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Research Article

MOLECULAR DOCKING STUDIES OF SIDDHA HERBAL FORMULATION *KUPPAIMENI CHOORNAM* ON HUMAN HISTAMINE RECEPTOR (3RZE)

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ABSTRACT

Background: Molecular docking has tremendous applications in the field of Siddha medicine especially herbal formulations were the interactions of the lead molecules of the formulation with that of receptors can be elucidated at the molecular level and furthermore to reach an assumption of its fundamental biochemical processes to which the formulation is targeting. *Kuppaimeni Choornam* (KC) is a simple herbal formulation used in Siddha medicine for urticaria and other skin allergies. As far as skin allergy is concerned Amino acids such as Asparagine (ASN), Tryptophan (Trp), Aspartate (Asp), Tyrosine (Tyr), Serine (Ser), Isoleucine (Ile), Lysine (Lys), Threonine (Thr), Phenylalanine (Phe) are the main core residues involved in mediating Human histamine receptor (3RZE). Binding of lead compounds with this core residue may inhibit the enzyme activity. **Aim & Objectives**: Molecular docking studies of Siddha herbal formulation KC and to screen the lead component interaction on the Human Histamine Receptor (3RZE). **Methodology**: Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. **Results and Conclusion**: The compounds present in KC like beta-sitosterol, apigenin, luteolin, cuminaldehyde, kaempferol, and triacetonamine showed maximum interactions with 3RZE when compared to that of the standard cetirizine. Hence, these compounds of test drug possess promising Human histamine 1 receptor (3RZE) inhibition activity. For prospective pharmacological validation of *Kuppaimeni Choornam*, the docking studies were an important step for its scientific justification.

KEYWORDS: Siddha medicine, *Kuppaimeni Choornam*, Human histamine receptor (3RZE), Molecular docking.

INTRODUCTION

Urticaria is a clinical condition characterized by wheal or angioedema or both anywhere in the body due to multiple factors.[1] The condition is broadly classified into two based on the duration of occurrence of the symptoms. Urticaria of sudden origin is most frequently a self-limited disorder caused by an allergic reaction to a food or drug and which have duration less than 6 weeks. If the symptoms persist continuously or show recurrences for more than 6 weeks, it is termed as chronic urticaria.[2] Chronic idiopathic urticaria (CIU) yet another common clinical presentation related to autoimmunity.[1] The main pathogenesis is better understood with the central role of histamine release and mast cell degranulation, which are the main triggering factors responsible for the sequence of clinical events occurring in urticaria.[1-3]

All the symptoms of urticaria are mostly mediated through H_1 receptors (Human histamine receptor- 3RZE) and therefore in a modern approach, drugs of non-sedating Anti-histamine category is effectively used for its management.[4] There are numerous amino acids responsible for mediating the 3RZE. The drugs or biomolecules that effectively targets the core residues block the receptor activity ultimately controls the symptoms of urticaria. [4, 5]

In traditional Siddha medicine, the clinical picture of urticaria is more or less correlated with the condition called '*Silvidam*'. Numerous herbal or herbomineral formulations are exclusively given for the condition. *Kuppaimeni Choornam* (KC) is one

among the herbal medicine used for classical urticarias and allergic conditions. The formulation is mentioned in the Tamil medical text *Agathiyar paripooranam* 400.[6] *Acalypha indica* and *Cuminum cyminum* are the main ingredients of this herbal combination. For a wider research perspective, the lead molecules of this formulation were interpreted for its Human histamine receptor - 3RZE inhibitor activity. This may help in wider acceptability of KC as an efficient Anti urticarial or anti- allergic herbal formulation.

Aim and Objectives

The main objective of the study is to carry out Molecular Docking (MD) studies of lead molecules from key ingredients of KC to find its interaction on Human Histamine Receptor (3RZE).

