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Admet, Biological Activity Prediction and Docking Studies of Main Phytoconstituents Present in Eucalyptus Using Computational Tools

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Abstract:- In-silico studies and computational tools are widely used in the field of drug discovery and drug design because of the advantages that they offer over traditional methods, such as reduced timeand cost.

Eucalyptus, scientific name *Eucalyptus globulus*, family Myrtaceae, has long been known to possess medicinal properties, evidenced by its inclusion in many herbal formulations by traditional systems of medicine. Its activity is mainly due to mixture of volatile oils, terprnoids esters ketones etc. In this study, the most active of the phytoconstituents were screened using multiple sources to analyze their suitability as new drug molecules.

First, the SMILES of the constituents were obtained using PubChem, an online chemical database. These SMILES were then used to predict the various parameters of the compounds using ADMETLab 2.0, which gave results about the constituents' physicochemical properties, medicinal chemistry, absorption, distribution, metabolism, excretion, and toxicity parameters. Then the molecules were screened in PASS Online, an online activity predictor, which indicated that 5 of the constituents were mainly antieczemic in their activity, with the remaining constituents showing mainly carminative activity and also some antineoplasticactivity.

To test the antieczemic activity of the compounds, they were imported into the docking software Maestro by Schrodinger, where they were prepared using LigPrep, and were docked against the human histamine receptor in complex with doxepin obtained from PDB (Protein ID:3RZE), using the compound flucloxacillin (-7.708) as a synthetic standard. Among the phytoconstituents assessed spathulenol showed highest antieczemic activity (-6.368) compared to the standard flucloxacillin.

***Keywords*:-** *Eucalyptus, In-Silico, ADME, Docking, Anti-Eczemic, Activity.*

I. INTRODUCTION

In-silico was a term that was first used to describe artificial life by Christopher Langton, an American computer scientist, at the Los Almos National Laboratory in 1987 [1]. The term was used to characterize the experiments of biological nature in a simulated environment in a computer. The first proper reference of this term in literature was by a team of French scientists in the year in 1991[2]. The first referenced chapter in a chapter format was in 1990, in a book authored by Hans B. Sieburg. Originally, the term was meant to indicate only computer simulations, but now, is also used to refer to any calculations done by the computer regarding the simulations. The term was also used to indicate the simulation of certain biological systems, but now it also applies to any biological or chemical data that is simulated in a computer. With the advent of the field of genomics, particularly the progress and the completion of the Human Genome Project, and the rise of Bioinformatics as a field of interest, in-silico methods began to see widespread use in multiple fields [3].

In the field of medicinal chemistry, the advent of in-silico studies implied the shortening of the immense time required in the process of drug discover and drug design [4]. Traditionally, the process of drug discovery was tedious and time consuming with each molecule having to synthesized individually and then also screened for activity in-vitro or in-vivo, and then be taken into clinical trials by a sponsor. Even if this process was relatively straight forward, there were still chances of the drug being rejected in clinical trials, not due to a lack of activity, but due to toxicity or some other adverse effect. With the aid of computational tools, the process of drug discovery can be greatly reduced, since a large number of molecules can be screened virtually, without any need for synthesis [5]. Newer tools also have the ability to predict the various parameters of the molecule such as its physicochemical properties, clinically relevant parameters such as its bioavailability, clearance, plasma binding tendency, and even its toxicity parameters. These predictions are not absolute however, since they are all made using algorithms based on existing compounds and

their chemical features. So it is entirely possible for a new molecule that has good activity and no toxicity to be completely missed by the software or for a molecule to be wrongly flagged as inactive [6]. However, with the increasing ability of the software, including the latest artificial intelligence powered algorithms and not to mention the growing size of chemical libraries, the predictions made by each generation of the computational software is getting more accurate [7].

With almost 900 species and subspecies, the enormous genus Eucalyptus (Eucalyptus spp.) belongs to the Myrtaceae family. The second-largest genus after acacia is this tall, evergreen endemic to Australia and Tasmania [8]. It has been effectively introduced into 90 different nations since the 1850s, where it is currently one of the most significant and commonly planted general. In the past, Aboriginal people employed the eucalyptus plant for a variety of uses, including food and medicine. The oil is produced from leaves, fruits, buds, and bark, and because it has antibacterial, antiseptic, antioxidant, anti-inflammatory, and anticancer properties, it is used to treat respiratory conditions like the common cold and influenza as well as sinus congestion. The leaf, stem, and root of E. globulus are rich sources of phytochemicals, including flavonoids, alkaloids, tannins, and propanoids [9]. In order to separate the phytoconstituents from the plant's organs, several studies were carried out: Numerous volatile substances, including 1,8-cineole (eucalyptol), aromadendrene, -gurjunene, globulol, ß-pinene, pipertone, ß-myrcene and terpinen-4-ol, and alloaromadendrene, were discovered in the leaves and shoots of the eucalyptus plant. Eucalyptol isthe primary and most significant substance. Asparagine, cysteine, glycine, glutamic acid, ornithine, and threonine were isolated from fruits [10]; borneol, caproic acid, citral, eudesmol, fenchone, p-menthane, myrcene, myrtenol, terpineol, verbinone, and asparagine, cysteine, glycine.

Despite the fact that essential oil contains more than 18 different components, eucalyptol accounts for 79.85% of the chemical composition. The essential oil also revealed a high concentration of oxygenated monoterpenes, which differ across each species of Eucalyptus and may have different medicinal characteristics [11]. Seasons and geographic location, for example, have an impact on the composition pattern of essential oils, which has an impact on biological processes. Many nations, including China, India, South Africa, Portugal, Brazil, and Tasmania, use Essential oil extensively in food and beverage preparation, perfumery,cosmetics,aromatherapy,and,phytotherapy,products[12].

In-silico studies, despite having been introduced in the early 1980s, did not see full acceptance in the pharmaceutical industry until the early 2000s (Agarwal et.al). It was only after the successful use of computational tools in the discovery and design of various new drugs, that there was a widespread adoption of these computational tools [13].

