EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 10
423-432
Year: 2017

SCREENING OF IMMUNOMODULATORY POTENTIAL OF HERBAL LEADS FROM SIDDHA FORMULATION POOVARASU NEI BY IN-SILICO DOCKING TECHNIQUE

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Article Received on 27/07/2017 **Article Revised on 16/08/2017** **Article Accepted on 05/09/2017**

ABSTRACT
Atopic dermatitis is a commonly occurring inflammatory skin disease in children's which is characterized by itching, redness, swelling,crusting and scaling. Siddha system of traditional medicine has several formulations made of herbs with bioactive phytocomponents which is effective against atopic dermatitis. The key mediators actively involved in inflammatory condition like dermatitis are histamine, IL6, TNF-alpha, Cyclooxygenase I and II. Hence the main aim of the present investigation is to screen the anti-allergic and anti-inflammatory potential of phytocomponents such as Kaempferol, Mansonone, Thespone present in the formulation *Poovarasu Nei* (PN) against target protein Histamine 1 receptor with PDB code 3RZE, IL6 Interleukin with PDB code 1P9M, TNF alpha with PDB 2AZ5, Cyclooxygenase II with PDB 6COX and Cyclooxygenase I with PDB 3KK6 along with their respective standards using computational docking analysis. The results of the study indicates that the lead kaempferol, thespone and mansonone possess significant COX I, TNF alpha and IL 6 inhibition activity. Further the lead kaempferol and thespone also possess significant inhibition of COX II and H1 receptors, whereas the compounds mansonone has no activity against these proteins. Based on the results of the In-silico screening analysis it was concluded that the compound's such as kaempferol, thespone and mansonone present in the siddha formulation PN possess significant inhibition of inflammatory mediators therefore this formulation may have promising immuno modulatory activity and may effective against atopic dermatitis.

KEYWORDS: Atopic dermatitis, Siddha system, PoovarasuNei, Histamine, IL6, TNF-alpha, Cyclooxygenase, Immuno modulatory activity.

1. INTRODUCTION
Atopic dermatitis (AD) is a common eczematous skin disorder affecting wide range of the general population of about 20% with age and ethnic differences.[1,2] AD is characterized by chronic cutaneous inflammation and dry skin with epidermal barrier dysfunction.[3-5] Intense pruritus is the major and burdensome symptom of AD.[6,7] Itch-induced scratching appears to exacerbate skin inflammation by accelerating cellular damage in the lesional skin.[8]

Approximately 80% of AD patients exhibit elevated levels of serum IgE.[9, 10] In contrast to normo-IgE and non-allergic intrinsic AD patients, extrinsic AD patients with hyper IgE levels are associated with increased disease severity,[11, 12] mutations in the FLG gene and impaired skin barrier function.[12-15]

The primary healthcare benefits of using such plant-derived siddha formulations are relatively safer when compared to allopathic drugs and offer profound therapeutic benefits.[16] Single and polyherbal preparations have diverse range of bioactive molecules and play a dominant role in the maintenance of human health since ancient times.[17] More than 1500 herbal preparations are sold as dietary supplements or ethnic traditional medicines.[18] The most frequently used type of herbal preparations is Nei. *PoovarasuNei* is one such preparations comprising of herbs with castor oil.

Computer aided drug discovery attains greater importance mainly because of the reliability in the results and also paves a new way for the research focus towards the alternative animal models. Molecular Docking continues to hold great promise in the field of Computer based drug design that screens small molecules by orienting and scoring them in the binding site of a protein. As a result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently

dock 70 – 80% of ligands correctly.[19] The main aim of the present investigation is to screen the anti-allergic and anti-inflammatory potential of phytocomponents such as Kaempferol, Mansonone, Thespone present in the formulation *PoovarasuNei* (PN) against target protein Histamine 1 receptor with PDB code 3RZE, IL6 Interleukin with PDB code 1P9M, TNF alpha with PDB 2AZ5, Cyclooxygenase II with PDB 6COX and Cyclooxygenase I with PDB 3KK6 along with their respective standards using computational docking analysis.