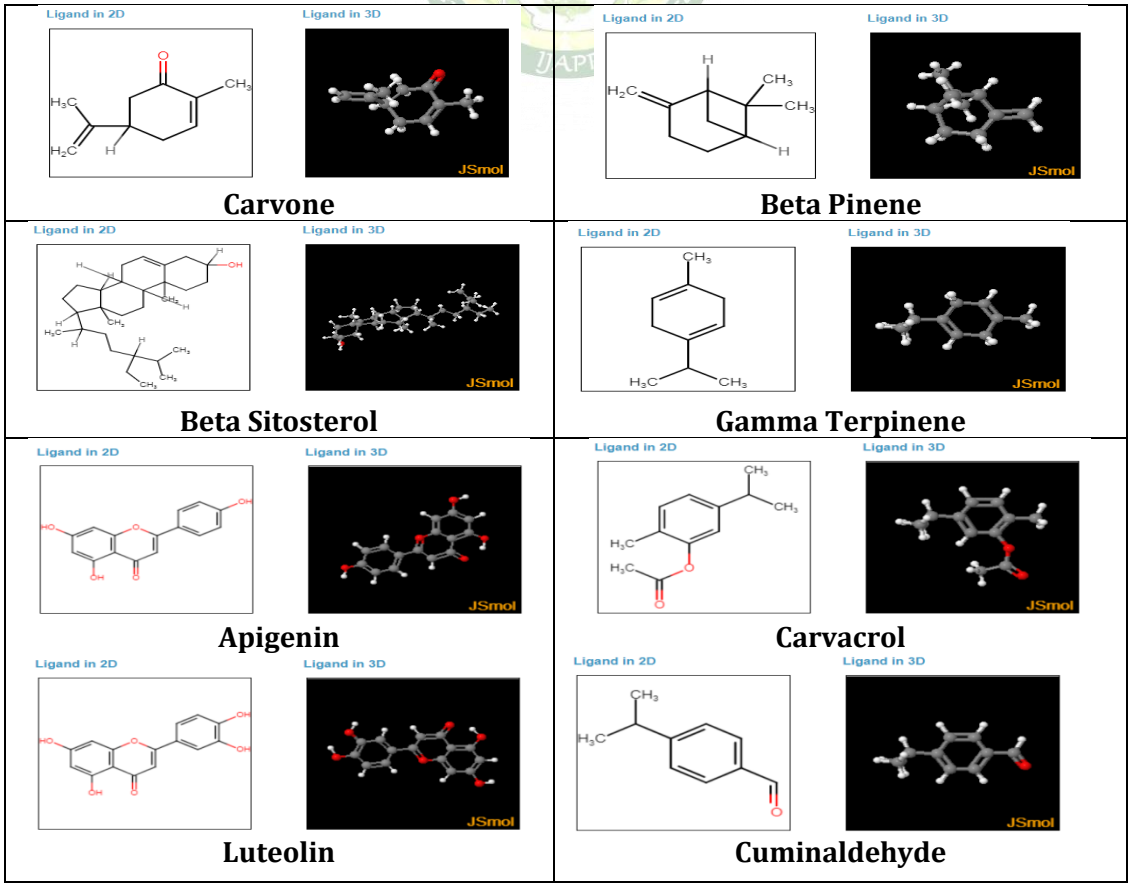
METHODOLOGY

Lead Molecules from KC

Docking calculations were carried out for the compounds retrieved from the herbal sources such as carvone, beta pinene, beta-sitosterol, gamma terpinene, apigenin, carvacrol, luteolin, cuminaldehyde, kaempferol, cymene, triacetonamine, and their respective standard cetirizine[7] against target protein model (Table. 1 & Fig A). The ligand molecular properties are illustrated in Table. 2.

Table: 1 List of Lead Molecules selected for docking studies

S.No	Name of the Herb	Phytocomponents
1.	*Acalypha indica* (Whole plant)	Beta Sitosterol
		Triacetonamine
2.	*Cuminum cyminum* (Seed)	Cuminaldehyde
		Beta Pinene
		Cymene
		Carvone
		Carvacrol
		Gamma-Terpinene
		Luteolin
		Kaempferol