In the field of drug discovery and design, computational tools are of main importance in the context of molecular screening (Rosales et.al). It aimed to reduce the time involved in the finding of a new lead molecule, and could also help in predicting, to a certain extent, the biological activity parameters of the compound and even its toxicity parameters. The quality of these predictions varies from one tool to another, since they use different algorithms and make different assumptions regarding molecular interactions [14].

One of the main tools that is used in screening the various pharmacokinetic properties of compounds, like its absorption, distribution, metabolism, excretion and toxicity, collectively called as ADMET properties, is an online tool called as ADMETLab, and more recently ADMETLab 2.0. This is in contrast to traditional methods, where the molecules ADMET properties would be screened only after verifying its activity on a receptor, which lead to the discovery of many compounds that were active in-vitro, but also highly toxic.

ADMETLab was a software that was developed by a team of Chinese scientists in 2018, (Xiong et.al) to be used as a web tool to quickly analyze the various properties of a molecule. Its predictions are based on experimental data and customized Quantitative Structure Property Relationship (QPSR) models [15].

This webserver tool can be used to calculate a total of 88 ADMET related properties including physicochemical properties, medicinal chemistry, and even toxicity parameters. It also had drawbacks, such as redundant compounds, or incomplete or unfinished relations/endpoints and in the earlier versions; the results were still up for interpretation. In the latest version ADMETLab 2.0, these shortcomings are overcome and it gives better results [16].

One of the properties that is of particular importance is the Lipinski's Rule of Five, also known as Pzifer's Rule of Five. It is a guideline that evaluates drug likeness to determine a compound's oral activity. It was first proposed by Christopher A. Lipinski in 1997 based on the common characteristic feature of most common orally active compounds, their lipophilicity. The five feature or rules that describe a molecule's activity are as follows: No more than 5 hydrogen bond donors, not more than 10 hydrogen bond acceptors, a molecular weight of less than 500 Daltons, a partition coefficient that does not exceed 5. These features or factors are known to affect the molecule's pharmacokinetic parameters. It is known however, that there are multiple exceptions to this rule, with many molecules obeying this rule, yet being orally inactive and others being activedespite deviating from this Rule [17].

The most probable activity of the molecules was screened for using PASS Online, (Filimonov et.al) a tool that uses structure descriptors and mathematical relations to predict the activity of a molecule by relating the structural features with a pre-existing library of molecules whose

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activity is confirmed. The result is displayed in a tabular format, with the values indicating the probability of the molecule having that activity [18].

After the activity of the molecules was identified using PASS Online, the activity was confirmed by carrying out docking studies in Maestro a software provided by Schrodinger. They were docked with the human histamine H1 protein (Protein ID:3RZE) obtained from PDB, an receptorthat has been identifies to play a key role in Eczema. Eucalyptus of the tribe Eucalypteae and genus Eucalyptus contains more than 700 species of tall trees and shrubs which is having ecological importance majorly found in Australia countries like Ethiopia, Malaysian, Brazil, Philippines, Indonesia, Tasmania and nearby island [19].

II. MATERIALS AND METHODS

➢ *Software and Programmes*

- *Chemsketch*

Phytoconstituents from literature review will be collected and their structure will be generated using Chemsketch software. An overview was available for specific phytoconstituent, including structure, molecular formula, molecular weight, canonical smiles, etc in databank.

The canonical smiles that were available in PubChem and pasted it into chem sketch. Structures for the particular drug were available. Similarly, this process was used to get structures for other compounds.

- *Open Babel*

Open Babel is computer software, a chemical expert system mainly used to interconvert chemical file formats. Different file formats are required by different computational software to perform studies. It has an input format in which the file is uploaded and the present file format and the desired output format must be specified before a convert command is given.

- *ADMET Lab 2.0*

By importing the SMILES to the ADMET Lab application, ADMET properties will be predicted. The ADMET property table includes endpoint, value and probability, respectively. The value is the predict labels. For example, for toxicity endpoints, the value "+" means Positive/Toxic while "-" means Negative/Nontoxic. The probability is related to the value, and it is generally higher than 50% because if the probability was less than 50%, it should have been predicted as the other result.

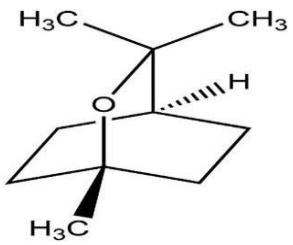
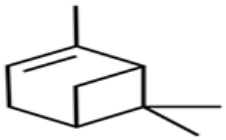
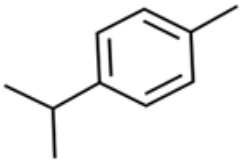
- *Pass Online*

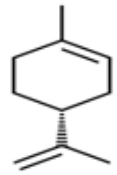
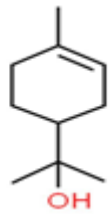
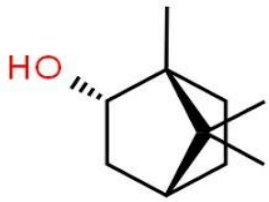
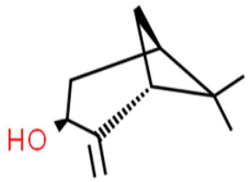
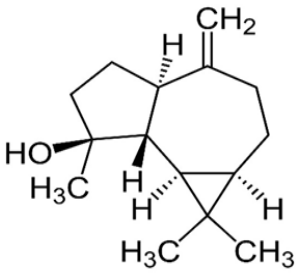
This is an online tool that is used to predict the biological activity of molecules. The SMILES of the compound are given as the input and the output includes a table of the various activities, given asprobabilities of being active.

- *Schrodinger Maestro*

This software, provided by Schrodinger was used to conduct the docking studies The SMILES of thecompounds were imported into the workspace and prepared using LigPrep, and then the protein/target was imported and processed and a receptor grid was generated. The final docking study was conducted in Glide and the results were interpreted using a standard as areference.