2. MATERIALS AND METHODS

2.1. Source of raw drugs

The *Poovarasam pattai* and *Nuna ver* is collected from southern zone of tamil nadu, Tiruvarur and other required raw drug is procured from a well reputed indigenous drug shop from Parrys corner, Chennai, Tamil Nadu, India. All raw drugs were authenticated by the Pharmacognosist, SCRI Chennai., Tamil Nadu, India.

2.1. Purification of raw drugs

Raw drugs are purified as mentioned in *Sikicharathna deepam Sarakku Suthi Muraigal*.[20]

2.2. Ingredients

The siddha formulation *PoovarasuNei* comprises of the following herbs as phyto ingredients

1.PoovarasampattaiSaaru (*Thespesiapopulnea*) -1.3 liters.
2.Sittramanakuennai (*Ricinuscommunis*) -1.3 liters
*3.*Kasakasapaal *(Papaver Somniferum)* -1.3 liters
*4.*Seeragam *(cuminumcyminum)*
*5.*Karunjeeragam *(Nigella sativa)* -10gms
*6.*Karpogarisi *(psoraleacorylifolia)*
*7.*Nunaver *(Morindatinctoria)*
*8.*Elarici *(Elettariacardamomum)*
*9.*Sadamanjil *(Nardostachysgrandiflora)* - 35gms
*10.*Manjal Karisalai Samulam *(wideliacalendulaceae)*
*11.*Kirambu *(syzygiumaromaticum)*
*12.*Sarkarai *(Saccharum officinarum)* - 200gms

2.3. Software's required

Several docking tools were been used in recent times which works behind structure-based drug design strategies one among which is auto dock 4 a componential software tools used to analyze the protein 3RZE, 1P9M, 2AZ5, 6COX, 3KK6 and to study the binding energy properties with the following lead component such as Kaempferol, Mansonone, Thespone along with standard Ibuprofen, Celecoxib,Diclofenac and Citrazine. Histamine 1 receptor with PDB code 3RZE, IL6 Interleukin with PDB code 1P9M, TNF alpha with PDB 2AZ5, Cyclooxygenase II with PDB 6COX and Cyclooxygenase I with PDB 3KK6 was obtained from protein data bank (www.pdb.org/pdb/). To get insight the intermolecular interactions, the molecular docking studies were done for the above mentioned phytoconstituents along with standard at the active site 3D space of enzyme of interest DPP-4 using online DOCKING SERVER web tool module.

2.4. Ligand preparation

The ligands such as Kaempferol, Mansonone, Thespone along with standard Ibuprofen, Celecoxib,Diclofenac and Citrazinewere built using Chemsketch and optimized using Docking server online web tool as shown in Figure 1 and 2 for docking studies by using Geometry optimization method MMFF94 and charge calculation was carried out based on Gasteiger method at PH 7 as shown in Table 1.

Table 1: Ligand Properties of the selected Lead.

Compounds	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds	Log P
Thespone	240.25	C15H12O3	0	3	0	3
Mansonone	228.291	C15H16O2	0	2	1	3.4
Kaempferol	286.23	C15H10O6	4	6	1	1.9
Cetirizine	388.89	C21H25ClN2O3	1	5	8	1.7
Diclofenac	296.14	C14H11Cl2NO2	2	3	4	4.4
Celecoxib	381.37	C17H14F3N3O2S	1	7	3	3.4