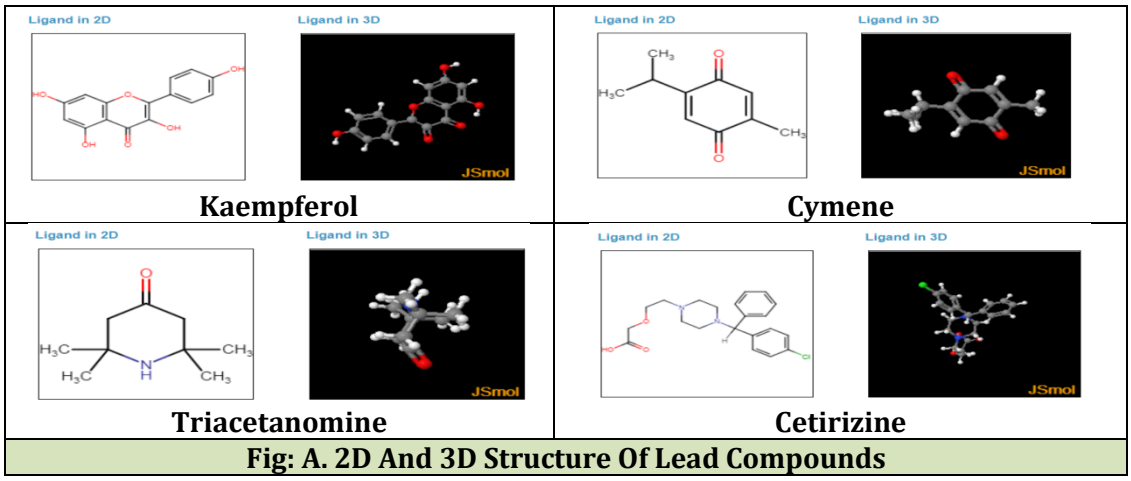


Fig: A. 2D And 3D Structure Of Lead Compounds

Table: 2 Ligand Properties of the compounds from KC selected for docking

Compound	Molecular Formula	Molar weight g/mol	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P
Carvone	$C_{10}H_{14}O$	150.221 g/mol	0	1	1	2.4
Beta Pinene	$C_{10}H_{16}$	136.238 g/mol	0	0	0	3.1
Beta Sitosterol	$C_{29}H_{50}O$	414.718 g/mol	1	1	6	9.3
Gamma Terpinene	$C_{10}H_{16}$	136.238 g/mol	0	0	1	2.8
Apigenin	$C_{15}H_{10}O_5$	270.24 g/mol	3	5	1	1.7
Carvacrol	$C_{10}H_{14}O$	150.221 g/mol	1	1	1	3.1
Luteolin	$C_{15}H_{10}O_6$	286.239 g/mol	4	6	1	1.4
Cuminaldehyde	$C_{10}H_{12}O$	148.205 g/mol	0	1	2	2.7
Kaempferol	$C_{15}H_{10}O_6$	286.239 g/mol	4	6	1	1.9
Cymene	$C_{10}H_{14}$	134.222 g/mol	0	0	1	4.1
Triacetanomine	$C_9H_{17}NO$	155.241 g/mol	1	2	0	0.5
Cetirizine	$C_{21}H_{27}C_{l3}N_2O_3$	461.808 g/mol	3	5	8	0

Target details and Receptor Structure

The crystalline structure of the target protein Human Histamine Receptor (3RZE)was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom was been added. (Fig. B) Different orientation of the lead molecules with respect to the target protein was evaluated by Auto-dock program and the best dock pose was selected based on the interaction study analysis.

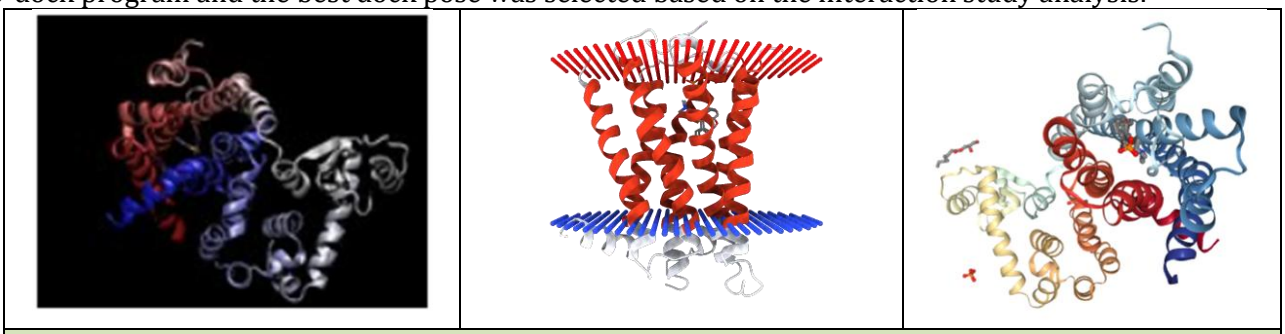


Fig. B.Target details and Receptor Structure of Human Histamine 1 Receptor – 3RZE

Tool for Study[8-9]

Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto-dock tools (*Morris, Goodsell, et al., 1998*). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (*Morris, Goodsell, et al., 1998*). Auto-dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively.

Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (*Solis and Wets, 1981*). Initial position, orientation, and torsions of the ligand

molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Results

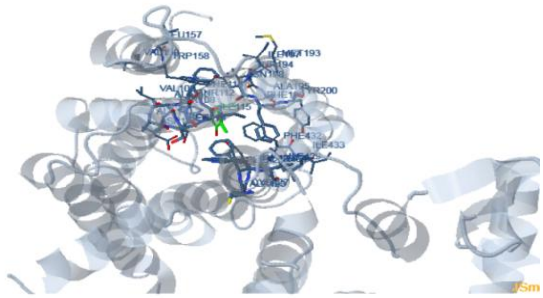
The compounds beta-sitosterol apigenin, luteolin, cuminaldehyde, kaempferol and triacetonamine have maximum interactions with when compared to that of the standard Cetirizine. Hence, these compounds of test drug possess promising Human histamine 1 receptor (3RZE) inhibition activity. The Summary of the molecular docking studies of the lead compounds and standard Cetirizine against 3RZEand its amino acid binding interactions have been tabulated in Table. 3 & 4, and Fig C.

