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In-Silico Study of Phytochemicals of *Asparagus Racemosa* and *Trifolium Pratense* for Anti-Cancer potential.

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Abstract: The investigation of natural compounds derived from medicinal plants for their anti-cancer potential has emerged as a promising strategy in modern drug discovery. Among these natural sources, Asparagus racemosa and Trifolium pratense have garnered attention due to their rich phytochemical composition and traditional medicinal uses. This abstract aims to provide an overview of the in silico studies conducted on the phytochemicals of Asparagus racemosa and Trifolium pratense, specifically focusing on their anti-cancer potential. Asparagus racemosa, commonly known as Shatavari, is a medicinal plant native to India and Sri Lanka. It has been traditionally used in Ayurvedic medicine for various health conditions, including cancer. Phytochemical analysis of Asparagus racemosa has revealed the presence of bioactive compounds such as saponins, flavonoids, and steroidal compounds, which have demonstrated cytotoxic effects against cancer cells in preclinical studies. However, the precise mechanisms underlying the anti-cancer activity of these phytochemicals remain to be fully elucidated. Similarly, Trifolium pratense, or red clover, has been recognized for its medicinal properties and therapeutic potential in various ailments, including cancer. Phytochemicals isolated from Trifolium pratense, including isoflavones, flavonoids, and phenolic compounds, have exhibited anti-cancer effects through multiple mechanisms, such as antioxidant, anti-inflammatory, and anti-proliferative activities. Several in silico studies have been conducted to explore the anti-cancer potential of phytochemicals from Asparagus racemosa and Trifolium pratense. These studies have employed molecular docking to predict the binding affinity of phytochemicals to key proteins involved in cancer pathways, such as kinases, transcription factors, and enzymes. Molecular dynamics simulations have further elucidated the dynamic behavior of protein-ligand complexes, providing insights into the stability and flexibility of binding interactions. Overall, in silico studies of phytochemicals from Asparagus racemosa and Trifolium pratense represent a valuable approach for accelerating the discovery of anti-cancer therapies from natural sources. By combining computational techniques with experimental validation and interdisciplinary collaborations, researchers can unlock the full therapeutic potential of phytochemicals, offering new avenues for cancer treatment and improving patient outcomes.

Continued research efforts are warranted to validate the findings of in silico studies and translate them into clinical practice, ultimately benefiting cancer patients worldwide. In the current study, the phytochemicals of Asparagus racemosa and Trifolium pratense with Anti-Cancer potential were studied. Utilizing PubChem and IMPPAT database, the 3D-SDF structures of Asparagus Racemosa and Trifolium Pratense phytochemicals were discovered. The PyMOL software was utilized to convert the ligand file format's 3D structure from SDF to PDB file format. Using SwissADME an excel sheet was created with the physicochemical properties of both Asparagus Racemosa and Trifolium Pratense phytochemicals. The physico-chemical properties considered are molecular weight, H-bond donor, H-bond acceptor, Clog P, Number of rotatable bond, TPSA. (Lipinski's Rule Analysis). Using SwissADME an excel sheet was created with the pharmacokinetics properties of both Asparagus Racemosa and Trifolium Pratense phytochemicals. The GI absorption, BBB permeant, P-gp substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9

inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, LOG Kp. The Hypotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, BBB barrier, eco toxicity, clinical toxicity, nutritional toxicity were analysed using ProTox Tool ((ProTox-3.0 - Prediction of TOXicity of chemicals (charite.de)).
Molecular docking of phytochemicals of Asparagus Racemosa and Trifolium Pratense was done against 5ILS protein retrieved from RCSB. (RCSB PDB: Homepage) . The lowest binding energies were analysed as there is a clear correlation between the proteins low binding energies and their high affinity for their substrates. The protein-ligand interaction was seen through PyMol tool (PyMOL | pymol.org) . The phytochemicals with the lowest binding energies can be excellent candidates for Anti-Cancer medicine development.

Keywords : Phytochemicals, inhibitor, lowest binding energy, binding affinity.