Table 1Phytoconstituents Assessed

Sl.no	Structure	Chemical constituents	SMILES
1		Eucalyptol (1,8-cineole)	CC1(C2CCC(O1)(CC2)C)C
2		Alpha-Pinene	CC1=CCC2CC1C2(C)C
3		P-Cymene	CC1=CC=C(C=C1)C(C)C

4		Limonene	CC1=CCC(CC1)C(=C)C
5		α-terpineol	CC1=CCC(CC1)C(C)(C)O
6		Borneol L	CC1(C2CCC1(C(C2)O)C)C
7		Trans- Pinocarveol	Trans- Pinocarveol
8		Spathulenol	CC1(C2C1C3C(CCC3(C)O)C(=C)CC2)C

III. RESULTS

The properties of the phytoconstituents were obtained using multiple online resources. The absorption, distribution, metabolism, excretion and toxicity parameters of the compounds were obtainedusing ADMET Lab. The potential activity of the molecules was obtained by screening the compounds in PASS Online, to obtain their most likely biological activity. Since a majority of the compounds showedantineoplastic activity, the same was screened for the compounds using the software by Schrodinger. The molecules were imported into the workspace and prepared to dock using LigPrep. The protein receptor chosen was the human Histamine H1 receptor in complex with doxepin from PDB site, (Protein ID: 3RZE). The receptor grid was then generated and the molecules were docked with 1 reference standard, a synthetic molecule fucloxacillin standard. The results were obtained as below:

ISSN No:-2456-2165

Table 2 Physicochemical Properties of Selected Phytoconstituents

Phytoconstituent	MolecularWeight	nHA	nHD	nHET	LogP
Eucalyptol(1,8cineole)	154.140	1	0	1	2.582
alpha-Pinene	136.130	0	0	0	4.125
p-cymene	134.110	0	0	0	3.994
Limonene	136.130	0	0	0	4.368
α-terpineol	154.140	1	1	1	3.084
Borneol L	154.140	1	1	1	2.719
Trans-Pinocarveol	152.120	1	1	1	2.049
Spathulenol	220.180	1	1	1	4.032

- **nHET**: Number of heteroatoms (optimal:1-15); **LogP:** Log of octanol-water partition coefficient **nHA:** number of hydrogen bond acceptors (optimal<10) **nHB**: number of hydrogenbond donors (optimal <5); **Molecular weight** (optimal <500)

Table 3 Medicinal Chemistry Properties of Selected Phytoconstituents

Phytoconstituent	Lipinski Rule	SA score	Np score	QED	Fsp3
Eucalyptol (1,8-cineole)	Accepted	3.953	2.776	0.521	1.000
alpha-Pinene	Accepted	4.273	2.714	0.449	0.800
p-cymene	Accepted	1.251	-0.717	0.553	0.400
Limonene	Accepted	3.165	2.359	0.485	0.600
α-terpineol	Accepted	3.214	2.436	0.575	0.800
Borneol L	Accepted	4.280	2.542	0.567	1.000
Trans-Pinocarveol	Accepted	4.694	3.129	0.526	0.800
Spathulenol	Accepted	4.302	3.307	0.620	0.867

- **QED**: A measure of &drug-likeness based on the concept of desirability (Attractive: > 0-67; unattractive: 0.49—0.67; too complex: < 0.34); **SA score:** Synthetic accessibility score is designed to estimate the ease of synthesis of drug-like molecules (SA score≥ 6, difficult tosynthesize. SA score<6 easy to synthesize); **NP score:** Natural product-likeness score (Range: -5to 5. The higher the score is, the higher the probability is that the molecule is a NP); **Fsp^3**, thenumber of sp3 hybridized carbons/total carbon count, is used to determine the carbon saturation of molecules and characterize the complexity of the spatial structure of molecules (excellent ≥0.45; poor < 0.45).

Table 4 Absorption Properties of Selected Phytoconstituents

Phytoconstituent	CACO-2 Permeability	Pgp Inhibitor	Pgp Substrate	HIA	F20
Eucalyptol (1,8)-cineole)	-4.414	0.002	0.0	0.002	0.01
alpha-Pinene	-4.303	0.0	0.0	0.004	0.102
p-cymene	-4.302	0.011	0.005	0.004	0.211
Limonene	-4.32	0.002	0.0	0.003	0.818
α-terpineol	-4.193	0.0	0.001	0.003	0.188
Borneol L	-4.442	0.0	0.002	0.005	0.017
Trans-Pinocarveol	-4.432	0.0	0.007	0.006	0.014
Spathulenol	-4.567	0.001	0.0	0.004	0.008

- **Caco-2 Permeability:** Optimal- higher than -5.15 Log unit; **Pgp- inhibitor:** Category l; Inhibitor; Category 0: Non-; **Pgp-substrate:** Category I: substrate; Category 0: Non- substrate; **HIA**: Human Intestinal Absorption (Category I: HIA+(HIA <30%); Category 0: HIA-(HIA < 30%); **F 20%:** 20% Bioavailability (Category I: F20%+ (bioavailability < 20%). Category 0: F20% bioavailability≥ 20%).

Table 5 Distribution Properties of Selected Phytoconstituents

Phytoconstituents	PPB	Vd	BBB Penetration	Fu	F20%
Eucalyptol (1,8-cineole)	90.09%	2.434	0.853	16.86%	0.01
alpha-Pinene	86.33%	1.7	0.896	12.59%	0.102
p-cymene	94.38%	2.139	0.728	6.087%	0.211
Limonene	86.38%	3.373	0.989	9.244%	0.818
α-terpineol	89.87%	1.307	0.981	13.68%	0.188

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Borneol L	77.67%	1.074	0.915	37.37%	0.017
Trans-Pinocarveol	39.19%	1.103	0.924	55.67%	0.014
Spathulenol	78.71%	00.919	0.978	18.37%	0.008

- **PPB**: Plasma Protein Binding (Optimal: <90%; **VD**: Volume Distribution (Optimal: 0.04- 20L/kg); **BBB Penetration:** Blood Brain Barrier Penetration (Category l: BBB+; Category O:BBB-; **Fu:** The fraction unbound in plasms (Low:<5% Middle:5-20%; High: >20%)

