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In-Silico Anti-Diarrhoeal Evaluation of Lagu Gangathara Chooranam

Research Article

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Abstract

Background: In siddha system of medicine diarrhoea can be compared with *kazhichal*. Diarrhoea results from several factors like intestinal hyper-motility, malabsorbtion of water, inflammation of GIT or use of laxatives. In siddha literature, the preparation named *lagu gangathara chooranam* is indicated for *kazhichal*. Aim: The study aims to perform the in-silico computational studies of phytoconstituents of the siddha formulation *lagu gangathara chooranam* targeted against m3 muscarinic acetyl choline receptor for anti-diarrhoeal activity. Methods: The retrieved phytocomponents were subjected for docking calculations against target enzyme m3 muscarinic acetylcholine receptor. Results: The computational analysis retrived 11 bio-active compounds from herbal formulation. It includes limonene, α-humulene, cyperolone, lupeol, beta amyrin, betulinic acid, mangiferin, quercetin, caryophyllene and luteolin. These compounds significantly bind against the target m3 muscarinic acetylcholine receptor. Hence these phytocompounds may produce significant anti-diarrheal activity by hindering the activity of m3 muscarinic acetylcholine receptor in the intestinal region which mediates the diarrhoea. Conclusion: Further clinical trials or experimental studies could be conducted to confirm the effectiveness of this siddha formulation *lagu gangathara chooranam* against diarrhoea.

Keywords: Molecular docking, *Lagu Gangathara Chooranam*, Antidiarrhoeal activity, M3 muscarinic Acetylcholine receptor, Siddha.

Introduction

Diarrhea is defined as the passage of stools of fluid consistency more frequently than in usual [usually more than 3 times per day]. The wet weight of the stool is increased (1). The approximate volume of water in stools is 200g/day in teenagers and adults whereas in children/infants it is 10 mL/Kg/day. Diarrhea is caused by the imbalance in the absorption of ions, other substrates and water by the small and large intestine (2). Based on the duration, diarrhea can be classified as acute and chronic diarrhea (3) . Infections or irritants cause acute diarrhea whereas chronic diarrhea results from many causes(1). Chronic diarrhea tends to be non-infectious due to malabsorption, inflammatory bowel disease and side effects of medications. (3). In young children Rota virus causes severe diarrhea globally. One fifth of the infectious diarrhea is caused by Norovirus in both children and adults. Noro virus is about to cause death in 200000 deaths in developing country annually(4). In India, the prevalence of diarrhea in 2007-2008 was estimated as 0.1-33.8% and in 2015-2018 it was 0.6-29.1% (5,6). As per WHO and UNICEF, every year 2.5 billion diarrheal cases and 1.9 million children below the age of 5 years die globally(7). Anti-diarrheal drugs stop diarrhea by decreasing the propulsive movement of GI smooth muscles or by reducing the secretions of the intestine (8). Due to contraindications, drug resistance and adverse effects of the currently available drugs there is a need for alternative medicines to treat diarrhea (9,10) . Siddha system is one of the unique traditional system of medicine with vast literatures. *Lagu Gangathara Chooranam* is one of the Siddha herbal formulations indicated for Kazhichal (11) . Kazhichal can be compared with diarrhoea in Siddha. Molecular docking can be defined as how the drug and enzyme or protein fit together. The phytocomponents bind with target's core amino acids [Ser151, Tyr529, Tyr506, and Trp503] using hydrogen bond to hinder the function of the M3 muscarinic acetylcholine receptor [PDB – 4U14] .This receptor is responsible for motility and peristalsis which mediates the diarrheal activity. Among the mAchR group, M3 subtype play many significant physiological functions such as glandular secretion and smooth muscle contraction (12-17). Thereby phytocomponents which 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. The aim of this study is to analyse the Anti-diarrheal activity of *Lagu Gangathara Chooranam* which is a siddha herbal

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formulation containing *Aegle marmelos L., Cyperus rotundus L., Wrightia tinctoria R.Br, Symplocos racemose Roxb, Bombax ceiba L., Saccharum officinarum L.*

Methodology

Based on the literature review, the phytochemicals from the medicinal plants possessing were retrieved (18-24). The retrieved phytocomponents were subjected for docking calculations against target enzyme M3 muscarinic acetylcholine receptor. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools [Affinity [grid] maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (25). AutoDock 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 (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.

Table 1: Details of Target

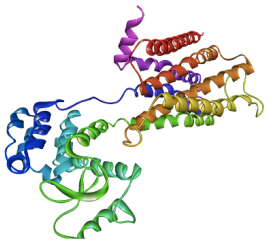
PDB	Name of the Target	Figure 1: M3 muscarinic acetylcholine receptor -PDB- 4U14
4U14	M3 muscarinic acetylcholine receptor	

Table 2: List of Phytocomponents selected for docking

Herbs	Phytochemicals
Aegle marmelos L.	· Limonene · α-Humulene
Cyperus rotundus L.	· Cyperolone
Wrightia tinctoria R.Br	· Lupeol · Beta Amyrin
Symplocos racemosa Roxb.	· Betulinic acid · Oleanolic acid
Bombax ceiba Linn	· Mangiferin · Quercetin
Woodfordia fruticosa Kurz	· Caryophyllene
Saccharum officinarum Linn	· Luteoline