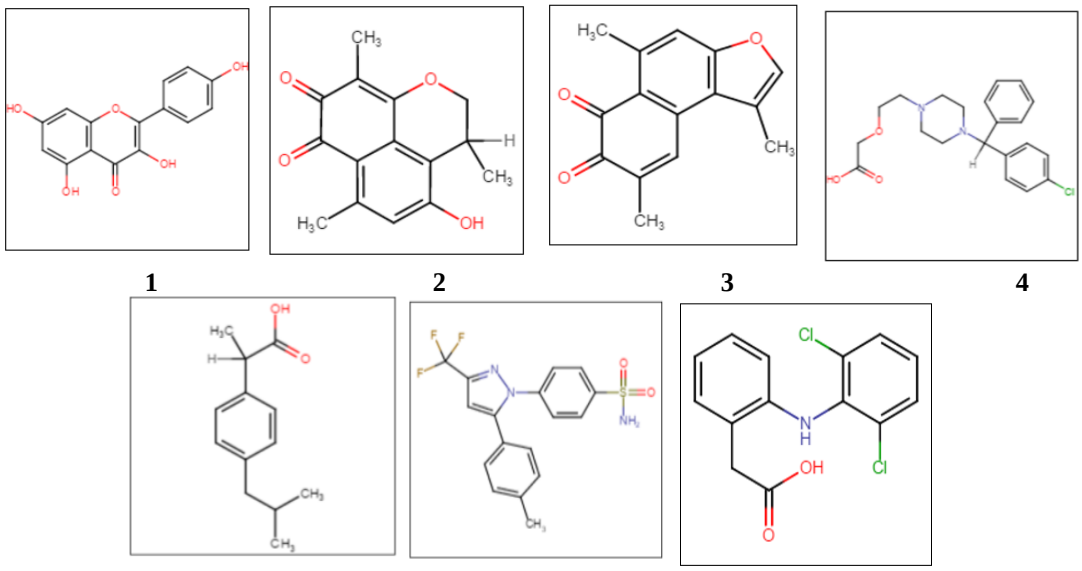


Fig 1: 2D Structure of lead 1.Kaempferol 2. Mansonone 3.Thespone 4.Citrazine 5.Ibuprofen 6.Celecoxib and 7.Diclofenac.

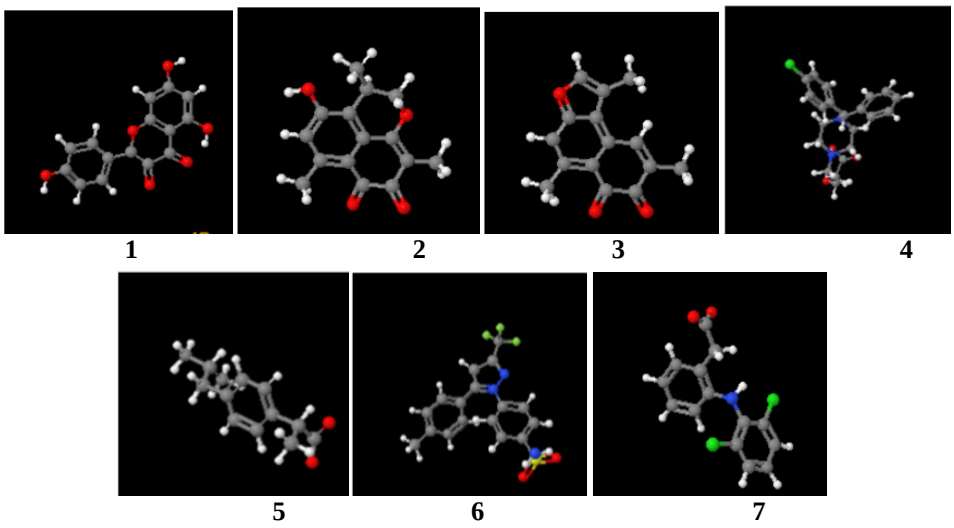


Fig 2: 3D Structure of lead 1.Kaempferol 2. Mansonone 3.Thespone 4.Citrazine 5.Ibuprofen 6.Celecoxib and 7.Diclofenac.

2.5. Active Site Prediction

Active site of enzyme was obtained by LIGSITE web server by using the automatic identification of pockets on protein surface given 3D coordinates of protein.The potential ligand binding sites in 3RZE, 1P9M, 2AZ5, 6COX, 3KK6 target protein is identified using grid space of 1 and probe of radius 5.0 angstrom.[21] Ligand site prediction was performed by using online tool GHECOM and the respective pockets calculations.[22,23]

2.6. Docking Methodology

Docking calculations were carried out using Docking Server.[24,25] Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out based on the binding free energy on the following compounds like Kaempferol, Mansonone, Thespone along with standard Ibuprofen, Celecoxib, Diclofenac, Citrazine and their binding affinity towards the target protein with PDB 3RZE, 1P9M, 2AZ5, 6COX, 3KK6, as shown in figure 3 to 7.

Fig 3:Target protein Histamine 1 receptor with PDB code 3RZE.

Fig 4:Target protein IL6 Interleukin with PDB code 1P9M.