Table 6 Metabolism Properties of Selected Phytoconstituents

Phytoconstituents	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**
	(Inhibitor/Substrate)				
Eucalyptol (1,8-cineole)	0.095/0.569	0.235/0.914	0.136/0.804	0.016/0.549	0.009/0.234
alpha-Pinene	0.469/0.368	0.267/0.867	0.312/0.846	0.012/0.786	0.045/0.263
p-cymene	0.941/0.0944	0.855/0.864	0.574/0.61	0.778/0.755	0.084/0.62
Limonene	0.678/0.652	0.223/0.834	0.06/0.804	0.02/0.874	0.057/0.253
α-terpineol	0.112/0.301	0.06/0.83	0.059/0.87	0.008/0.311	0.018/0.207
Borneol L	0.083/0.496	0.052/0.902	0.113/0.841	0.005/0.776	0.023/0.213
Trans-Pinocarveol	0.052/0.122	0.023/0.767	0.041/0.689	0.003/0.591	0.009/0.231
Spathulenol	0.139/0.587	0.085/0.895	0.227/0.604	0.009/0.802	0.095/0.357

- **CYP1A2/CYP2C19/CYP2C9/CYP2D6/ CYP3A4 inhibitor:** Category 1: Inhibitor; Category O: Non-inhibitor **CYP1A2/CYP2C19/CYP2C9/CYP2D6/ CYP3A4 substrate**: Category I: Substrate;Category 0: Non-substrate.

Table 7 Excretion Properties of Selected Phytoconstituent

Phytoconstituents	**Clearance**	**T1/2**
Eucalyptol (1,8-cineole)	8.066	0.352
alpha-Pinene	15.022	0.114
p-cymene	7.38	0.276
Limonene	11.57	0.233
α-terpineol	8.942	0.527
Borneol L	13.693	0.276
Trans-Pinocarveol	9.502	0.116
Spathulenol	14.582	0.064

- **Cl:** Clearance (High>15mL/min/kg; moderate: 5-15mL/min/kg; low:<5mL/min/kg) $\boldsymbol{T_{1/2}}$:Half -life (Category 1: long half-life; Category0 :short half-life; long half-life>3h; short half-life:<3h)

Table 8 Toxicity Properties of Selected Phytoconstituents

Phytoconstituents	**H-HT**	**DILI**	**AmesTest**	**Carcinogenicity**	**RespiratoryToxicity**
Eucalyptol (1,8-cineole)	0.448	0.046	0.01	0.7	0.182
alpha-Pinene	0.916	0.023	0.002	0.056	0.825
p-cymene	0.037	0.201	0.018	0.386	0.03
Limonene	0.69	0.037	0.007	0.922	0.216
α-terpineol	0.087	0.034	0.003	0.71	0.03
Borneol L	0.055	0.031	0.011	0.071	0.89
Trans-Pinocarveol	0.146	0.026	0.003	0.112	0.954
Spathulenol	0.238	0.116	0.015	0.065	0.863

- **H-HT:** Human hepatotoxicity (Category 1:H-HT positive (+) category 0 :H-HT negative (-));
- **DILI:** Drug Induced Liver Injury (Category 1: drugs with high risk of DILI; Category 0:drugs with norisk of DILI); **AMES Toxicity**: Category 1: Ames positive(+), Category 0: Ames negative(-
- ;**Carcinogenicity:** Category 1: carcinogens, Category 0: noncarcinogens; **Respiratory Toxicity**: Category 1: respiratory toxicants, Category 0: respiratory non toxicants.

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Table 9 Most Probable Activity of Phytoconstituents:

Phytoconstituent	Pa	Pi	Activity
Eucalyptol (1,8-cineole)	0.927	0.00	Alcohol dehydrogenase substrate
	0.898	0.00	CYPC12 substrate
	0.833	0.022	Phobic disorder treatment
	0.814	0.012	Acylcarnitine hydrolase inhibitor
	0.793	0.004	Hepatic disorder treatment
alpha-Pinene	0.863	0.012	Testosterone 17beta-dehydrogenase (NADP+)inhibitor
	0.844	0.012	CYP2J substrate
	0.821	0.004	Cardiovascular analeptic
	0.792	0.009	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenaseinhibitor
	0.780	0.001	Alpha-pinene-oxide decyclase inhibitor
p-cymene	0.928	0.004	Ubiquinol-cytochrome-c reductase inhibitor
	0.919	0.004	Mucomembranous protector
	0.881	0.002	Carminative
	0.884	0.006	Antieczemic
	0.863	0.005	Membrane permeability inhibitor
Limonene	0.961	0.001	Carminative
	0.934	0.000	Retinol dehydrogenase inhibitor
	0.896	0.005	Antieczemic
	0.881	0.001	Alpha-pinene-oxide decyclase inhibitor
	0.816	0.007	Apoptosis agonist
α-terpineol	0.862	0.005	Respiratory analeptic
	0.837	0.003	Carminative
	0.853	0.023	CYP2C12 substrate
	0.825	0.014	Antieczemic
	0.804	0.014	Analeptic
Borneol L	0.962	0.002	Testosterone 17beta-dehydrogenase (NADP+)inhibitor
	0.954	0.002	Acylcarnitine hydrolase inhibitor
	0.947	0.002	Alkylacetylglycerophosphatase inhibitor
	0.918	0.003	Cardiovascular analeptic
	0.872	0.003	Vaso protector
Trans- Pinocarveol	0.917	0.004	Antieczemic
	0.881	0.009	Testosterone 17beta-dehydrogenase (NADP+)inhibitor
	0.837	0.006	Respiratory analeptic
	0.835	0.010	Acylcarnitine hydrolase inhibitor
	0.775	0.007	Antineoplastic
Spathulenol	0.826	0.013	Antieczemic
	0.774	0.004	MMP9 expression inhibitor
	0.749	0.005	Dermatologic
	0.753	0.018	Antineoplastic
	0.761	0.036	Testosterone 17beta-dehydrogenase (NADP+)inhibitor

