADMET properties and thus can be very beneficial for COVID-19 treatment (11). Glycyrrhizin also showed very strong binding with the virus enzymes Mpro , PLpro, and Nucleocapsids and this data gives an understanding of how the natural compounds could be used to counter SARS-CoV-2 infection (12).
Several studies have been reported on the inhibitors of Interleukin-6 that are obtained from natural sources. The downregulation of IL-6 by curcumin has been reported by Ghandadi, M (2017). The inhibition of IL-6 signaling by curcumin suggests the anti-inflammatory effects of curcumin and can be considered as a potential therapy against IL-6-involved pathological diseases (13).

Zhong, M.et al.,(1999) studied that Reveratrol dose-dependently inhibits IL-6 release in cultured macrophages induced by A23187 and fMLP. It inhibits IL-6 release by blocking the calcium ion influx into cells (14).

The inhibition of IL-6 secretion in mouse macrophages by andrographolide derivative compounds with the structure of 12-hydroxy-14-dehydroandrographolide showed better inhibitory activity than the compounds with the structure of is andrographolide. (15)

IL-6 and TNF-alpha secretion were moderately reduced by the compounds apigenin, capsaicin, chrysin, diosmetin, kaempferol, luteolin, naringenin, quercetin, and resveratrol (16). Epigallocatechin-3-gallate (EGCG), an anti-inflammatory compound found in green tea, inhibits IL-1beta-induced IL-6 production has been reported by Salahuddin, et al.,(2008)(17).

Vedin, et al.,(2008) reported that there was an increase in DHA in the plasma of the AD patients treated with DHA-rich n-3 FAs supplementation and it also reduced the release of IL-6. (18). Treatment of bipolar patients with Lithium increased IL-beta and decreased IL-6 production. They also tried LiCl treatment and the results showed that the treatment decreased IL-1beta production and had minimal effects on IL-6 production (19). These studies conclude that the inhibition of IL-6 plays a very important role in managing various diseases.

IL-6 overexpress in most tumor types. The bond between inflammation and cancer is reflected by the high levels of IL-6 levels and it also promotes tumorigenesis by regulating multiple signalling pathways, including apoptosis, survival, proliferation, angiogenesis, invasiveness and metastasis, and, most importantly, metabolism. so blocking IL-6 could be a potential anticancer therapy (20). Various plant compounds show the better killing of cancer cells in in vitro studies.Zea mays leaves are proven to kill cancer cells and also help to improve the chemotherapeutic action of Etoposide (21). These studies prove that natural compounds could be a good source to inhibit IL-6.

CONCLUSION:

The phytochemical Secoisolariciresinol has significantly interacted with active residues LEU 63A, LYS 67A, MET 68 A, and ARG 169A of Interleukin-6. The compound exhibited a significant G-score as well as the predicted ADME properties and drug-likeness were also noteworthy. It is also an interleukin -6 inhibitor and it is used in treating cancer-related diseases and belongs to the class 4 toxicity group. Therefore, the compound should further be analyzed for stability in the interaction with the targeted protein using molecular dynamics studies.

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CONFLICTS OF INTEREST:
The authors declare there are no competing interests.

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