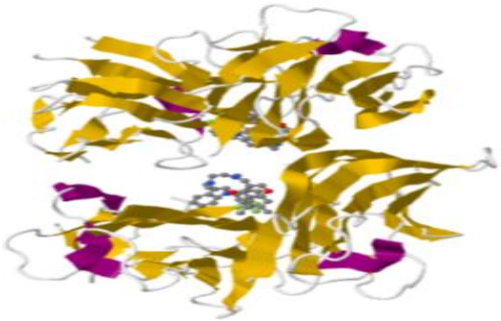


Fig 5:Target protein TNF alpha with PDB 2AZ5.

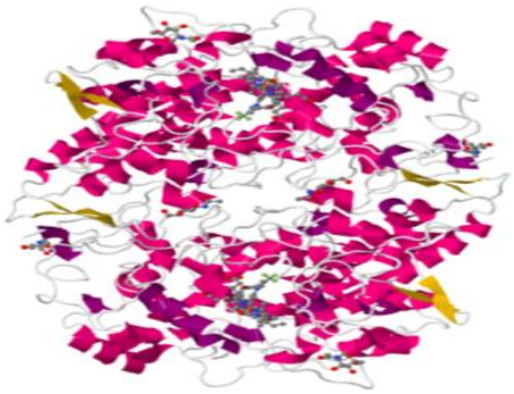


Fig 6:Target protein Cyclooxygenase II with PDB 6COX.

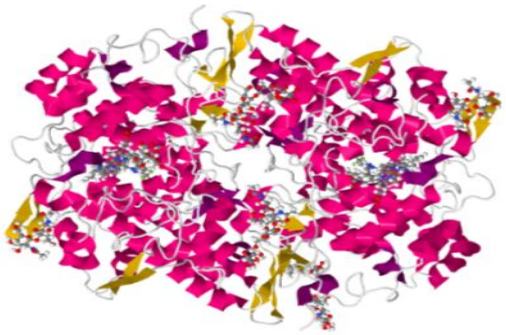


Fig 7:Target protein Cyclooxygenase I with PDB 3KK6

Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of Å grid points and 0.375 Å spacing were generated using the Autogrid program. 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 and Wets local search method.[26] 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.[27]

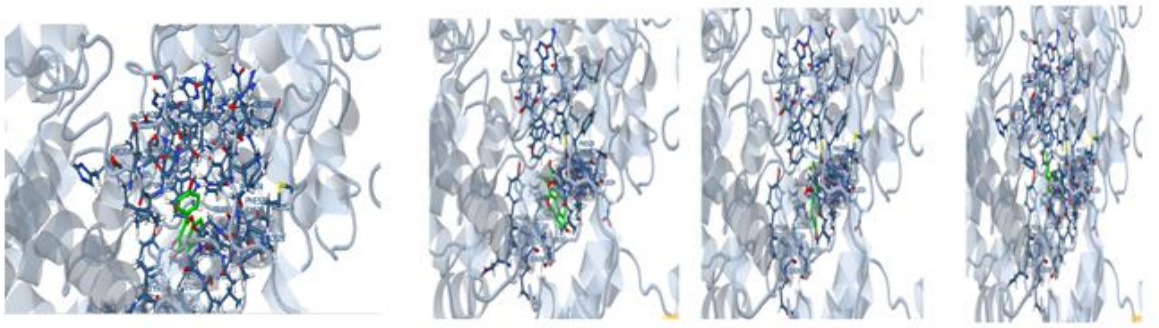
3. RESULTS

3.1. Dock scores

The result of binding interactions of the ligand with Cyclooxygenase 1 has revealed that out of three compound's kaempferol has 9 interactions (90%) similar to that of the standard ibuprofen hence it has promising COX I inhibition activity similarly other two compounds thespone and mansonone has 50% percentage similar interaction to that of the standard ibuprofen hence all three compounds has COX I inhibition activity as shown in table 2 and fig 8.