I: INTRODUCTION:

Worldwide, the fight against cancer has been ongoing, despite significant advancements in treatment and prevention. One of the hallmarks of the illness is the body's cells growing uncontrollably, making it impossible to stop. Consequently, malignant cell tumors that have the ability to spread [(1)]. Chemotherapy, radiation, and medications generated from chemicals are currently used as therapies. Chemotherapy is one of the treatments that can cause patients great stress and significant harm to their health. As a result, emphasis is placed on employing complementary medicines and treatments to combat cancer [(2)]. Herbal remedies have been the mainstay of medical care for many years, and they continue to be so in underdeveloped nations. Since plants naturally contain antibacterial qualities, they have been employed in medicine. As a result, studies are now looking into the possible qualities and applications of extracts from terrestrial plants for the creation of possible medications based on nanomaterials that could treat illnesses like cancer [(3)] .There are now numerous plant species being used to treat or stop the growth of cancer. Many plant species with anticancer qualities have been found by researchers, with particular attention paid to those that have been utilized in herbal therapy in underdeveloped nations [(1,4–5).]
The process by which cancer develops is influenced by changes in epigenetic mechanisms and their dysregulation [(6)]. Cancer cells have dysregulated control over the hypermethylation of tumor-suppressor genes on CpG islands. Tumor-suppressor gene inactivation and gene silencing may arise from this [(7).] In recent years, medications that can block or reverse epigenetic changes have been developed [(6).]
Using all plant parts—including the stem, leaf, root, and bark—in the treatment should help to promote the sustainability of medicinal plants in underdeveloped nations. Additional techniques for conservation include tissue culture, cryopreservation, germplasm conservation, keeping viable seeds, propagating plants in sterile circumstances, and swiftly creating mature plants and clones of uncommon species [(9-10)]. In wealthy nations, these preservation techniques will also enable industrial use [(9).]
To meet the growing need for natural alternatives to pharmaceuticals, various medicinal plants are being widely cultivated in developed regions including Europe, India, and portions of China[(9)] . Plant extinction may be avoided and strain on other wild species may be reduced by cultivating sustainable species. Plants with anti-cancer potential have long been the focus of research due to their rich diversity of bioactive compounds. These natural compounds, often referred to as phytochemicals, exhibit various biological activities that can inhibit the growth and spread of cancer cells.
One such plant is Asparagus racemosa, which contains phytochemicals like saponins, flavonoids, and steroidal compounds. These compounds have demonstrated cytotoxic effects on cancer cells, inducing apoptosis and inhibiting proliferation. Similarly, Trifolium pratense, commonly known as red clover, contains isoflavones, flavonoids, and phenolic compounds, which have shown promise in preventing and treating cancer through antioxidant, anti-inflammatory, and anti-proliferative mechanisms.
Trifolium pratense contains a number of isoflavones, including genistein and daidzein, which are well-known for their anti-cancer properties. These substances exhibit estrogenic action and have been shown to have inhibitory effects on malignancies that are hormone-dependent, such as tumors of the breast and prostate. It has been demonstrated that genistein, in particular, inhibits the growth and spread of tumors by suppressing angiogenesis, causing apoptosis, and inhibiting tumor cell proliferation.
Trifolium pratense contains flavonoids with anti-inflammatory and antioxidant characteristics, like formononetin and biochanin A, which are important for both the prevention and treatment of cancer. These substances lower oxidative stress, scavenge free radicals, and alter inflammatory pathways linked to the development of cancer.

Asparagus Racemosa **(Plant with anti-cancer potential)** –

Selected phytochemicals of Asparagus Racemosa for study of Anti-Cancer Potential are: Cyanin, Quercitin, Sarsasapogenin, Hyperoside, Rutin, Bita-Sitosterol, Stigmasterol, D-Glucose, L-Rhamnose, beta-Sitosterol-beta-D-glucoside, Diosgenin, D-Galacturonic Acid, Kaempferol, 3-(Galactosyloxy)-3',4',5,7-tetrahydroxyflavylium chloride, Saccharin, Quercetin 3-O-glucuronide.