Table 10 Docking Study Scores of Selected Phytoconstituent

Compound	Docking Score	Glide LigandEfficiency	Glide LigandEfficiency SA
Flucloxacillin	-7.708	-0.257	-0.798
Spathulenol	-6.368	-0.398	-1.003
Limonene	-6.084	-0.608	-1.311
alpha-terpineol	-5.944	-0.540	-1.202
p-cymene	-5.933	-0.593	-1.278
Trans-Pinocarveol	-5.273	-0.479	-1.066
alpha-Pinene	-4.989	-0.499	-1.075
Eucalyptol (1,8-cineole)	-4.684	-0.426	-0.947

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Table 11 Ligand Interaction with Human Histamine H1 Protein (PDB Id:3RZE)

Sl.no	Ligand	Hydrophobic reaction withligand	Polar interactionwith ligand	H-bond	Positively charged	Negativelycharged
1.	Flucloxacill in	TRP103,ILE454,TYR458, TRP428,TYR431,PHE432, PHE435,TYR108,PHE184, TRP158,ALA195,ILE115, PHE424,PHE199	ASN198, THR194, THR112,SER111	ASP107	LYS191	ASP107
2.	Spathulenol	TRP428,TYR108,TYR431, PHE432,PHE435,ALA195, TRP158,PHE199,ILE115	THR112 , SER111, ASN198, THR194		LYS191	ASP107
3.	Limonene	TYR108,TRP158,PHE424, ILE115,TRP428,TYR431, PHE432,PHE435,ALA195, PHE199	ASN198, THR194, THR112, SER111			
4.	Alpha- terpineol	TYR431,PHE432,PHE435, ALA195,TRP158,TYR108	THR112 , SER111 , ASN198, THR194		LYS191	ASP107
5.	p-cymene	TRP158,TYR108,ALA195 ,PHE199,PHE424,TRP428 ,TYR431,PHE432,ILE115	THR112,SER111,TH R194,ASN198			
6.	Trans- pinocarveol	TYR108,TRP158,ALA195 ,TRP428,TYR431,PHE432	SER111,T HR112,AS N198,THR 194	THR112		ASP107
7.	Alpha- pinene	PHE424,PHE199,TRP428, ILE115,TYR431,PHE432, PHE435,ALA195,TYR108	ASN198, THR194, SER111, THR112			ASP107
8.	Eucalyptol(1,8cineole)	PHE432,TYR431,TRP428, TRP158,PHE199,TYR108	ASN198,SER111,TH R112			ASP107