Table 2: Summary of the molecular docking studies of compounds against Cyclooxygenase I Receptor

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thespone	-8.00	1.37	-0.02	-8.00	602.22
Mansonone	-4.71	351.0	-0.30	-5.01	602.20
Kaempferol	-2.33	19.58*	-0.12	-2.92	531.95
Ibuprofen	-6.33	23.05	-0.02	-7.55	524.81



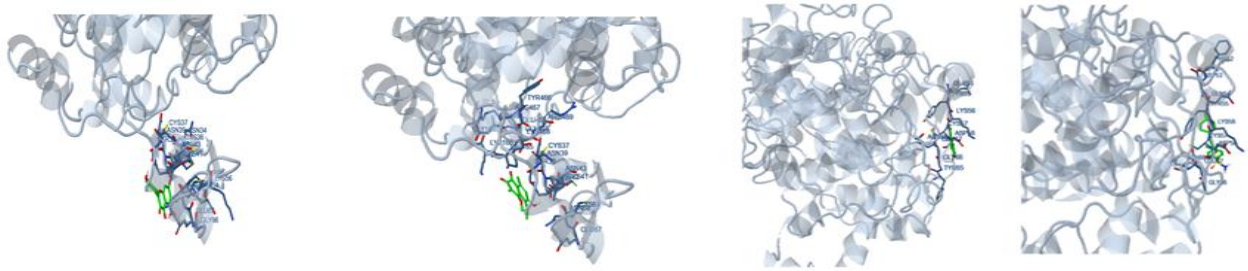
Kaempferol **Mansonone** **Thespone** **Ibuprofen**

Fig 8: Possible ligand binding pockets on the surface of target Cyclooxygenase I with PDB 3KK6. Pockets calculated by GHECOM.

The result of binding interactions of the ligand with Cyclooxygenase 2 has revealed that out of three compound's kaempferol has 2 interaction (40%) similar to that of the standard Celecoxib hence it has promising COX 2 inhibition activity similarly other compound thespone has 20% percentage similar interaction to that of the standard hence both compounds has COX 2 inhibition activity. Compound mansonone has no COX2 inhibition activity as shown in table 3 and fig 9.

Table 3: Summary of the molecular docking studies of compounds against Cyclooxygenase II Receptor

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thespone	-4.64	399.11	-0.05	-4.64	413.71
Mansonone	-4.23	790.94	-0.15	-4.53	467.00
Kaempferol	-3.53	2.57*	-0.05	-4.08	449.57
Cetirizine	-3.70	1.95*	-0.09	-5.13	427.00



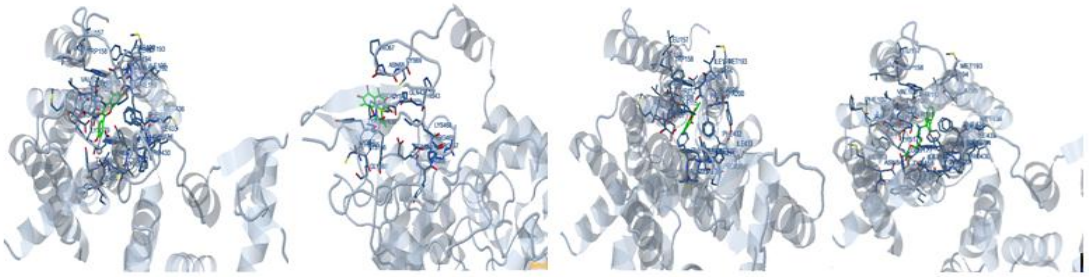
Kaempferol **Mansonone** **Thespone** **Celecoxib**

Fig 9 : Possible ligand binding pockets on the surface of target Cyclooxygenase II with PDB 6COX . Pockets calculated by GHECOM.

The result of binding interactions of the ligand with Histamine 1 Receptor has revealed that out of three compound's kaempferol has 9 interactions (60%) similar to that of the standard Cetirizinehence it has promising H1 receptor blocking activity similarly other compound thespone has 46% percentage similar interaction to that of the standard hence both compounds has H1 receptor blocking activity. Compound mansonone has no H1 receptor blocking activity as shown in table 4 and fig 10.

Table 4: Summary of the molecular docking studies of compounds against Histamine 1 receptor.