Trifolium Pratense **(Plant with Anti-Cancer potential)-**

Selected phytochemicals of Trifolium Pratense for study of Anti-Cancer Potential are: Pratenol B, Pratenol A, Coumarin, 1-Phenylethanol, Acetophenone, Methyl cinnamate, Salicylate, Longifolene, cis-3-Hexenyl acetate, Soyasapogenol B, Rothindin, Genistin, , D-Glucuronic Acid, Pseudobaptigenin, Pratensein, Calycosin, Ononin, Isochavibetol, 2-Ethoxy-1-methoxy-4-(1-propenyl)benzene, Biochanin A, Formononetin, Soyasapogenol E, Trifolian, Soyasapogenol D, Trifolin, Caffeine, Cannabinol, 2-Ethylhexanal, , Medicagol, Coumestrol, Daidzein, Stearic Acid, 2-Phenylethanol,, Isocoumarin, Palmitic acid, , Formononetin, 3-Hydroxycoumarin, Trifolirhizin, Soyasapogenol F, Arachidic acid, Hyocine, 2-Hexenal, cis-3-Hexen-1-ol, 2-Hexen-1-OL.

II.METHODOLOGY

The procedures that were performed are mentioned as follows:

2.1 : Information Source :

To obtain the *Asparagus Racemosa* and *Trifolium Pratense* phytochemicals and conventional medicinal molecules, the IMPPAT database (https://cb.imsc.res.in/imppat/home) and PubChem were utilized (3D PDB). Utilizing PubChem and IMPPAT database, the 3D-SDF structures of Asparagus Racemosa and Trifolium Pratense phytochemicals were discovered. The PyMOL software was utilized to convert the ligand file format's 3D structure from SDF to PDB file format. The PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was searched for the 3D structures of ligands. The ligand structures in .sdf files (structure dimension files) were optimized, and converted to PDB file (protein data bank file) using PyMol software (https://pymol.org/2).

2.2 Calculation of ADMET properties:

Using SwissADME an excel sheet was created with the physicochemical properties of both *Asparagus Racemosa* and *Trifolium Pratense* phytochemicals. The physico chemical properties considered are molecular weight, H-bond donor, H-bond acceptor, Clog P, Number of rotatable bond, TPSA. (Lipinski's Rule Analysis)

Using SwissADME an excel sheet was created with the pharmacokinetics properties of both *Asparagus Racemosa* and *Trifolium Pratense* phytochemicals. The GI absorption, BBB permeant, P-gp substrate, CYP1A2 inhibitor, CYP2C19 inhibitor, CYP2C9 inhibitor, CYP2D6 inhibitor, CYP3A4 inhibitor, LOG Kp.

2.3 ProTox II Tool :

Protox II is a computational tool developed by the United States Environmental Protection Agency (EPA) for predicting the potential toxicity of chemicals, specifically herbicides, to non-target organisms, particularly plants. It employs quantitative structure-activity relationship (QSAR) models to estimate the likelihood of a chemical's herbicidal activity by analyzing its molecular structure and comparing it to known herbicides. The tool focuses on assessing the chemical's potential to inhibit the enzyme protoporphyrinogen oxidase (Protox), a crucial enzyme in the heme biosynthesis pathway of plants. By evaluating various molecular descriptors, Protox II generates predictions of the chemical's toxicity to plants, typically expressed as an EC50 value.

The Hypotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, BBB barrier, eco toxicity, clinical toxicity, nutritional toxicity were analysed using ProTox Tool (ProTox-3.0 - Prediction of TOXicity of chemicals (charite.de))

2.4 Molecular Docking:

The molecular docking using Autodock was done using phytochemicals of Asparagus Racemosa with 5ILS protein-
Cyanin, Quercitin, Sarsasapogenin, Hyperoside, Rutin, Bita-Sitosterol, Stigmasterol, D-Glucose, L-Rhamnose, beta-Sitosterol-beta-D-glucoside, Diosgenin, D-Galacturonic Acid, Kaempferol, 3-(Galactosyloxy)-3',4',5,7-tetrahydroxyflavylium chloride, Saccharin.
The molecular docking using Autodock was done using phytochemicals of Trifolium Pratense with 5ILS protein- 1-Phenylethanol ,2-Ethylhexanal, 2-Hexen-1-OL, 2-Phenylethanol, Acetophenone , Caffeine, cis-3-Hexen-1-ol, Coumarin, Coumestrol, Daidzein, D-Glucuronic Acid, Formononetin, Genistin, Isochavibetol, Isocoumarin, Methyl cinnamate, Trifolian.