Table: 3 Summary of the molecular docking studies of the DC lead compounds against Human histamine 1 receptor (3RZE)

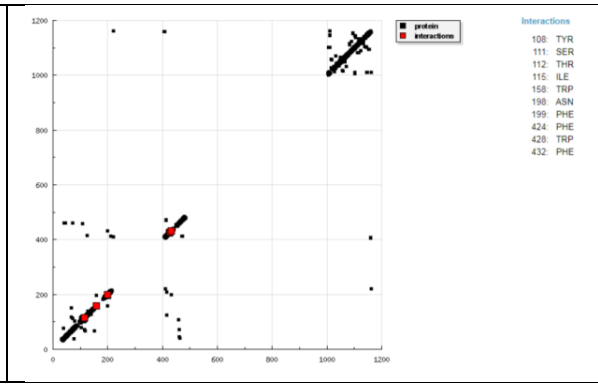
Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)(**nM)	Total Intermolecular energy (Kcal/mol)	Total Interaction Surface
Carvone	-6.54	16.12	-0.01	-6.84
Beta Pinene	-6.26	25.66	0.01	-6.26
Beta Sitosterol	-5.79	57.06	-0.09	-8.07
Gamma Terpinene	-5.91	46.68	0	-6.21
Apigenin	-8.28	853.19**	-0.28	-8.82
Carvacrol	-7	7.46	-0.13	-7.86
Luteolin	-9.01	248.17**	-0.45	-9.29
Cuminaldehyde	-5.91	46.63	-0.02	-6.51
Kaempferol	-7.25	4.83	-0.28	-7.81
Cymene	-6.47	18.09	0	-6.77
Triacetonamine	-5.93	44.91	1.4	-5.93
Cetirizine	-10.56	18.16**	-0.72	-12.71

No of Interactions	Lead / Standard	Amino Acid Residue- Binding													
6	CARVONE	108 TYR	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	432 PHE				
5	BETA PINENE	108 TYR	111 SER	112 THR	115 ILE	198 ASN	199 PHE	424 PHE	432 PHE						
10	BETA SITOSTEROL	103 TRP	107 ASP	108 TYR	111 SER	112 THR	158 TRP	179 LYS	194 THR	195 ALA	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR
5	GAMMA TERPINENE	108 TYR	111 SER	112 THR	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	432 PHE						
8	APIGENIN	107 ASP	108 TYR	111 SER	112 THR	198 ASN	199 PHE	424 PHE	428 TRP	431 TYR	432 PHE	454 ILE	458 TYR			
7	CARVACROL	107 ASP	108 TYR	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	431 TYR	432 PHE			
9	LUTEOLIN	107 ASP	108 TYR	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	431 TYR	432 PHE	454 ILE	458 TYR	
15	CETIRIZINE	84 ASN	103 TRP	107 ASP	108 TYR	111 SER	115 ILE	179 LYS	194 THR	424 PHE	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR
5	CUMINALDEHYDE	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	432 PHE						
9	KAEMFEROL	107 ASP	108 TYR	111 SER	112 THR	158 TRP	194 THR	195 ALA	198 ASN	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR	
6	CYMENE	107 ASP	108 TYR	111 SER	112 THR	115 ILE	199 PHE	428 TRP	431 TYR	432 PHE						
9	TRIACETONAMINE	84 ASN	103 TRP	107 ASP	108 TYR	179 LYS	428 TRP	431 TYR	454 ILE	458 TYR						

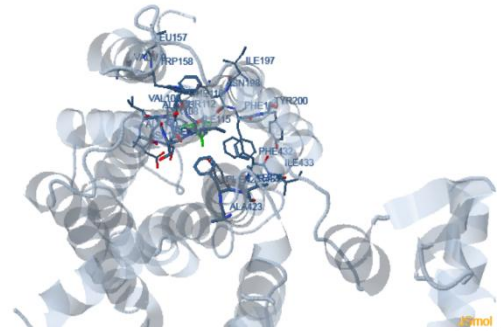
Fig: C. Amino acid Residue Interaction of Lead and Standard against Human histamine 1 receptor (3RZE)



Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)



Carvone with Human histamine 1 receptor (3RZE)