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thespone	-7.33	4.2	-0.06	-7.33	605.56
Mansonone	-4.49	512.09*	-0.30	-4.79	518.36
Kaempferol	-6.78	10.66	-7.09	-7.31	699.24
Cetirizine	-0.77	272.11*	0.01	-5.23	931.84



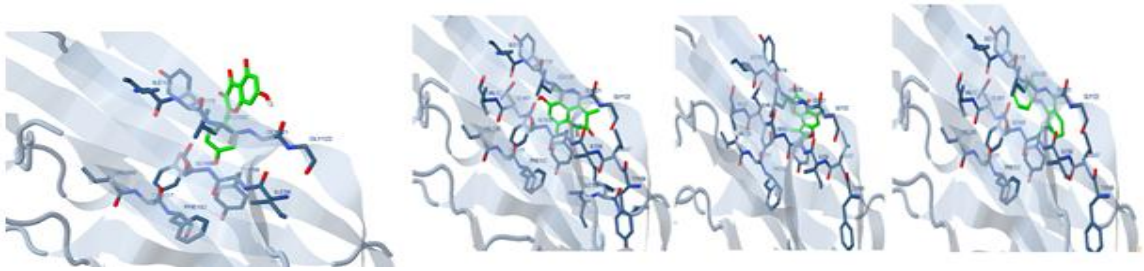
Kaempferol Mansonone Thespone Cetirizine

Fig 10: Possible ligand binding pockets on the surface of target enzyme Histamine 1 receptor with PDB code 3RZE. Pockets calculated by GHECOM.

The result of binding interactions of the ligand with TNF alpha has revealed that out of three compound's mansonone has all 5 interactions (100%) similar to that of the standard diclofenachence it has Excellent TNF alpha inhibition activity similarly other two compounds thespone has 90% percentage and kaemferol has 75 % similar interaction to that of the standard hence all three compounds has promising TNF alpha inhibition activity as shown in table 5 and fig 11.

Table 5: Summary of the molecular docking studies of compounds against TNF Alpha Receptor

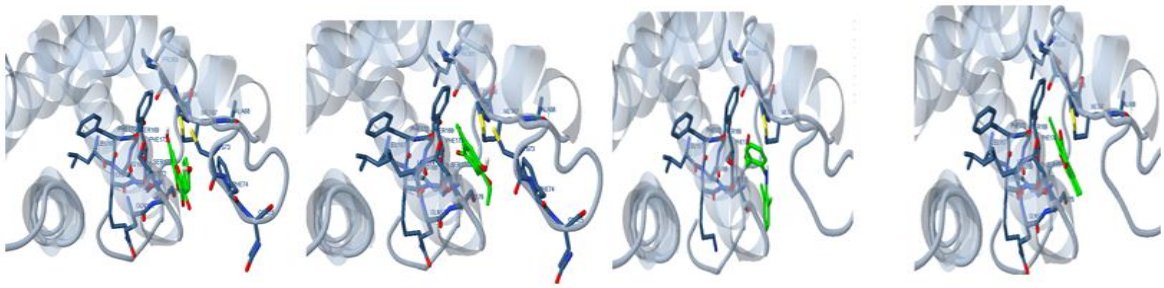
Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thespone	-5.57	82.10	-0.03	-5.57	450.77
Mansonone	-5.23	147.57	-0.08	-5.52	456.28
Kaempferol	-4.07	1.05*	-0.13	-4.61	414.87
Cetirizine	-0.77	272.11*	0.01	-5.23	931.84



Kaempferol Mansonone Thespone Diclofenac

Fig 11 : Possible ligand binding pockets on the surface of target TNF alpha with PDB 2AZ5. Pockets calculated by GHECOM.

The result of binding interactions of the ligand with IL 6 has revealed that out of three compound's mansonone and kaempferol has all 4 interactions (100%) similar to that of the standard diclofenac hence it has Excellent IL6 inhibition activity similarly other compound thespone has 75% percentage similar interaction to that of the standard hence all three compounds has promising IL 6 inhibition activity as shown in table 6 and fig 12.



Kaempferol Mansonone Thespone Diclofenac

Fig 12 : Possible ligand binding pockets on the surface of target IL6 Interleukin with PDB code 1P9M. Pockets calculated by GHECOM.

Table 6: Summary of the molecular docking studies of compounds against IL 6 Receptor.