➢ *Docking Figures*

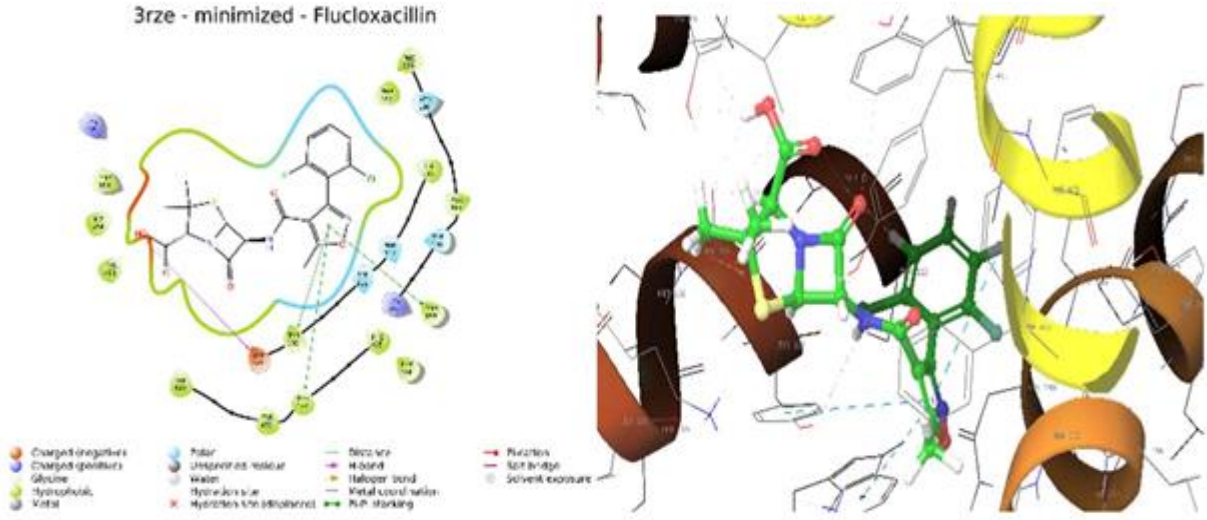

Fig 1 Interaction of Flucloxacillin with 3RZE

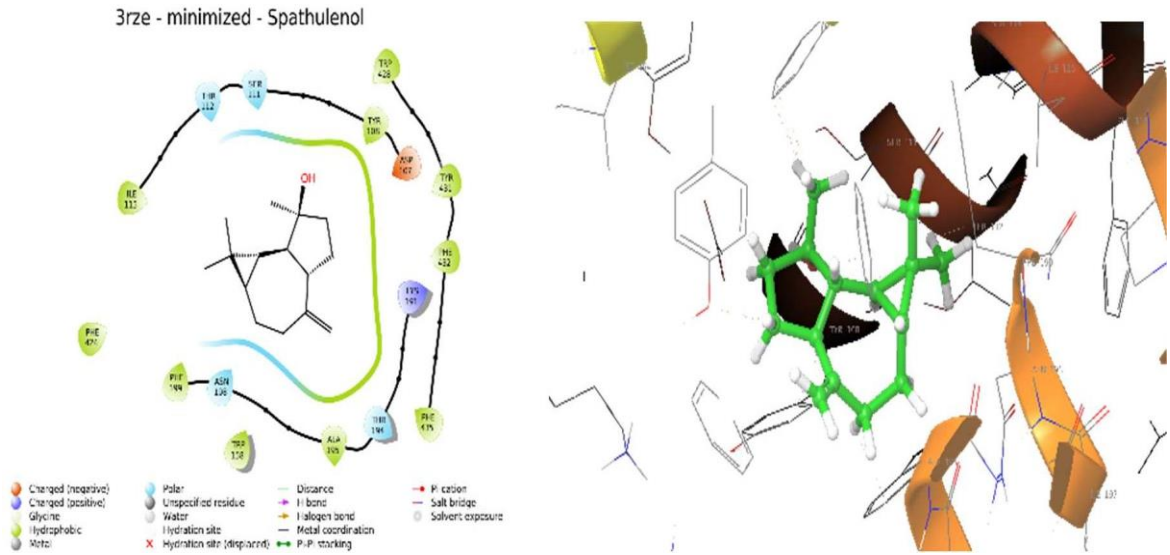

Fig 2 Interaction of Spathulenol with 3RZE

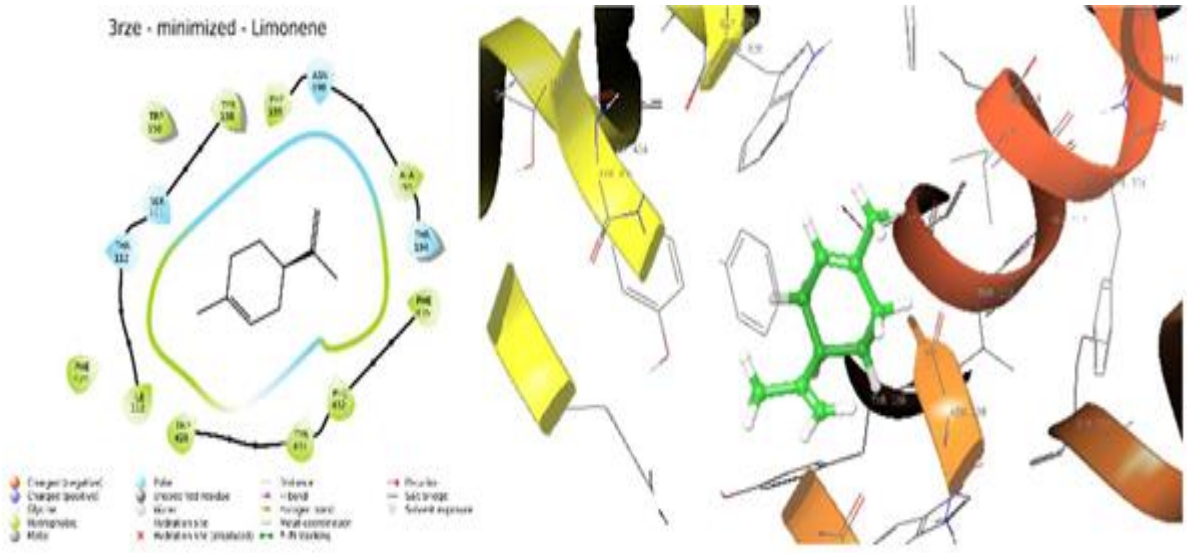

Fig 3 Interaction of Limonene with 3RZE

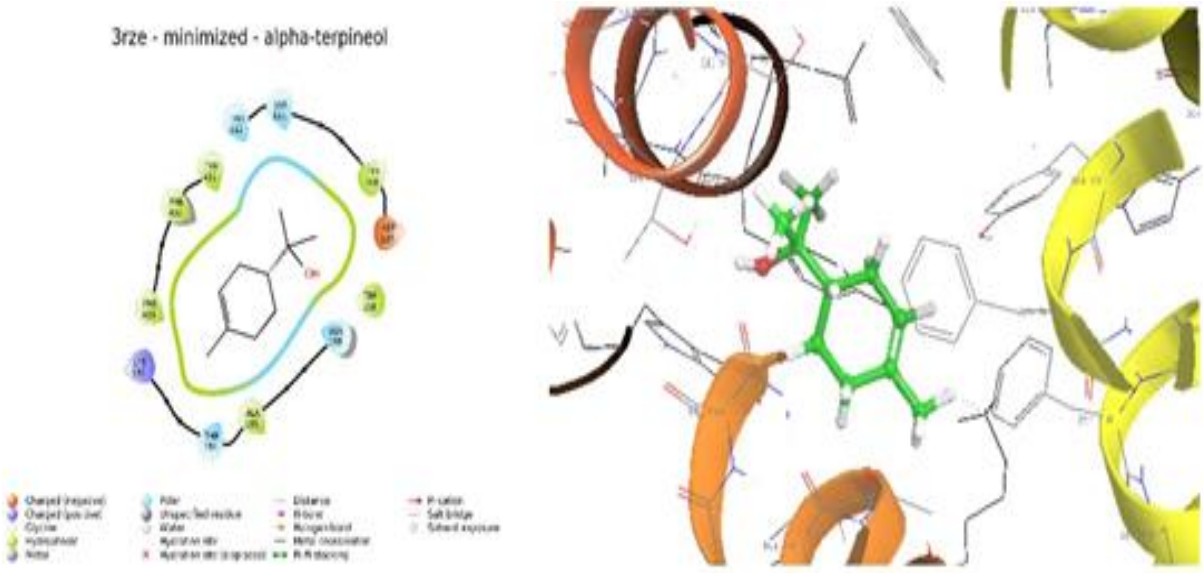

Fig 4 Interaction of Alpha-Terpineol with 3RZE

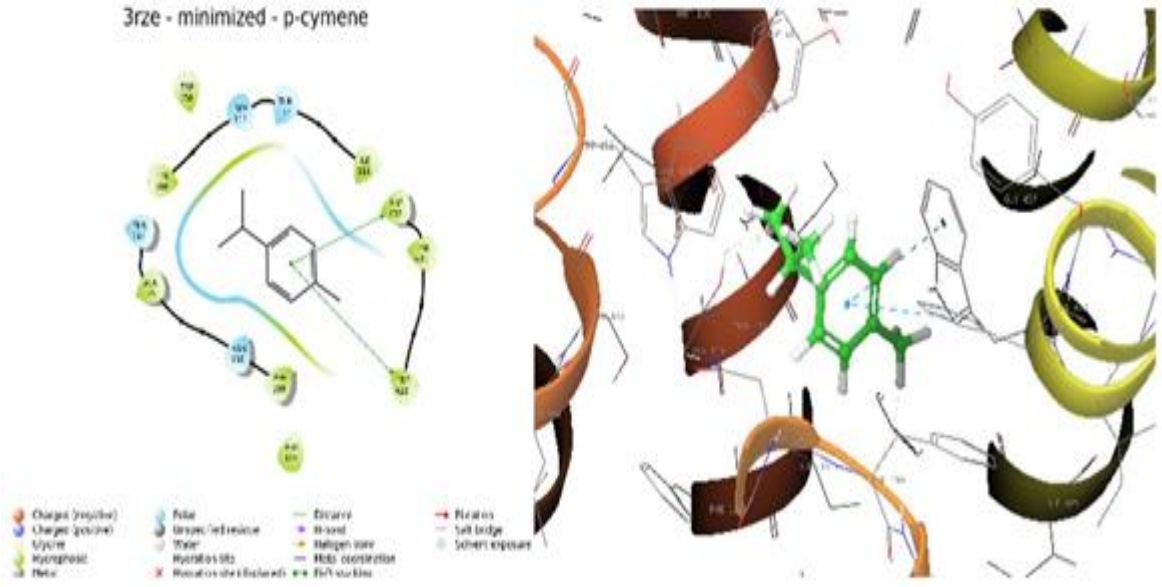

Fig 5 Interaction of P-Cymene 3RZE

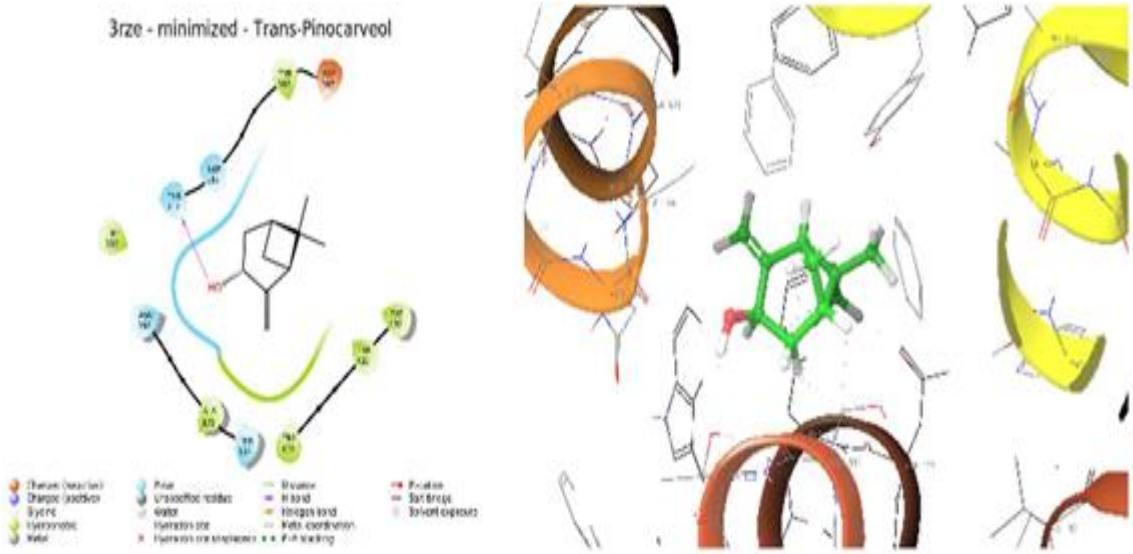

Fig 6 Interaction of Trans-Pinocarveol with 3RZE

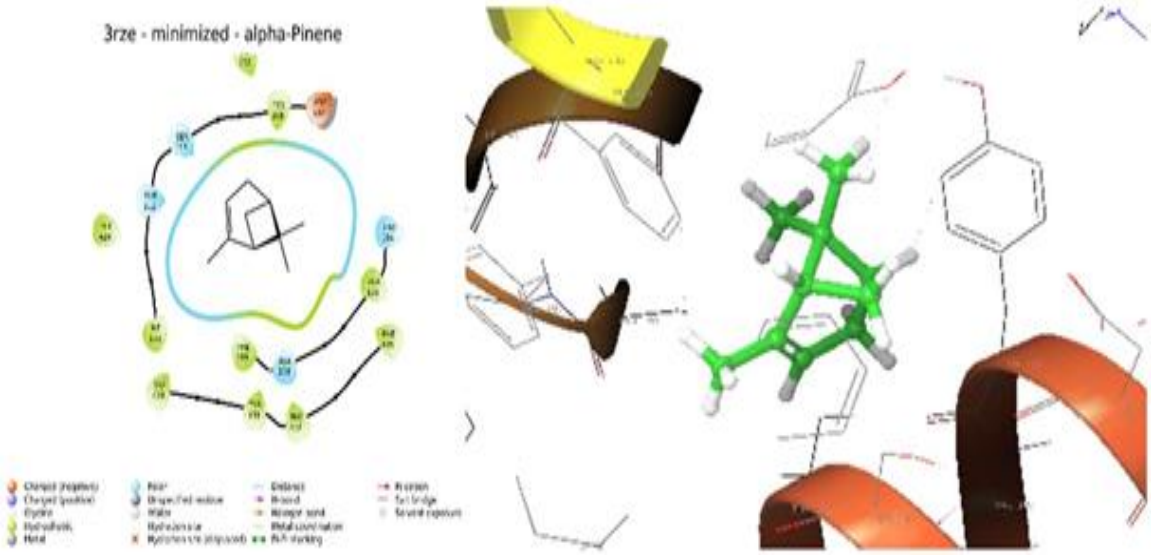

Fig 7 Interaction of Alpha Pinene with 3RZE

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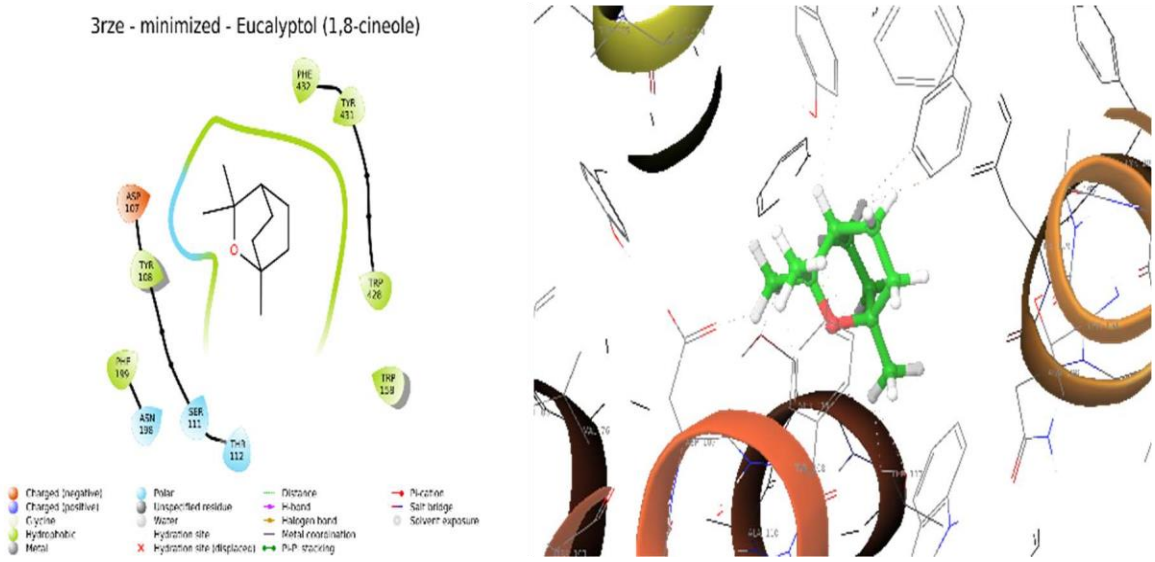

Fig 8 Interaction of Eucalyptol (1,8-Cineole) with 3RZE

IV. DISCUSSION

➢ *Generation of Smiles and Structures*

All the phytoconstituents structures of the Eucalyptus plant were generated by using Chemdraw and developed Smiles of these structures.

➢ *Validation of Lipinski Rule of Five and Physicochemical/Pharmacokinetic Properties*

Lipinski's rule of five (Pfizer's rule of five) is a rule to evaluate the drug-likeness and pharmacokinetics (ADME)/ pharmacological activity. The rule states that orally active drugs should not have any violation of five rules i.e., not more than 5 Hydrogen bond donors; not more than 10 Hydrogen bond acceptors; molecular weight less than 500 Daltons, and an Octanol-water partition coefficient (log *P*) value less than 5.

All the phytoconstituents have no violations and therefore they obey Lipinski's rule of five. Physicochemical properties of Eucalyptus have no violation except alpha-pinene, p-cymene and limonene for nHET, Log P.

