Short Report

Molecular docking analysis to validate the efficacy of Polyherbal Siddha Formulation Seethabedhi Chooranam for Anti-diarrhoeal activity

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Abstract: Siddha Medicine is considered one of the oldest traditional systems of medicine in the world. Herbal remedies play a significant role in Siddha Medicine. The system utilizes a wide range of herbs, metals, minerals, marine, and animal origin to prepare medicines. Polyherbal formulations are common, and the combination of ingredients is believed to enhance therapeutic effects. Molecular docking analysis is a computational approach to predict the binding affinity and interactions between small molecules and target proteins. This technique plays a vital role in the process of drug discovery and design, helping researchers understand the interactions between potential drug compounds and their target proteins. The M3 muscarinic acetylcholine receptor was used as the target receptor in docking calculations for recovered phytocomponents. Five bioactive lead compounds were retrieved from the herbs present in the formulation *Seethabedhi Chooranam*. According to documented data on the herbs, the main constituents, namely β-caryophyllene, Quercetin and Eugenol have a 100% binding efficacy when they interact with the core target amino acids (Ser151, Tyr529, Tyr506, and Trp503) that are present on the target, on the other hand Cinnamic acid and Kaempferol have a 90% binding efficacy with target amino acid when compared with the standard Loperamide which has a 100 % binding efficacy on the M3 muscarinic acetylcholine receptor (PDB- 4U14). The bio-active compounds were found to exhibit significant binding against the target protein, as indicated by computational analysis results. This suggests that the compounds may have promising anti-diarrheal properties by impeding the activity of the M3 muscarinic acetylcholine receptor, which is present in the intestinal region that mediates the diarrhea.

Keywords: *Siddha Medicine, in-silico, Docking study, Seethabedhi chooranum, M3 muscarinic acetylcholine receptor*

1. INTRODUCTION

The Siddha system of medicine has its origins in India, particularly in the Tamil region. It is one of the traditional system of medicine practiced in South India and Sri Lanka. Siddha medicine is believed to have ancient roots and is associated with the Siddhars. The Siddha system is considered a part of the Indian traditional medicine systems, along with Ayurveda and Unani. The Siddha system of medicine utilizes a diverse range of substances for preparing medicinal formulations. These substances can be broadly categorized into plant-based, mineral-based, metal-basted, marine-based and animal-based origins. The Siddha practitioners believe in the therapeutic properties of these natural substances and their ability to bring about healing and balance in the body. Polyherbal formulations are common, and the combination of ingredients is believed to enhance therapeutic effects.

The term “In-silico” refers to computational models. In-vitro models are typically used alongside with In-silico approaches. They have been successful in achieving numerous advancements in a range of pharmacological areas. There are clarification of absorption, distribution, metabolism, excretion and toxicity properties, the discovery and optimization of novel molecules and physicochemical characterization.

Molecular docking analysis is a computational approach to predict the binding affinity and interactions between small molecules and target proteins. This technique plays a vital role in the process of drug discovery and design, helping researchers understand the interactions between potential drug compounds and their target proteins.

2. MATERIALS AND METHOD

Seethabedhi Chooranam (SC) was taken for docking study from the Siddha Authentic Literature "*Kannusaamy Paramparai Vaithiyam*" which was written by S. Kannusamy Pillai.[1]

The M3 muscarinic acetylcholine receptor (PDB – 4U14) which is responsible for motility and peristalsis which mediates the diarrheal activity will be inhibited by phytocomponents binding to the target's core amino acids (Ser151, Tyr529, Tyr506, and Trp503) through the formation of a hydrogen bond. Therefore, it would be preferable to inhibit and establish the anti-diarrhoeal activity with phytocomponents that inhibit the target muscarinic acetylcholine receptor by occupying the residual active amino acids.

The binding of phytocomponents with the core amino acids (Ser151, Tyr529, Tyr506, and Trp503) of the target by forming a hydrogen bond will hinder the function of the M3 muscarinic acetylcholine receptor (PDB – 4U14) which is responsible for motility and peristalsis which mediates the diarrheal activity. Thereby phytocomponents that inhibit the target muscarinic acetylcholine receptor by occupying the residual active amino acids could preferably block the intestinal motility and thereby establish the anti-diarrhoeal activity.

PDB	Name of the Target
4U14	M3 muscarinic acetylcholine receptor

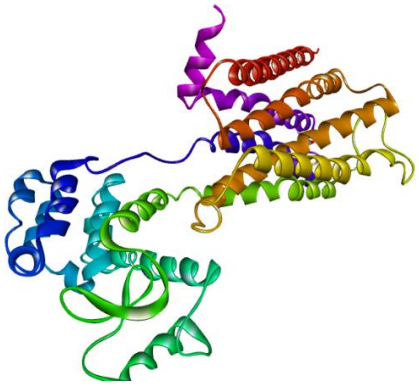

Figure 1: M3 muscarinic acetylcholine receptor - PDB- 4U14

2.1 Docking Methodology

Docking calculations were carried out for retrieved phytocomponents against target enzyme M3 muscarinic acetylcholine receptor. 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 Auto grid 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.