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki µM (*mM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Thespone	-4.58	437.64	-0.24	-4.58	485.15
Mansonone	-3.03	6.01*	-0.13	-3.33	496.46
Kaempferol	1.33	0	-0.25	0.67	548.13
Diclofenac	-0.24	0	-0.16	-2.11	504.15

Table 7: Interaction of lead compounds with active site amino acid residue of Cyclooxygenase I Receptor

Compound	Target binding Amino acid residue						
Ibuprofen	205 PHE	209 PHE	348 TYR	352 LEU	381 PHE	385 TYR	387 TRP
Kaempferol	205 PHE	209 PHE	344 VAL	348 TYR	349 VAL	352 LEU	375 ASN
Mansonone	349 VAL	352 LEU	385 TYR	387 TRP	518 PHE	523 ILE	527 ALA
Thespone	349 VAL	381 PHE	384 LEU	385 TYR	387 TRP	518 PHE	523 ILE

Table 8: Interaction of lead compounds with active site amino acid residue of Cyclooxygenase II Receptor.

Compound	Target binding Amino acid residue						
Celecoxib	54 GLN	55 TYR	56 LYS	57 CYS	67 GLU		
Kaempferol	35 PRO	38 SER	40 PRO	42 GLN	55TYR	67GLU	68 ASN
Mansonone	38 SER	40 PRO	42 GLN	68 ASN	165 VAL	166 LYS	465 GLU
Thespone	67 GLU						

Table 9: Interaction of lead compounds with active site amino acid residue of Histamine 1 receptor.

Compound	Target binding Amino acid residue														
Citrazine	84 ASN	103 TRP	107 ASP	108 TYR	111 SER	158 TRP	179 LYS	194 THR	424 PHE	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR
Kaempferol	107 ASP	108 TYR	111 SER	112 THR	158 TRP	179 LYS	195 ALA	198 ASN	431 TYR	432 PHE	454 ILE	458 TYR			
Mansonone	No interaction														
Thespone	107 ASP	108 TYR	111 SER	115 ILE	198 ASN	199 PHE	424 PHE	428 TRP	431 TYR	432 PHE					

Table 10: Interaction of lead compounds with active site amino acid residue of TNF Alpha Receptor.

Compound	Target binding Amino acid residue					
Diclofenac	57 LEU	59 TYR	61 GLN	119 TYR	151 TYR	
Kaempferol	59 TYR	119 TYR	151 TYR			
Mansonone	57 LEU	59 TYR	61 GLN	119 TYR	151 TYR	155 ILE
Thespone	59 TYR	61 GLN	119 TYR	151 TYR		

Table 11: Interaction of lead compounds with active site amino acid residue of IL 6 Receptor.

Compound	Target binding Amino acid residue						
Diclofenac	66 LYS	168 ARG	169 SER	172 GLU			
Kaempferol	66 LYS	67 MET	74 PHE	168 ARG	169 SER	172 GLU	176 SER
Mansonone	66 LYS	67 MET	168 ARG	169 SER	172 GLU	173 PHE	176 SER
Thespone	66 LYS	67 MET	169 SER	172 GLU	173 PHE	176 SER	

4. DISCUSSION

Due to the rising ethical issues on the usage of laboratory animals against screening of drugs made researcher to acquire alternate high precision techniques like virtual screening. Molecular Docking continues to hold great promise in the field of Computer based drug design, which screens small molecules by orienting and scoring them in the binding site of a protein. So result novel ligands for receptors of known structure were designed and their interaction energies were calculated using the scoring functions. Dock score was used to estimate the ligand-binding energies. Apart from these, other input parameters for docking are also considered for evaluating the compounds inhibition efficacy. It is estimated that docking programs currently dock 70 - 80% of ligands correctly.[28]

The molecular docking approach can be used to model the interaction between a small molecule and a protein at the atomic level, which allow us to characterize the behavior of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical

processes.[29,30] The docking process involves two basic steps: prediction of the ligand conformation as well as its position and orientation within these sites (usually referred to as *pose*) and assessment of the binding affinity. These two steps are related to sampling methods and scoring schemes, respectively, which will be discussed in the theory section.