Medicinal chemistry parameter interpretation; SA (synthetic accessibility) score have been reported as easy to synthesize the drug-like molecules i.e., SA score < 6, NP (natural product- likeness) score values are moderately good except p-cymene (-0.717), QED values of the phytoconstituents show unattractive to the desirability, and the values of Fsp^3 are greater than 0.45 except p-cymene which shows the least carbon saturation (which may also predict for solubility).

➢ *ADMET Analysis*

ADMET analysis is produced by ADMET Lab 2.0 by providing smiles. GI absorption ofall phytoconstituents are poor. All are prominently Pgp-inhibitors and Pgp-substrate. Human Intestinal Absorption (HIA) is an indicator of intestinal absorption where Eucalyptol (1,8-cineole) has a maximum of 0.002. Spathulenol has a maximum of 0.008 F20% value, indicating human oral bioavailability.

Trans-Pinocarveol has the best therapeutic index, where it has the least Plasma protein binding (PPB) affinity of 39.9% and p-cymene has maximum PPB. The volume distribution was found to be in the range of 0.04 - 20 L/Kg. Compounds did not show any Blood-Brain Barrier Penetration. Efficacy of drug is seen when the fraction of unbound in plasma is higher i.e., Fu >20%. Trans-Pinocarveol shows the highest of all (55.67%) with a least of 6.087% p-cymene.

The majority of the drug is metabolized by cytochrome P450 enzymes. p-cymene inhibits the enzyme CYP1A2 and CYP2C19; and CYP2C19, CYP2C9 were found to be active substrate for metabolism of all the compounds.

Excretion of the phytoconstituents indicates that alpha-pinene and spathulenol have high clearance and only α-terpineol having least half-life (t1/2) of a drug in the blood.