2.5 Visualisation of Protein ligand interaction using PyMol Tool :

The dlg files obtained from the docking of Phytochemicals of Asparagus Racemosa and Trifolium Pratense were converted to PNG images using PyMol.

2.6 Protein-Ligand interaction using PLIP-

PLIP (PLIP - Welcome (tu-dresden.de))is a free and open-source web server and command-line utility designed for structural bioinformatics-based protein-ligand interaction study. To predict, annotate, and visualize the interactions between proteins and small molecule ligands, such as medications, metabolites, and cofactors, it incorporates a number of algorithms and datasets. Analyzing protein-ligand complexes and determining the precise interactions between the protein and ligand molecules is one of PLIP's main tasks. PLIP uses a range of computational techniques, including as geometric criteria, hydrophobic contacts, hydrogen bond analysis, and electrostatic interactions, to identify the important residues involved in ligand binding and define the binding interface.

III. RESULTS :

The docking of phytochemicals of *Asaparagus Racemosa* (Cyanin, Quercitin, Sarsasapogenin, Hyperoside, Rutin, Bita-Sitosterol, Stigmasterol, D-Glucose, L-Rhamnose, beta-Sitosterol-beta-D-glucoside, Diosgenin, D-Galacturonic Acid, Kaempferol, 3-(Galactosyloxy)-3',4',5,7-tetrahydroxyflavylium chloride, Saccharin, Quercetin 3-O-glucuronide) and *Trifolium Pratense* (1-Phenylethanol, 2-Ethylhexanal , 2-Hexen-1-ol , 2-Phenylethanol, Acetophenone, Caffeine, cis-3-Hexen-1-ol, Coumarin, Coumestrol, Daidzein, D-Glucuronic Acid, Formononetin, Genistin, Isochavibetol, Isocoumarin , Methyl cinnamate, Trifolian) was done with 5ILS protein. The lowest binding energies were obtained. The lowest binding energies of the proteins and their high affinity for their substrates are directly connected.

Following table shows the lowest binding energies obtained-

S.no	Phytochemical of Asparagus Racemosa	LBE	Run
1	3-(Galactosyloxy)-3',4',5,7-tetrahydroxyflavylium chloride	-5.67	8
2	Beta-Sitosterol-beta-D-glucoside	-8.54	6
3	Bita-Sitosterol	-8.26	1
4	Cyanin	-3.66	13
5	D-Galacturonic Acid	-5.09	20
6	D-Glucose	-4	6
7	Diosgenin	-7.5	19
8	Hyperoside	-5.32	12
9	Kaempferol	-6.23	11
10	L-Rhamnose	-4.37	19
11	quercetin 3-O-glucuronide	-6.7	1
12	Quercitin	-5.84	17
13	Rutin	-4.31	16
14	Saccharin	-6.03	6
15	Sarsasapogenin	-7.34	4
16	Stigmasterol	-8.35	6

S.no	Phytochemicals of Trifolium Pratense	LBE	Run
1	1-Phenylethanol	-4.76	11
2	2-Ethylhexanal	-4.22	15
3	2-Hexen-1-OL	-3.94	2
4	2-Phenylethanol	-4.62	5
5	Acetophenone	-4.76	17
6	Caffeine	-5.2	16
7	cis-3-Hexen-1-ol	-4.05	15
8	Coumarin	-5.6	16
9	Coumestrol	-7.08	8
10	Daidzein	-6.62	9
11	D-Glucuronic Acid	-5.52	2
12	Formononetin	-7.09	8
13	Genistin	-6.28	8
14	Isochavibetol	-5.91	6
15	Isocoumarin	-5.39	14
16	Methyl cinnamate	-5.43	2
17	Trifolian	-7.51	2

Through the results taken from docking the phytochemical of *Asparagus Racemosa* ; Beta-Sitosterol-beta-D-glucoside showed the lowest binding energy -8.54 towards 5ILS protein.
The phytochemical of *Trifolium Pratense*; Trifolian showed the lowest binding energy -7.51 towards 5ILS protein.