Figure 2: 2D and 3D Structure of Selected Ligands

Figure 2.1: Limonene

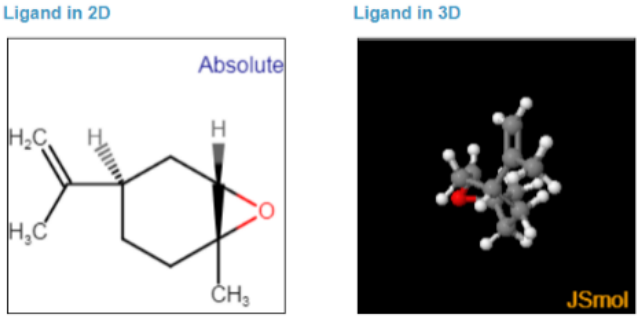


Figure 2.2: α-Humulene

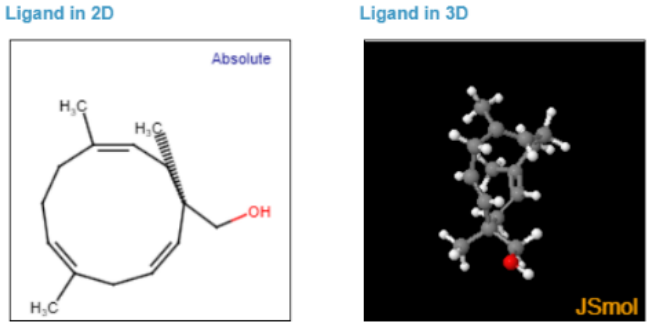


Figure 2.3: Cyperolone

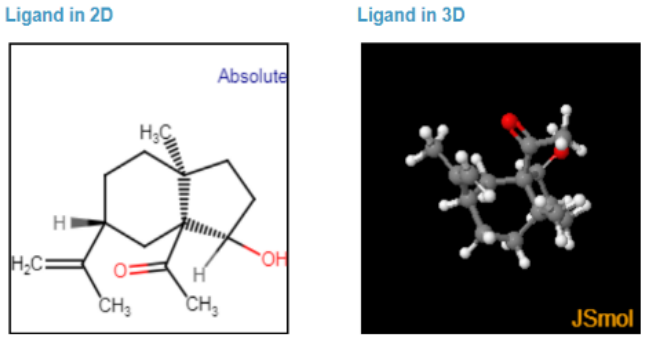


Figure 2.4: Lupeol

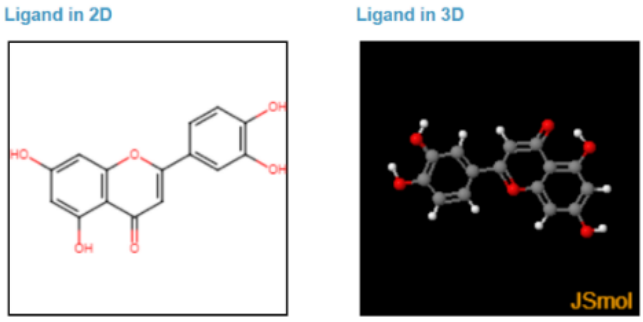


Figure 2.5: Beta Amyrin

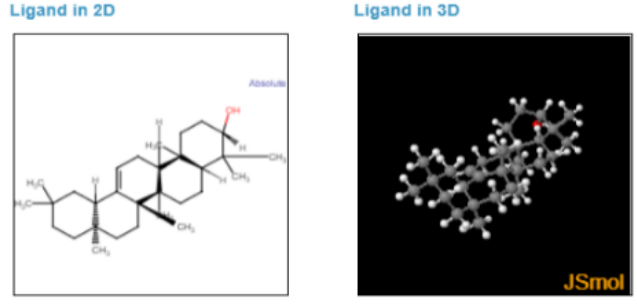


Figure 2.6: Betulinic acid

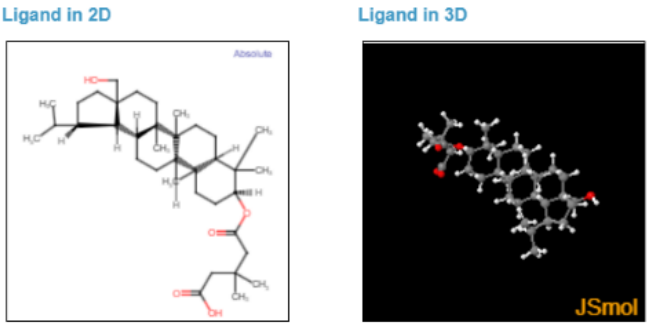


Figure 2.7: Oleanolic acid

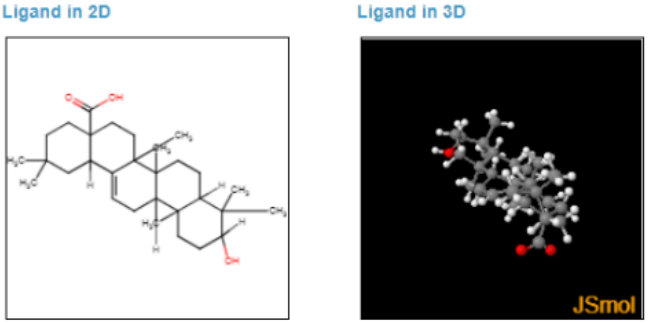


Figure 2.8: Mangiferin

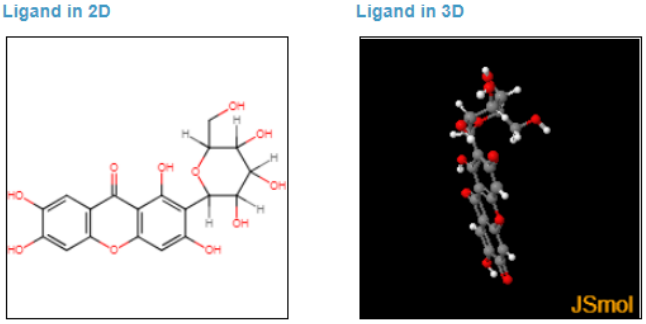


Figure 2.9: Quercetin

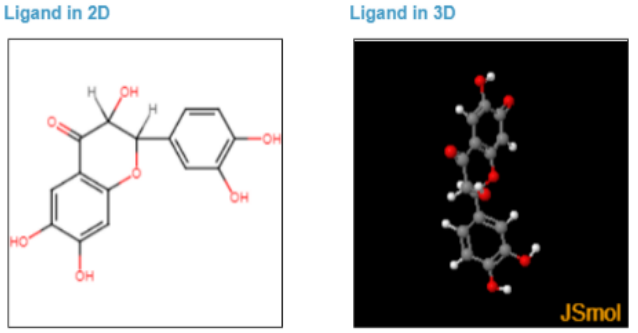


Figure 2.10: Caryophyllene

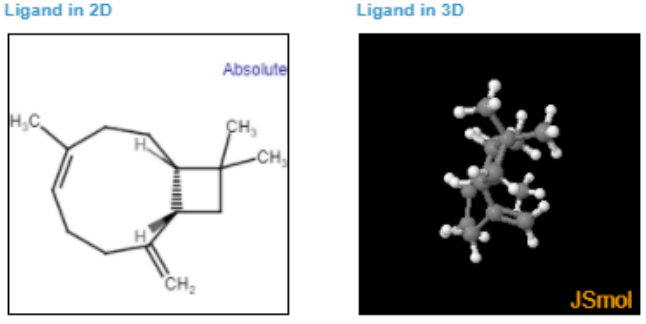


Figure 2.11: Luteolin

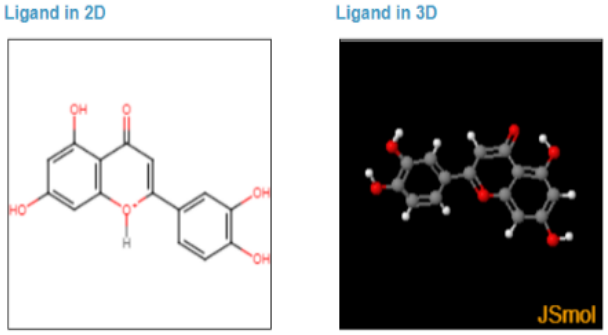
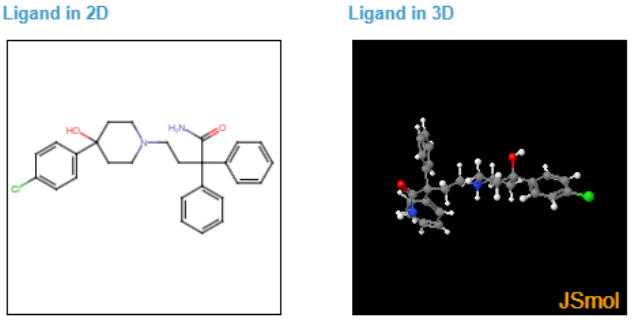


Figure 2.12: Loperamide



Results and Discussion

Lagu Gangathara Chooranam is a polyherbal formulation containing 6 raw drugs. Among those, *Aegle marmelos L* possesses Anti-inflammatory, Antibacterial and Anti-diarrhoeal activity (29). *Cyperus rotundus L.* have Antidiarhoeal and Anti-emetic activity (30). *Symplocos racemose Roxb* possess antimicrobial, Analgesic, anti-inflammatory and anti-diarrheal activity (31,32). *Wrightia tinctoria R.Br* possess antibacterial activity (33). *Woodfordia fruiticosa [L] Kurz* possessess anti-inflammatory, antibacterial, anti-ulcer and antimicrobial activity(34). *Bombax ceiba L* have anti-microbial and anti-inflammatory avtivity (35). From data of the herb, 11 lead compounds such as Limonene, α-Humulene, Cyperolone, Lupeol, Beta Amyrin, Betulinic acid, Mangiferin, Quercetin, Caryophyllene and Luteolin possess significant binding efficacy by interacting with the core target amino acids. Loperamide exhibits significant antidiarrheal activity by reducing the gut motility. Loperamide acts via the peripheral opioid receptors with limited access to CNS(36). Loperamide is considered as a standard drug to compare the efficacy of the test drug *Lagu Gangathara Chooranam*. Cyperolone has highest binding affinity of -7.63 kcal/mol Caryophyllene has binding affinity of -7.57 kcal/mol, followed by α -Humulene with -7.48 kcal/mol, Quercetin with -7.26 kcal/mol, Luteolin – 7.24kcal/mol, betulinic acid with-6.86 kcal/mol, beta amyrin with -6.31 kcal/mol, Limenone with -5.50 kcal/mol, Mangiferin with -5.45 kcal/mol, Lupeol with -3.12 kcal/mol and Oleanolic acid with -2.53 kcal/mol with the core aminoacids Ser151, Tyr529, Tyr506, and Trp503 of the M3 muscarinic acetylcholine receptor. Loperamide has the binding affinity of about -7.57 kcal/mol. But the cyperolone possess higher binding energy of about