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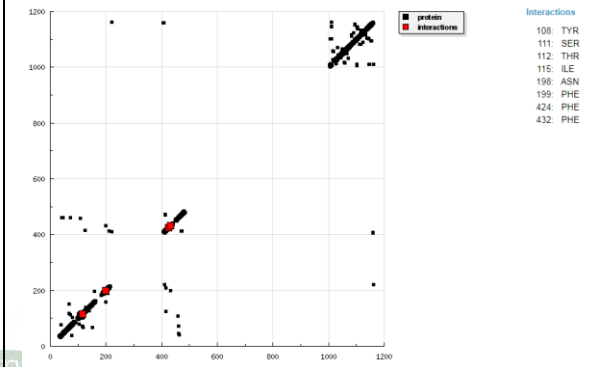
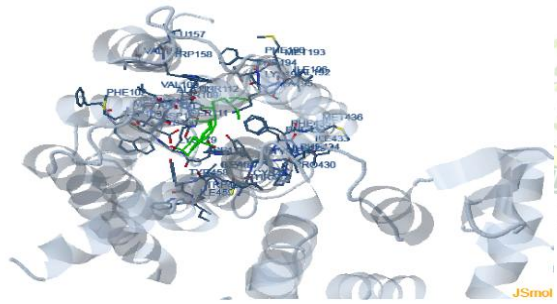


Pinenewith Human histamine 1 receptor (3RZE)



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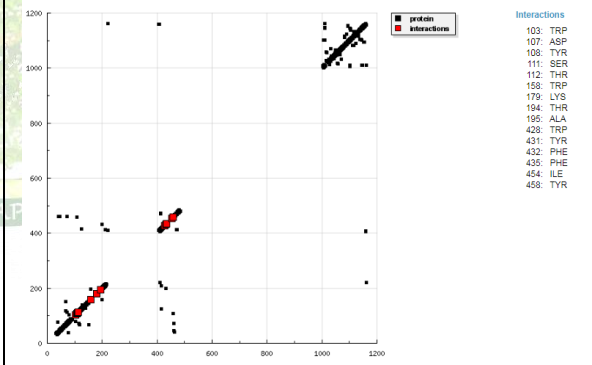
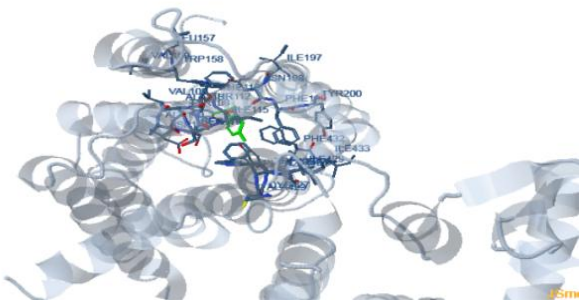


Betasitosterol with Human histamine 1 receptor (3RZE)



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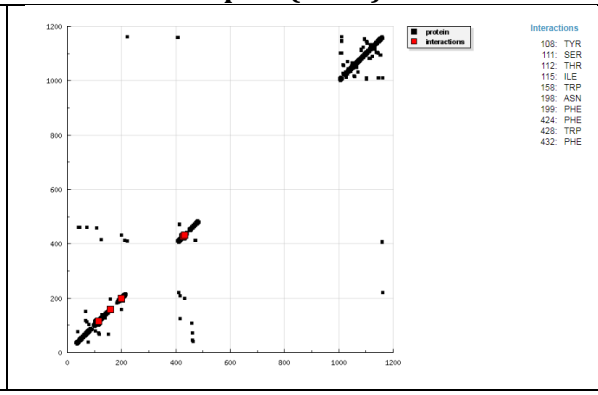
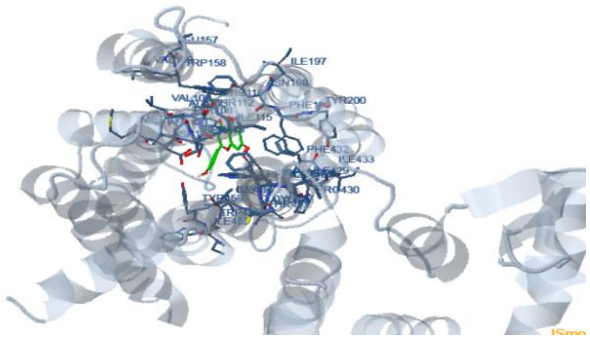
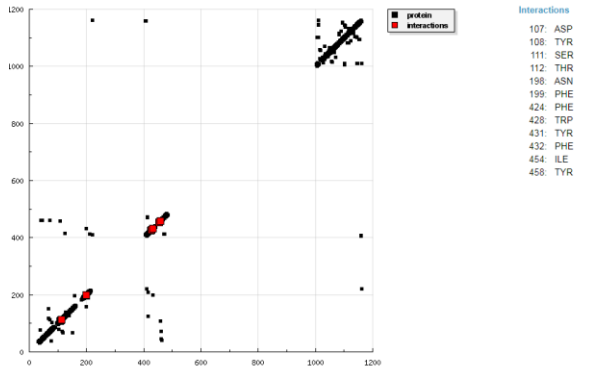