Table 1: Ingredients of SC

Vernacular Name (Tamil)	Botanical Name
Kirambu	*Syzygium aromaticum*
Elavangap paddai	*Cinnamomum verum*
Kadukkai Poo	*Terminalia chebula*

Table 2: List of Phytocomponents Selected for docking

Botanical Name of herbs	Phytochemicals
Syzygium aromaticum	• Quercetin • Kaempferol • Eugenol • β-caryophyllene
Cinnamomum verum	• Cinnamic acid
Terminalia chebula	• Gallic acid • Maslinic acid

3. OBSERVATION AND INFERENCE

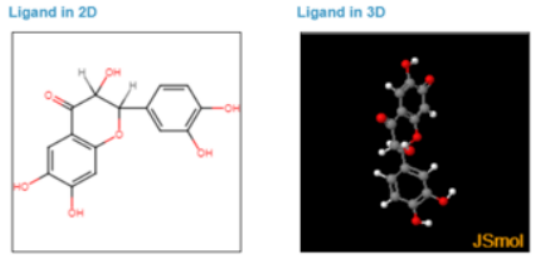

Figure 1: Quercetin

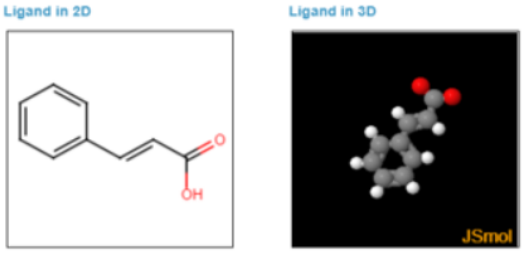

Figure 5: Cinnamic acid

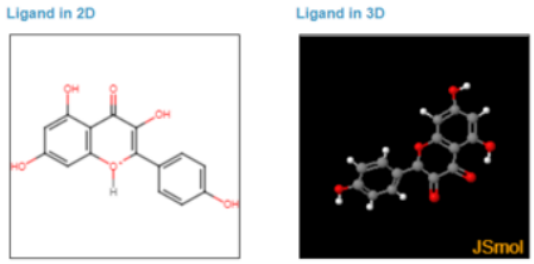

Figure 2: Kaempferol

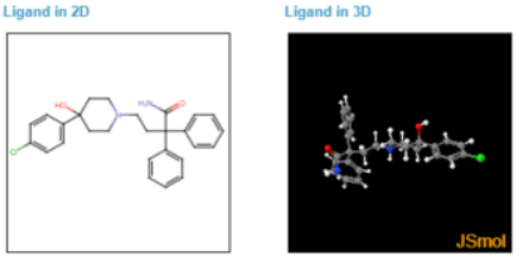

Figure 6: Loperamide

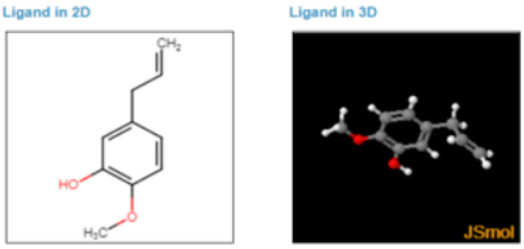

Figure 3: Eugenol

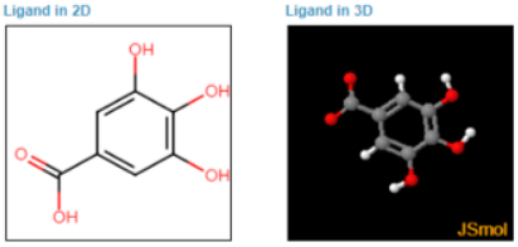

Figure 7: Gallic acid

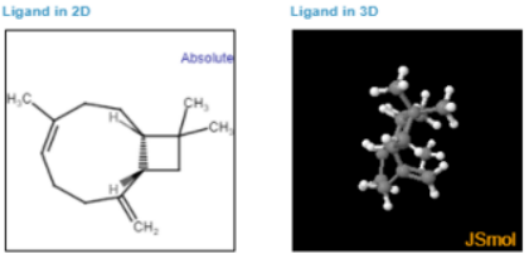

Figure 4: β-caryophyllene

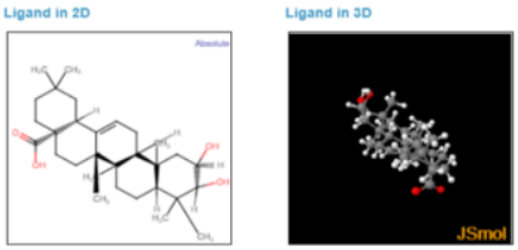

Figure 8: Maslinic acid

Table 3: Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Quercetin	302.23 g/mol	C15H10O7	5	7	1
Kaempferol	286.23 g/mol	C15H10O6	4	6	1
Eugenol	164.2 g/mol	C10H12O2	1	2	3
β-caryophyllene	204.35 g/mol	C15H24	0	0	0
Cinnamic acid	148.16 g/mol	C9H8O2	1	2	2
Loperamide	477 g/mol	C29H33ClN2O2	1	3	7
Gallic acid	170.12 g/mol	C7H6O5	4	5	1
Maslinic acid	472.7 g/mol	C30H48O4	3	4	1