-7.63 kcal/mol than loperamide [-7.57 kcal/mol]. But the other drugs possess anti-microbial, antibacterial and anti-diarhoeal activity that makes the drug more effective in treating diarrhea. Among the 11 compounds, Limonene, Cyperolone, Betulinic acid, Mangiferin, Quercetin, Caryophyllene showed 4 interactions with amino acid residue [Ser151, Tyr529, Tyr506, and Trp503]. α-Humulene and Beta Amyrin showed 3 interactions with the core amino acid residue Ser151, Tyr506, and Trp503. Lupeol and luteolin showed 3 interactions with the core amino acid residue Tyr529, Tyr506, and Trp503. Oleonolic acid showed 3 interactions with the core amino acid residue Ser151, Tyr506. Loperamide possess 4 interactions with the core aminoacid residue Ser151, Tyr506,Trp503 and TYR529. In this drug, Cyperolone possess 4 interactions as same as loperamide . Quercitin was reported to have protection and benefits against chronic inflammation of the intestine (37). Added quercitin affects the progression of the diseases such as IBD and colitis (38). Luteolin possess strong anti-inflammatory activity and have therapeutic efficacy against IBD (39,40,41) . Betulinic acid and oleanolic acid have gastroprotective and anti-ulcer activity (42). A study reported that both betulinic acid and oleanolic acid have the ability to suppress the diarrhea induced by Lt[Heat labile enterotoxin] at 4mM, which is less than LD50 in mice (43). Oleanolic acid and betulinic acid binds with heat labile enterotoxin [LT] with the binding energy -11.76 kcal/mol and -10.99 kcal/mol respectively. Oleonolic acid formed hydrophobic bonds with Gly33, Trp88, Lys 91and Lys34 whereas betulinic acid binds with Gly33, Trp88, Lys 91 and Gln56 residues of LTB (44). In a study done in Male Swiss albino rats, diarrhoea was induced using castor oil. Administration of minimal doses of limonene reduced the intestinal content volume by 28.52% (45). Electrolyte balance in the intestine cells is essential for gut health in animals. In animals, ricinolic acid increases the permeability of electrolytes by inhibiting Na^+/K^+ ATPase thereby increases the severity of diarrhea. Luteolin was found to be effective in increasing the activity of Na^+/K^+ ATPase and increases the concentration of Na^+ and K^+ in the small intestine. In molecular docking study, luteolin binds with the amino acids TYR-443, TYR-32 of Na^+/K^+ ATPase. This indicates that luteolin possess potent antidiarrheal activity (46). In a docking study for antidiarrhoeal activity, Loperamide binds with kappa and delta opioid receptor with the binding energy of about -6.6 and -10.1 kcal/mol. While, beta amyrin had binding energy of about -4.3 to -7.9 kcal/mol with human-kappa opioid receptor (47). But in this study beta amyrin binds with M3 muscarinic acetylcholine receptor with the binding energy of about -6.31 kcal/mol. Finally, this study states that 11 lead components bind with core amino acids [Ser151, Tyr529, Tyr506, and Trp503] on the target with 75-100% binding efficacy with the target receptor M3 muscarinic acetylcholine receptor -PDB- 4U14. This shows that the siddha preparation *Lagu Gangathara Chooranam* possess promising antidiarrhoeal activity.

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
Limonene	136.23 g/mol	C10H16	0	0	1
α-Humulene	204.35 g/mol	C15H24	0	0	0
Cyperolone	236.35 g/mol	C15H24O2	1	2	2
Lupeol	426.729 g/mol	C30H50O	1	1	1
Beta Amyrin	426.7 g/mol	C30H50O	1	1	0
Betulinic acid	456.7 g/mol	C30H48O3	2	3	2
Oleanolic acid	456.711 g/mol	C30H48O3	2	3	1
Mangiferin	422.342 g/mol	C19H18O11	8	11	2
Quercetin	302.23 g/mol	C15H10O7	5	7	1
Caryophyllene	204.35 g/mol	C15H24	0	0	0
Luteolin	286.24g/mol	C15H10O6	4	6	1
Loperamide	477 g/mol	C29H33ClN2O2	1	3	7

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
Limonene	-5.50 kcal/mol	93.68 Um	-0.01 kcal/mol	-5.79 kcal/mol	434.098
α-Humulene	-7.48 kcal/mol	3.27 Um	-0.22 kcal/mol	-7.48 kcal/mol	635.225
Cyperolone	-7.63 kcal/mol	2.56 uM	-0.12 kcal/mol	-7.79 kcal/mol	612.282
Lupeol	-3.12 kcal/mol	5.20 Mm	-0.08 kcal/mol	-3.71 kcal/mol	974.617

Beta Amyrin	-6.31 kcal/mol	23.87 Um	-0.21 kcal/mol	-5.46 kcal/mol	738.836
Betulinic acid	-6.86 kcal/mol	9.40 Um	-0.18 kcal/mol	-6.40 kcal/mol	719.055
Oleanolic acid	-2.53 kcal/mol	13.91 mM	-0.03 kcal/mol	-3.17 kcal/mol	958.768
Mangiferin	-5.45 kcal/mol	100.95 uM	-0.16 kcal/mol	-4.32 kcal/mol	815.729
Quercetin	-7.26 kcal/mol	4.74 uM	-0.13 kcal/mol	-6.45 kcal/mol	707.124
Caryophyllene	-7.57 kcal/mol	2.84 uM	-0.09 kcal/mol	-7.57 kcal/mol	584.688
Luteolin	-7.24 kcal/mol	4.94 uM	-0.14 kcal/mol	-7.33 kcal/mol	698.265
Loperamide	-7.57 kcal/mol	2.84 uM	-0.15 kcal/mol	-7.57 kcal/mol	585.278