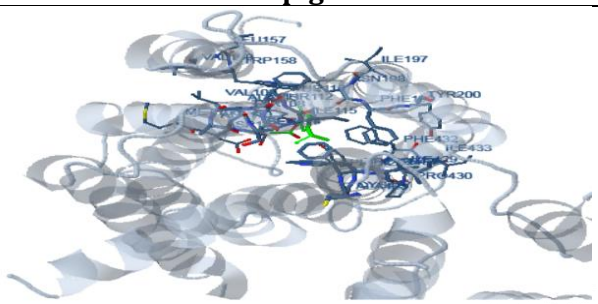
Gamma triterpene with Human histamine 1 receptor (3RZE)

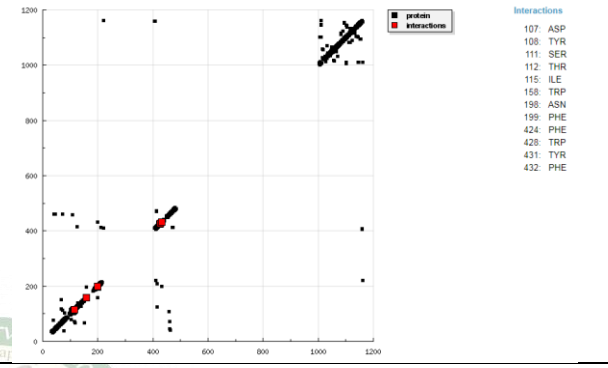




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

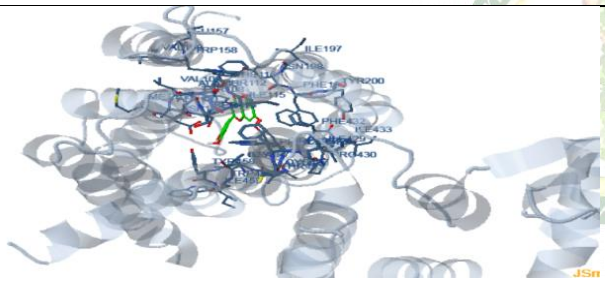
Apigenine with Human histamine 1 receptor (3RZE)

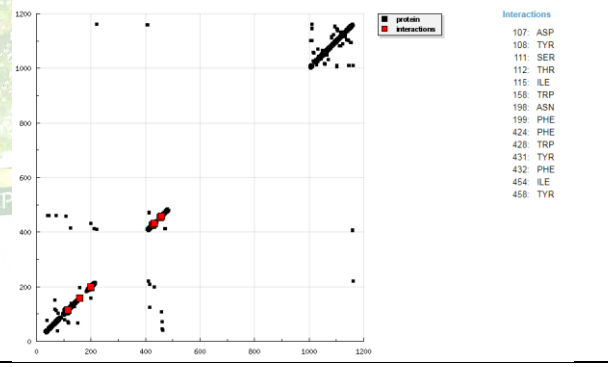




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

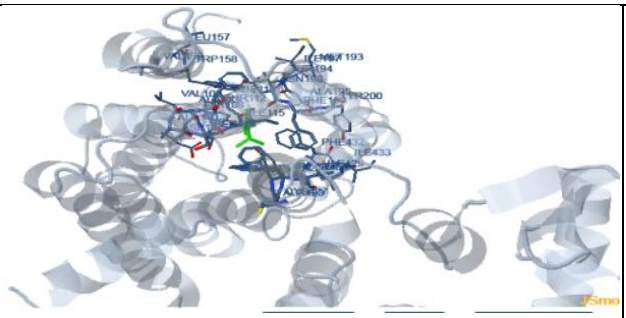
Carvacrol with Human histamine 1 receptor (3RZE)

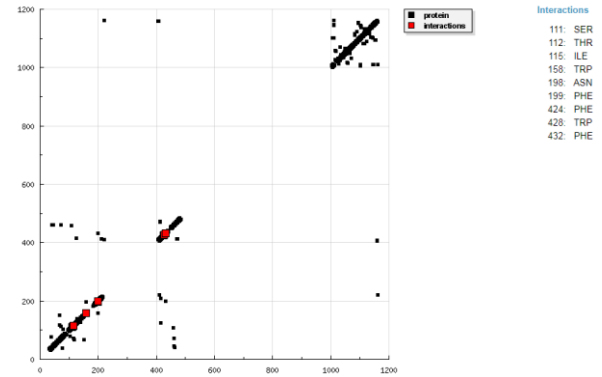




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

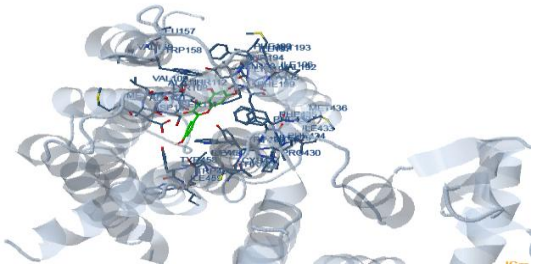
Luteolin with Human histamine 1 receptor (3RZE)