Table 4: Summary of the molecular docking studies of compounds against M3 muscarinic acetylcholine receptor -PDB- 4U14

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Quercetin	-7.29 kcal/mol	4.54 uM	-0.13 kcal/mol	-6.46 kcal/mol	704.945
Kaempferol	-6.41 kcal/mol	20.16 uM	-0.06 kcal/mol	-6.81 kcal/mol	687.712
Eugenol	-5.18 kcal/mol	159.81 uM	-0.04 kcal/mol	-5.65 kcal/mol	464.477
β-caryophyllene	-7.57 kcal/mol	2.84 uM	-0.19 kcal/mol	-7.57 kcal/mol	585.278
Cinnamic acid	-4.80 kcal/mol	303.47 uM	-0.02 kcal/mol	-5.40 kcal/mol	465.96
Loperamide	-7.57 kcal/mol	2.84 uM	-0.15 kcal/mol	-7.57 kcal/mol	585.278
Gallic acid	-5.01 kcal/mol	210.92 uM	-0.14 kcal/mol	-4.55 kcal/mol	414.82
Maslinic acid	-1.21 kcal/mol	130.05 mM	-0.04 kcal/mol	-1.05 kcal/mol	984.931

Table 5: Amino acid Residue Interaction of Lead and Standard against M3 muscarinic acetylcholine receptor -PDB- 4U14

Molecule	Interactions	Amino Acid Residue- Binding														
Quercetin	4	116 ILE	148 TYR	151 SER	225 LEU	231 THR	234 THR	239 PHE	503 TRP	506 TYR	507 ASN	529 TYR	532 CYS	533 TYR		
Kaempferol	3	115 SER	199 TRP	231 THR	234 THR	235 ALA	238 ALA	503 TRP	506 TYR	510 VAL	529 TYR	532 CYS	533 TYR			
Eugenol	4	116 ILE	147 ASP	148 TYR	151 SER	503 TRP	506 TYR	529 TYR	532 CYS	533 TYR						
β-caryophyllene	4	116 ILE	147 ASP	148 TYR	151 SER	503 TRP	506 TYR	529 TYR	532 CYS	533 TYR						
Cinnamic acid	3	239 PHE	503 TRP	506 TYR	507 ASN	529 TYR	532 CYS	533 TYR								
Loperamide	4	148 TYR	151 SER	152 ASN	155 VAL	199 TRP	225 LEU	231 THR	234 THR	238 ALA	239 PHE	503 TRP	506 TYR	507 ASN	510 VAL	529 TYR
Gallic acid	2	148 TYR	151 SER	152 ASN	155 VAL	199 TRP	234 THR	235 ALA	238 ALA	503 TRP						
Maslinic acid	2	148 TYR	222 ILE	225 LEU	231 THR	234 THR	238 ALA	239 PHE	503 TRP	506 TYR	507 ASN	510 VAL	525 TRP			

The bio-active compounds like Eugenol, β-caryophyllene, Quercetin, Cinnamic acid and Kaempferol present in the *Seethabedhi Chooranam*. According to documented data on the herbs, the main constituents, namely β-caryophyllene, Quercetin and Eugenol have a 100% binding efficacy when they interact with the core target amino acids (Ser151, Tyr529, Tyr506, and Trp503) that are present on the target, on the other hand Cinnamic acid and Kaempferol have a 90% binding efficacy with target amino acid when compared with the standard Loperamide which has a 100 % binding efficacy on the M3 muscarinic acetylcholine receptor (PDB- 4U14).

4. CONCLUSION

The bio-active compounds like Eugenol, β-caryophyllene, Quercetin, Cinnamic acid and Kaempferol present in the *Seethabedhi Chooranam*. According to documented data on the herbs, the main constituents, namely β-caryophyllene, Quercetin and Eugenol have a 100% binding efficacy when they interact with the core target amino acids (Ser151, Tyr529, Tyr506, and Trp503) that are present on the target, on the other hand Cinnamic acid and Kaempferol have a 90% binding efficacy with target amino acid when compared with the standard Loperamide which has a 100 % binding efficacy on the M3 muscarinic acetylcholine receptor (PDB- 4U14). The bio-active

compounds were found to exhibit significant binding against the target protein, as indicated by computational analysis results. This suggests that the compounds may have promising anti-diarrheal properties by impeding the activity of the M3 muscarinic acetylcholine receptor, which is present in the intestinal region that mediates the diarrhea.

6. REFERENCES

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