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

Compounds	Interactions	Amino acid Residues													
Limonene	4	116 ILE	147 ASP	148 TYR	151 SER	503 TRP	506 TYR	529 TYR	532 CYS	533 TYR					
α-Humulene	3	148 TYR	151 SER	152 ASN	199 TRP	235 ALA	238 ALA	239 PHE	503 TRP	506 TYR	507 ASN	532 CYS			
Cyperolone	4	147 ASP	148 TYR	151 SER	503 TRP	506 TYR	529 TYR	532 CYS	533 TYR						
Lupeol	3	148 TYR	222 ILE	225 LEU	231 THR	235 ALA	238 ALA	239 PHE	503 TRP	506 TYR	507 ASN	510 VAL	525 TRP	529 TYR	
Beta Amyrin	3	148 TYR	151 SER	152 ASN	199 TRP	222 ILE	225 ILE	231 THR	234 THR	235 ALA	238 ALA	503 TRP	506 TYR	507 ASN	510 VAL	525 TRP
Betulinic acid	4	148 TYR	151 SER	152 ASN	199 TRP	222 ILE	225 ILE	231 THR	235 ALA	238 ALA	503 TRP	506 TYR	507 ASN	510 VAL	525 TRP	529 TYR
Oleanolic acid	2	148 TYR	151 SER	152 ASN	199 TRP	222 ILE	225 ILE	231 THR	235 ALA	238 ALA	239 PHE	506 TYR	507 ASN	510 VAL	525 TRP	
Mangiferin	4	148 TYR	151 SER	152 ASN	199 TRP	225 ILE	231 THR	238 ALA	239 PHE	503 TRP	506 TYR	529 TYR				
Quercetin	4	116 ILE	148 TYR	151 SER	225 ILE	231 THR	234 THR	503 TRP	506 TYR	507 ASN	529 TYR	532 CYS	533 TYR			
Caryophyllene	4	116 ILE	147 ASP	148 TYR	151 SER	503 TRP	506 TYR	529 TYR	532 CYS	533 TYR						
Luteolin	3	116 ILE	147 ASP	148 TYR	225 ILE	231 THR	234 THR	503 TRP	506 TYR	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

Docking Pose

Figure 3.1: Limonene with M3 muscarinic acetylcholine receptor -PDB- 4U14

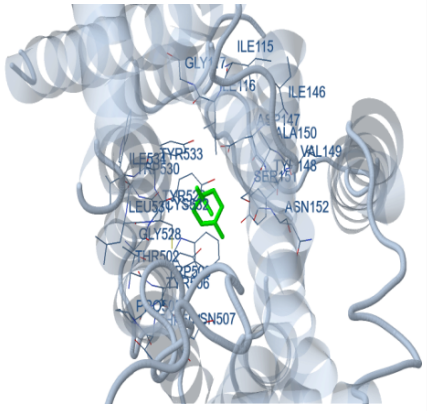
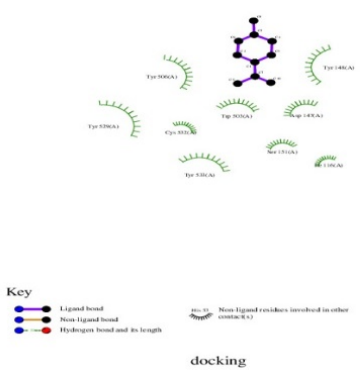
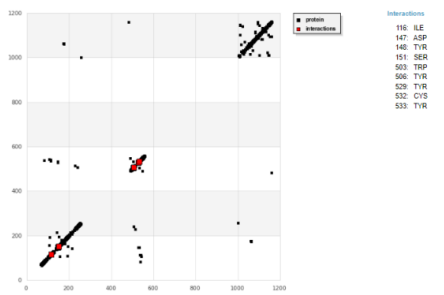
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.2: α-Humulene with M3 muscarinic acetylcholine receptor -PDB- 4U14

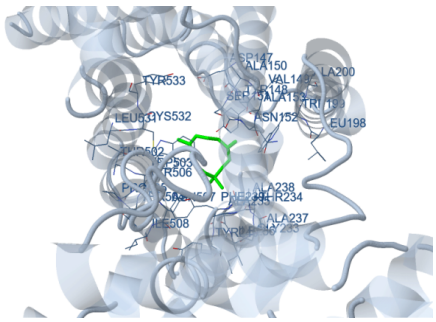
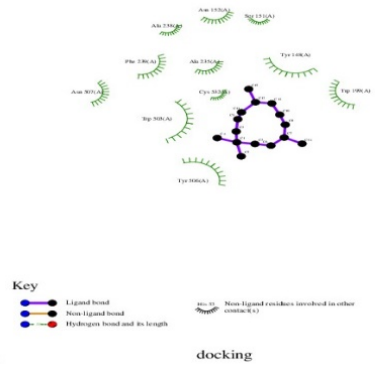
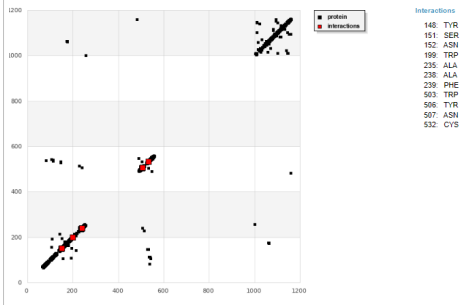
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.3: Cyperolone with M3 muscarinic acetylcholine receptor -PDB- 4U14