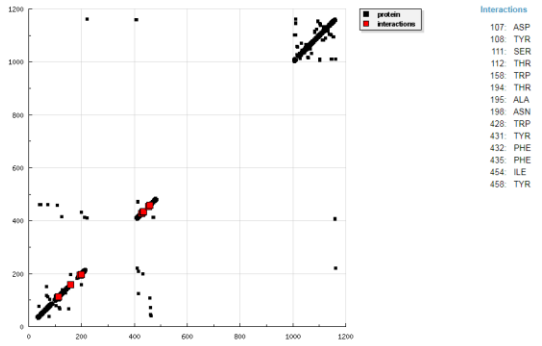




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

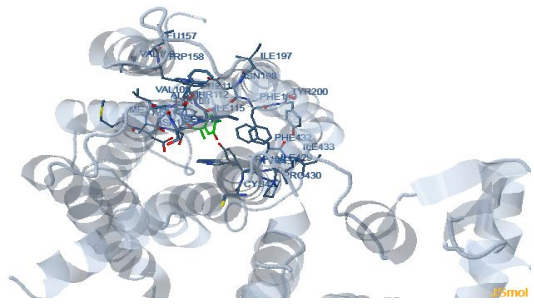
Cuminaldehyde with Human histamine 1 receptor (3RZE)

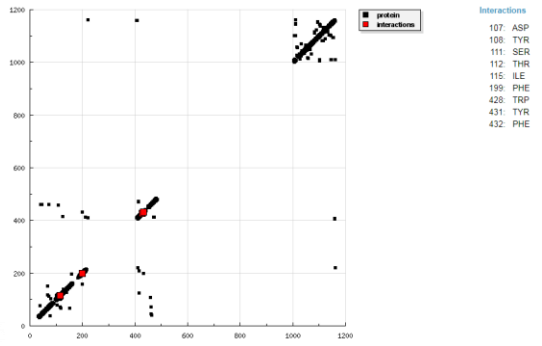




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

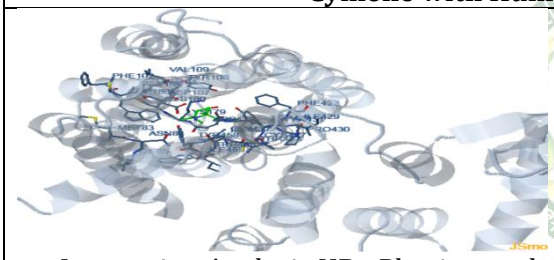
Kaempferol with Human histamine 1 receptor (3RZE)

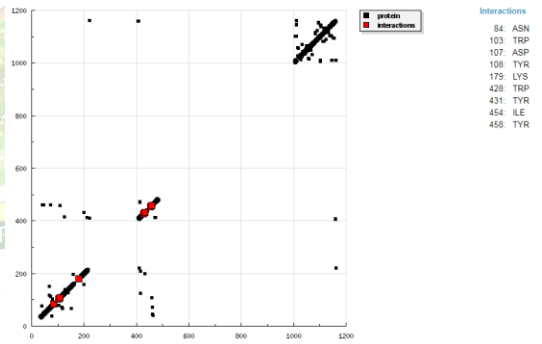




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

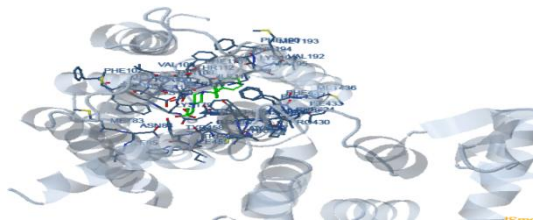
Cymene with Human histamine 1 receptor (3RZE)

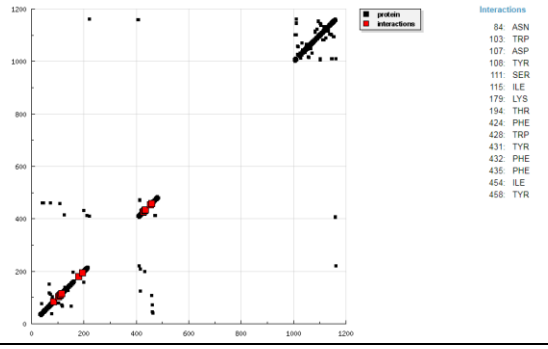




Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

Triacetonamine with Human histamine 1 receptor (3RZE)