IV: DISCUSSION

It has long been known that plants are abundant in bioactive substances, many of which have anti-cancer qualities. These substances, referred to as phytochemicals, have a variety of chemical configurations and modes of action. Alkaloids, flavonoids, terpenoids, and polyphenols are a few examples. Researchers can investigate the binding interactions of these phytochemicals with certain protein targets linked to the initiation and progression of cancer by using molecular docking experiments.
Numerous bioactive substances found in these plants have the ability to interact with particular proteins involved in cancer pathways. Comprehending the correlation between these compounds' binding energies and their affinity for proteins linked to cancer is crucial for the creation of efficacious anti-cancer medications.
Curcumin from turmeric and resveratrol from grapes are two well-known examples. Turmeric has a strong bioactive component called curcumin, whose anti-cancer properties have been the subject of much research. Gupta and Aggarwal's investigation used docking simulations of curcumin with a number of cancer-related proteins, including COX-2, STAT3, and NF-κB. According to the research, curcumin interacts with these proteins to decrease their function, which has encouraging anti-cancer properties. (11)
In the current study, the docking of Anti-Cancerous phytochemicals of Asaparagus Racemosa (Cyanin, Quercitin, Sarsasapogenin, Hyperoside, Rutin, Bita-Sitosterol, Stigmasterol, D-Glucose, L-Rhamnose, beta-Sitosterol-beta-D-glucoside, Diosgenin, D-Galacturonic Acid, Kaempferol, 3-(Galactosyloxy)-3',4',5,7-tetrahydroxyflavylium chloride, Saccharin, Quercetin 3-O-glucuronide) and Trifolium Pratense (1-Phenylethanol, 2-Ethylhexanal , 2-Hexen-1-ol , 2-Phenylethanol, Acetophenone, Caffeine, cis-3-Hexen-1-ol, Coumarin, Coumestrol, Daidzein, D-Glucuronic Acid, Formononetin, Genistin, Isochavibetol, Isocoumarin , Methyl cinnamate, Trifolian) was done with 5ILS protein. The lowest binding energies were obtained. The lowest binding energies of the proteins and their high affinity for their substrates are directly connected. Through the results taken from docking the phytochemical of Asparagus Racemosa ; Beta-Sitosterol-beta-D-glucoside showed the lowest binding energy -8.54 towards 5ILS protein.
The phytochemical of Trifolium Pratense; Trifolian showed the lowest binding energy -7.51 towards 5ILS protein.

There are various phases involved in the creation of medications derived from plants, such as preclinical research, clinical trials, and regulatory approval. Preclinical research uses in vitro and in vivo models to evaluate the safety, effectiveness, and mode of action of phytochemicals. After then, promising candidates move on to clinical studies so that human participants can test their safety and effectiveness. The approval process is supervised by regulatory bodies like as the FDA and EMA, who make sure that pharmaceuticals derived from plants meet strict requirements related to safety, quality, and effectiveness.
Medications manufactured from plants may have fewer adverse effects than those made from synthetic materials. Over millennia, a multitude of phytochemicals have developed in harmony with human physiology, increasing the probability of their compatibility with the human body (12). Furthermore, chemicals originating from plants frequently display advantageous pharmacokinetic characteristics, like increased bioavailability and metabolic stability, which can augment their therapeutic efficiency (13).
The creation of pharmaceuticals derived from plants is also consistent with the values of environmental stewardship and sustainability. Plant-derived medications are generated from renewable botanical sources that can be grown in environmentally friendly methods, as opposed to synthetic substances, which frequently need substantial chemical synthesis and may cause toxic consequences (14). In addition to lessening the environmental impact of medication development, this sustainable strategy boosts regional economies and encourages the preservation of biodiversity.

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