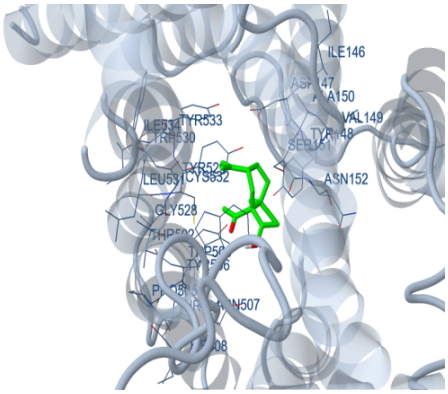
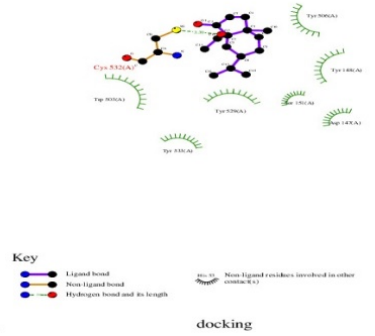
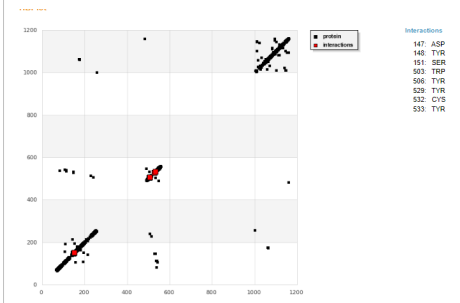
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.4: Lupeol with M3 muscarinic acetylcholine receptor -PDB- 4U14

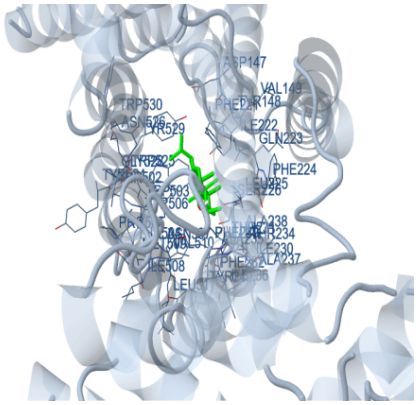
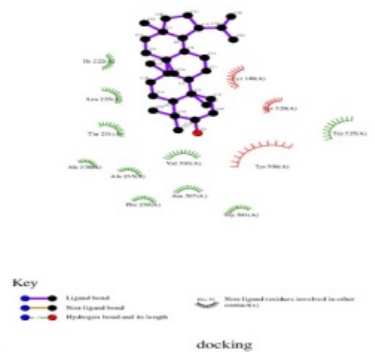
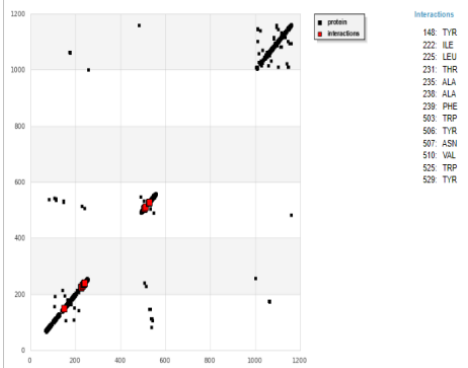
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.5: Beta Amyrin with M3 muscarinic acetylcholine receptor -PDB- 4U14

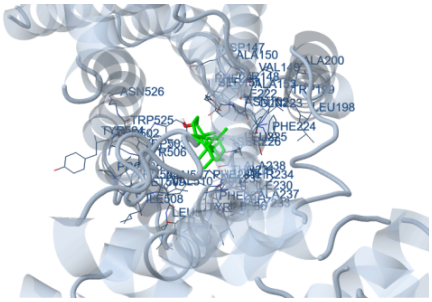
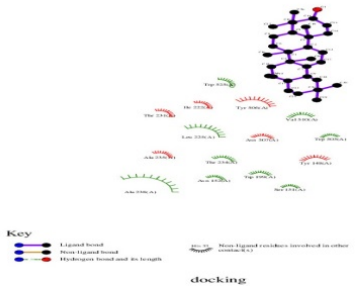
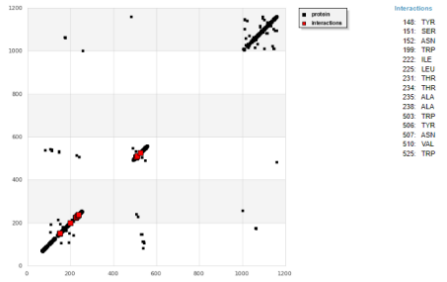
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.6: Betulinic acid with M3 muscarinic acetylcholine receptor -PDB- 4U14

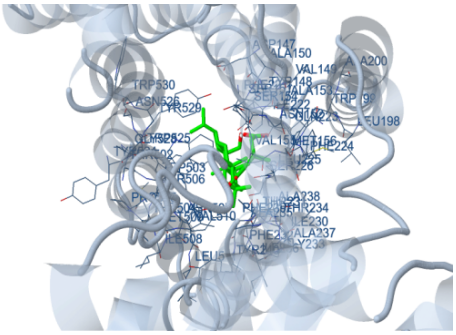
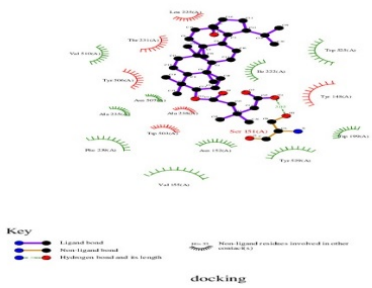
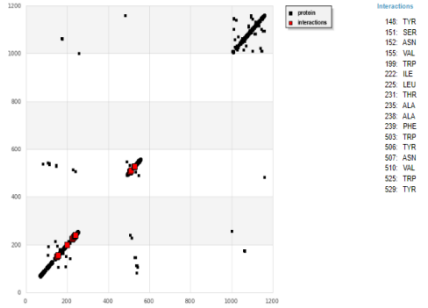
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.7: Oleanolic acid with M3 muscarinic acetylcholine receptor -PDB- 4U14