Interaction Analysis HB - Plotting analysis (Hydrogen Bond formation)

Cetirizine with Human histamine 1 receptor (3RZE)

Fig. D. Amino acid interactions of Lead molecules of DC and standard

DISCUSSION

Urticaria is a common clinical manifestation occurring due to a multitude of factors and mostly termed as a mast cell-driven disease. As symptomatic relief is the primary aim of its management, the fundamental of the drug therapy in modern medicine is to reduce the effects of mediators of mast cells such as histamine, platelet aggravating factors, and others on the target organs. The biogenic amine called histamine exerts its effect by eventually acting on the four G- Protein-Coupled Receptors (GPCR). Histamine receptors (H_1R) are one among the important GPCR located in the endothelial cells and sensory nerves

that has a crucial role in regulating inflammatory and allergic responses occurring in the human system. Due to its prime importance, H_1R is the main target of drugs included in the Anti-histamine category. Many of the second-generation nonsedating H_1receptor antagonists like cetirizine and levocetirizine are very popular due to their safety profile and been used as the first line of management in most of the urticarial conditions. [10 - 11]

In traditional siddha medicine (TSM), there are numerous herbal formulations, which are used as first order preferences in most of the urticarial conditions. Urticarial symptoms are more or less correlated with the clinical picture of Siddha diagnostical term *Silvidam*, characterized by the sudden or progressive development of inflammatory rashes (wheals) throughout the body or in particular places with accompanying itching, burning or even pain. The etiology is multidimensional mostly occurring due to toxic bites, adverse drug reactions, food incompatibilities, and allergies. Single or compound formulations are prescribed accordingly with the severity. The ancient concepts of drug administration are truly based on body nature (*Deham*), nature of pulse (*Nadi*) and the clinical presentations of the particular condition. The type and form of medicines are selective, considering the above criteria. [12-14]

Kuppaimeni Choornam (KC) is a simple herbal combination indicated for classical urticarias. Dried *Kuppaimeni* (*Acalypha indica*- whole plant) and *Jeeragam* (*Cuminum cyminum*) are the key ingredients. In the previous clinical background, the drug in powder form was observed effective at doses 2-5 g thrice or four times a day for reducing the symptoms of most of the skin allergies, rashes due to insect bites or urticarial conditions due to unknown reasons.[6] Acalypha and cuminum has been proved for its Anti-inflammatory effects in many of the recent studies.[15-19]

Molecular docking is a widely accepted tool, which allows the researcher to predict the pharmacological efficiency of a drug or formulations. This approach has tremendous applications in the field of Siddha medicine especially herbal formulations were the interactions of the lead molecules of the formulation with that of receptors can be elucidated at the atomic level and furthermore to reach an assumption of its fundamental biochemical processes to which the formulation is targeting. [20-21] As far as skin allergy is concerned amino acids such as asparagine (Asn), tryptophan (Trp), aspartate (Asp), tyrosine (Tyr), serine (Ser), isoleucine (Ile), lysine (Lys), threonine (Thr), phenylalanine (Phe) are the main core residues involved in mediating Human histamine receptor (3RZE). Binding of lead compounds with this core residue may inhibit the enzyme activity. The lead molecule from both the ingredients was selected for the docking studies with comparative standard cetirizine. Most of the molecules have proven records as an anti-allergic, antihistamine, anti-prostaglandin or anti-inflammatory [22-24] (Table 4). Almost all the lead compounds had shown maximum interactions with H_1R.

Table: 4. Pharmacological significance of lead molecules from *Kuppaimeni Choornam*

S.No	Name of the Compound	Pharmacological Activity
1.	Carvone	Anti-inflammatory
2.	Beta Pinene	Anti-inflammatory
3.	Beta Sitosterol	Anti- Prostaglandin
4.	Gamma Terpinene	Anti-inflammatory
5.	Apigenine	Anti-histaminic, Anti-inflammatory
6.	Carvacrol	Prostaglandin inhibitor, Anti-inflammatory
7.	Luteolin	Anti-allergic, Anti-histaminic
8.	Cuminaldehyde	Anti-inflammatory
9.	Kaempferol	Anti-allergic, Anti histaminic

CONCLUSION

For prospective pharmacological validation of *Kuppaimeni Choornam*, the docking study was an important step for its scientific justification. The H_1R antagonist effect of *Kuppaimeni choornam* further opens its wide clinical possibilities further supporting its traditional claim as a simple herbal remedy for classical urticarias.

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