Human hepatotoxicity (H-HT) indicates probability of adverse hepatic effects. Eucalyptol, alpha-pinene & limonene were found to be less probability of H-HT. In DILI (Drug Induced Liver Injury) test, all the compounds were toxic. In Ames test for mutagenicity, all were close to being toxic. From the TD50 values, Eucalyptol and limonene were found to be non-carcinogens. Eucalyptol, p-cymene, limonene, α-terpineol possess a high risk of drug induced respiratory toxicity.

➢ *Pass Online Prediction and Docking Score*

Pass-Online provides the prediction of most probable activity of different pharmacological actions, interaction with metabolic enzymes, toxic effects, etc. with average accuracy based on the SAR studies. It was found that limonene, α-terpineol, Trans–pinocarveol and spathulenol had shown maximum anti-eczemic activity and some other phytoconstituents have shown carminative and antineoplastic activity.

Using computer software Maestro (Schrodinger), docking study of the phytoconstituents was compared with standard Flucloxacillin in Human intestine H1 receptor protein (PDB ID: 3RZE) was performed.

Spathulenol showed docking score -6.368 better than trans–pinocarveol -5.273 when compared with standard compound Flucloxacillin -7.708.

V. CONCLUSION

The selected phytoconstituents of *Eucalyptus globulus* were analyzed and screened using computational tools like ADMETLab 2.0 and PASS Online, to assess their physicochemical properties and other relevant pharmacokinetic parameters.

From the study, it was revealed that trans-pinocarveol has the best QED activity asobtained from ADMETLab 2.0 , and also good anti-eczemic activity from PASS Online.

The docking studies also indicated that spathulenol has the best anti-eczemic activity compared to Flucloxacillin with the Human Intestine H1 receptor protein.

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