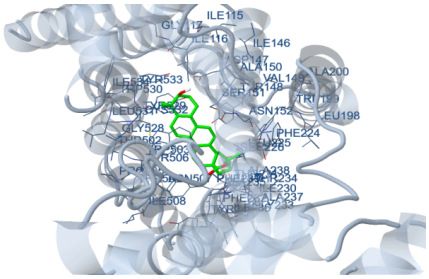
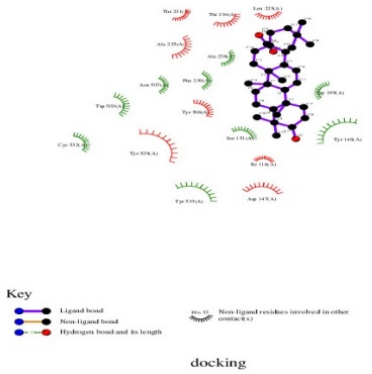
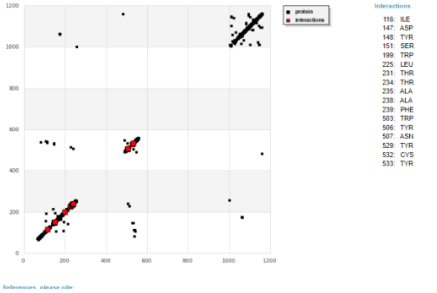
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.8: Mangiferin with M3 muscarinic acetylcholine receptor -PDB- 4U14

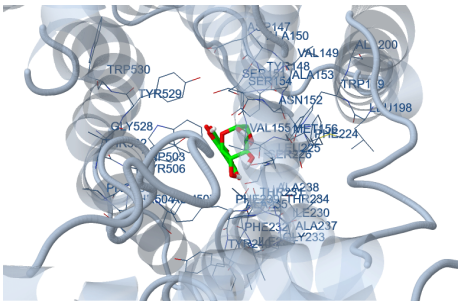
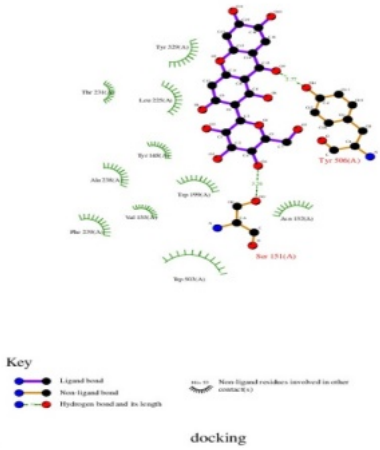
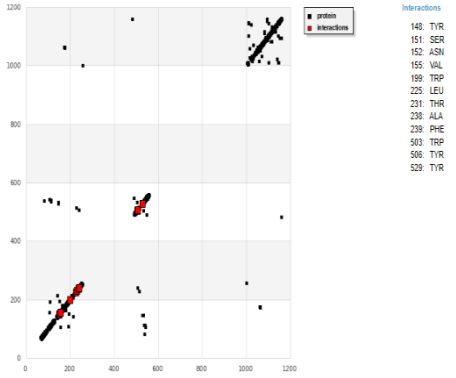
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.9: Quercetin with M3 muscarinic acetylcholine receptor -PDB- 4U14

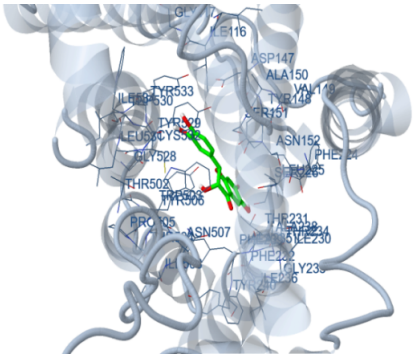
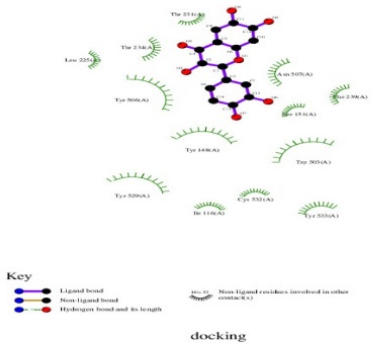
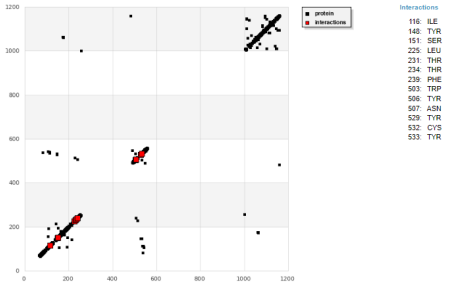
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.10: Caryophyllene with M3 muscarinic acetylcholine receptor -PDB- 4U14