Siddha system of medicine is practiced in south Indian region especially in the state of Tamil Nadu. It has close affinity to Ayurveda yet it maintains a distinctive identity of its own. This system has come to be closely identified with Tamil civilization. The term '*Siddha*' has come from '*Siddhi*'- which means achievement. *Siddhars* were the men who achieved supreme knowledge in the field of medicine, yoga or *tapa* (meditation).[31]

Histamine is a major mediator in allergic diseases, and has multiple effects that are mediated by specific surface receptors on target cells. Four types of histamine receptors have now been recognized pharmacologically and the first three are located in the gut. The ability of histamine receptor antagonists to inhibit mast cell degranulation suggests that they might be developed as a group of mast cell stabilizer.[32] The result of binding interactions of the ligand with Histamine 1 Receptor has revealed that out of three compound's kaempferol has 9 interactions (60%) similar to that of the standard Cetirizine hence it has promising H1 receptor blocking activity similarly other compound thespone has 46% percentage similar interaction to that of the standard hence both compounds has H1 receptor blocking activity. Compound mansonone has no H1 receptor blocking activity.

Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which originated in Southern parts of India. Siddha medicine invented from Dravidian culture and is grown in the time of Indus valley civilization. Chinese alchemy, Taoism, and Taoist Patrology are considered as a main source of inspiration for Siddha alchemy.[33] It is believed that in ancient time, the system was developed by eighteen siddhar (a class of Tamil sages). Though Siddha system of medicine resembles with Ayurveda in many aspects it has own philosophy and concept, holistic approach, and lifestyle oriented measures.[34]

Two isoenzymes of COX, COX-1 (constitutive form) and COX-2 (inducible form) have been identified. The classical NSAIDs inhibit both isoenzymes and their use is often accompanied by gastrointestinal intolerance due to a decreased production of protective prostaglandin E2 in the stomach.[35] The result of binding interactions of the ligand with Cyclooxygenase 1 has revealed that out of three compound's kaempferol has 9 interactions (90%) similar to that of the standard ibuprofen hence it has promising COX I inhibition activity similarly other two compounds thespone and mansonone has 50% percentage similar interaction to that of the standard ibuprofen hence all three compounds has COX I inhibition activity. The result of binding interactions of the ligand with Cyclooxygenase 2 has revealed that out of three compound's kaempferol has 2 interaction (40%) similar to that of the standard Celecoxib hence it has promising COX 2 inhibition activity similarly other compound thespone has 20% percentage similar interaction to that of the standard hence both compounds has COX 2 inhibition activity. Compound mansonone has no COX2 inhibition activity.

Cytokines such as TNF alpha and IL-6 seems to have strong etiology in almost all the incidence of inflammation and its associated process. Research finding further justifies that blockage of these cytokines are known to offers better clinical management in allergy and AD. The result of binding interactions of the ligand with TNF alpha has revealed that out of three compound's mansonone has all 5 interactions (100%) similar to that of the standard diclofenac hence it has Excellent TNF alpha inhibition activity similarly other two compounds thespone has 90% percentage and kaemferol has 75 % similar interaction to that of the standard hence all three compounds has promising TNF alpha inhibition activity. The result of binding interactions of the ligand with IL 6 has revealed that out of three compound's mansonone and kaempferol has all 4 interactions (100%) similar to that of the standard diclofenac hence it has Excellent IL6 inhibition activity similarly other compound thespone has 75% percentage similar interaction to that of the standard hence all three compounds has promising IL 6 inhibition activity.

5. CONCLUSION

The results of the present investigation indicates that the lead kaempferol, thespone and mansonone possess significant COX I, TNF alpha and IL 6 inhibition activity. Further the lead kaempferol and thespone also possess significant inhibition of COX II and H1 receptors, whereas the compounds mansonone has no activity against these proteins. Based on the results of the In-silico screening analysis it was concluded that the compound's such as kaempferol, thespone and mansonone present in the siddha formulation PN possess significant inhibition of inflammatory mediators therefore this formulation may have promising immuno modulatory activity and may effective against atopic dermatitis. Further studies have to be carried with special emphasis on molecular biology aspect of the drug and its target receptor in the biological system in near future.

ACKNOWLEDGEMENT

I wish to acknowledge my thanks to The Noble research solutions, Chennai for their technical support for this research work.

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