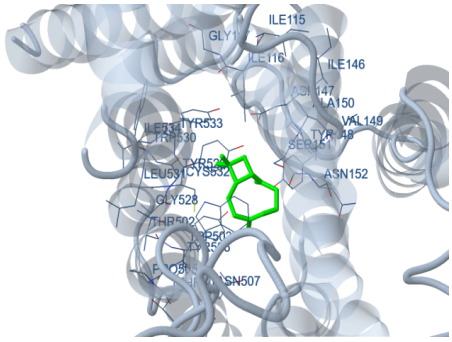
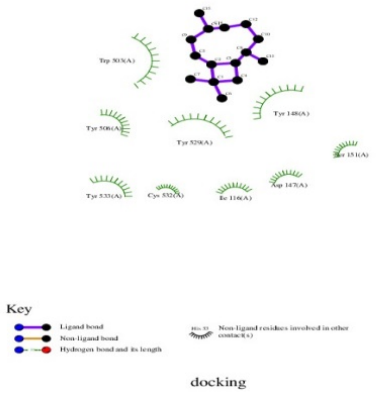
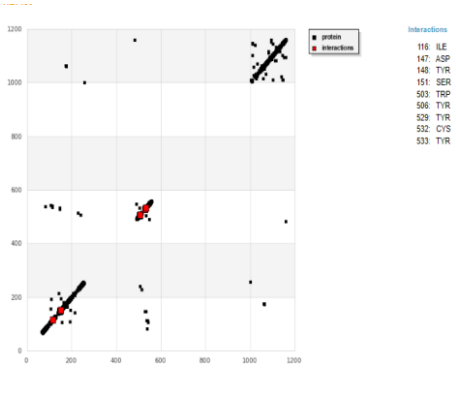
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.11: Luteolin with M3 muscarinic acetylcholine receptor -PDB- 4U14

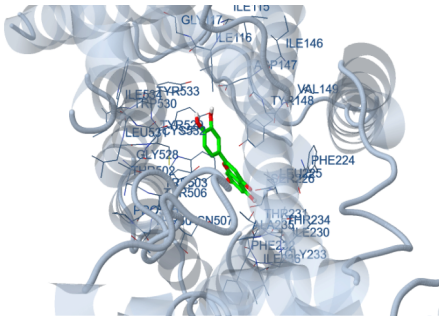
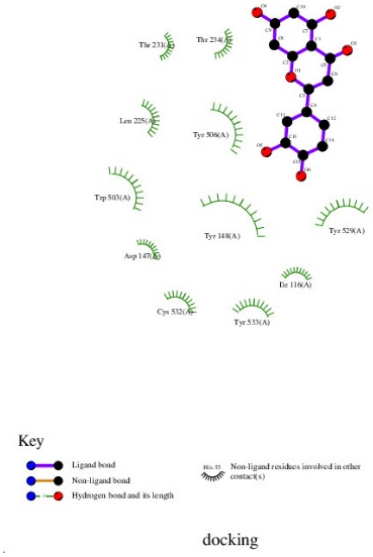
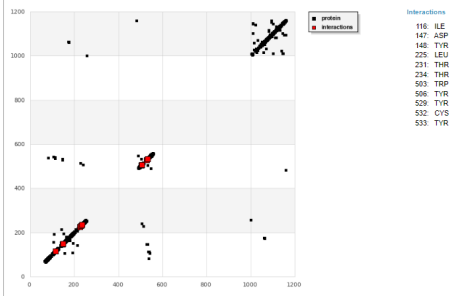
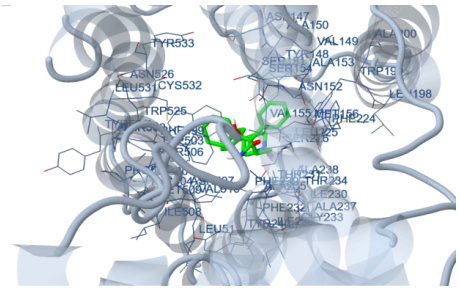
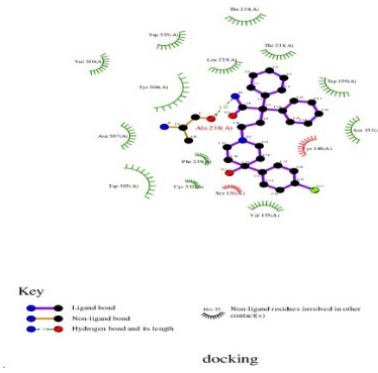
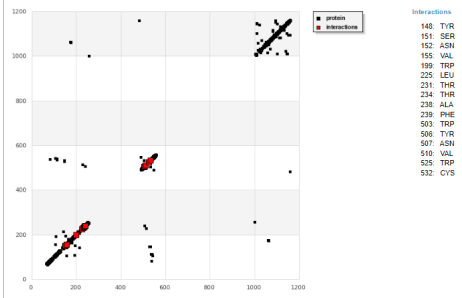
	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Figure 3.12: Loperamide with M3 muscarinic acetylcholine receptor -PDB- 4U14

	2D Interaction Plot	Hydrogen bond plotting Analysis with core amino acid
		

Conclusion

The computational analysis states that the herbal preparation with bio-active compounds like Limonene, α-Humulene, Cyperolone, Lupeol, Beta Amyrin, Betulinic acid, Mangiferin, Quercetin, Caryophyllene and Luteolin exhibit effective binding capacity against the target protein. Hence, it was concluded that the phytocompounds have promising anti-diarrheal activity by hindering the activity of M3 muscarinic acetylcholine receptor present in the intestinal region that mediates the diarrhoea.

Conflict of Interest: Nil

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