IJPSR (2024), Volume 15, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 August 2023; received in revised form, 22 October 2023; accepted, 30 December 2023; published 01 March 2024

POTENTIAL ANTI-ULCER ACTIVITY OF SIDDHA POLY HERBAL FORMULATION *PANCHA MOOLA KUDINEER CHOORANAM*: INSIGHTS FROM MOLECULAR DOCKING

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Keywords:

Glycyrrhizin, *Helicobacter pylori*, Molecular docking, *Pancha Moola Kudineer Chooranam*, Ulcer

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ABSTRACT: The Siddha medicine system, predominantly practiced in South India, encompasses a diverse range of medicinal formulations. Among them, *Pancha Moola Kudineer Chooranam* (PMKC) from the Therayar Maha Karisal literature is claimed to be effective against ulcers. *Helicobacter pylori*, causes gastric and duodenal ulcers through various transmission routes. It colonizes the duodenal epithelial cells, leading to gastritis, gastric atrophy, and potentially gastric carcinoma. This study provided valuable insights on the potential anti-ulcer activity of root extracts of the formulation PMKC, which includes *Glycyrrhiza glabra*, *Chukrasia tabularis*, *Terminalia chebula*, *Terminalia bellirica*, and *Phyllanthus emblica* against *Helicobacter pylori* through molecular docking. Eight bioactive compounds were identified which underwent docking calculations. The results indicated that these phytochemicals interacted with the active amino acid residues of *H. pylori* urease, suggesting potential inhibitory effects on the enzyme. Among the compounds tested, Glycyrrhizin demonstrated the highest inhibition against *H. pylori* urease, followed by Gallic acid. Maslinic acid and Chuktabrin showed minimal inhibition. In conclusion, the active components of PMKC exhibited significant binding affinity to *H. pylori* urease, indicating their potential as therapeutic agents for managing ulcers. These findings underscore the anti-ulcer activity of the phytochemicals present in PMKC's root extracts, emphasizing the importance of further research in this field.

INTRODUCTION: Among the diverse traditional systems of medicine practiced globally, the Siddha system, predominantly followed in southern India, holds a prominent position. In siddha system there are various literatures containing a vast collection of medicinal formulation and the disease it can be treated with. *Pancha Moola Kudineer Chooranam* (PMKC) [1] is one such siddha herbal formulation from the literature *Therayar Maha Karisal*.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(3).979-85

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(3).979-85

The saint Therayar has mentioned that this formulation can be used for the treatment of *kunmam* (ulcer) [2]. There are 4,448 diseases explained by the siddhars one of them is called *kunmam*, whose symptom is relatable to ulcer.

Since, one of the major causes of ulcer is *H. pylori*, molecular docking against *Helicobacter pylori* is done *H. pylori* is a gram negative, spiral shaped bacteria discovered in the year of 1984 by Dr. Barry J. Marshall and Dr. J. Robin Warren. Generally, 90% of duodenal ulcer cases, 70% of gastric ulcer cases [3] and in 57% of Mucosa Associated Lymphoid Tissue lymphoma (MALT) *H. pylori*w as detected [4]. The transmission of infection occurs through faecal-oral, gastric-oral, oral-oral route [5].

H. pylori infection transmission is faster in conditions of poor hygiene and sanitation. In majority cases it does not show any symptoms while in minor cases symptoms like abdominal pain, nausea, vomiting and dyspepsia are present.

One of the noticeable morphological features in *H. pylori* is that it has multiple flagella at one end, which helps in its motility and adherence. The colonisation occurs in epithelial cells with the help of adhesion molecule called BabA. The prime region of colonisation is the duodenum in association with gastric metaplasia. It causes gastritis by provoking the local inflammation in the underlying epithelial cells [3]. The complication caused by *H. pylori* ranges from mild pan gastritis to gastric atrophy and hypochlorhydria predisposing to the development of gastric carcinoma. In certain cases, it also causes hypergastrinemia, the sequence of development of carcinoma begins with gastritis, atrophy, intestinal metaplasia, dysplasia and ends in carcinoma [5].

MATERIALS AND METHODS:

Active Components from PMKC: Docking calculations were conducted for the active components Glabridin, Glycyrrhizin, Chuktabrin B, Gallic acid, Maslinic acid, Chebuloside, Betulonic acid and Ellagic acid retrieved from the ingredients of the PMKC against the target protein molecule. The respective siddha herbal source of the phytoconstituents is listed below.

TABLE 1: DOCKING ANALYSIS OF SELECTED PHYTOCOMPONENTS

Botanical name	Tamil name	Family	Phyto component
Glycyrrhiza glabra	*Adhimadhuram*	Fabaceae	Glabridin [6] Glycyrrhizin [6]
Chukrasia tabularis	*Aayilpattai*	Meliaceae	Chuktabrin B [7,8]
Terminalia chebula	*Kadukkai*	Combretaceae	Gallic acid [9] Masilinic acid [9]
Terminalia bellirica	*Thandrikai*	Combretaceae	Chebuloside [10]
Phyllanthus embelica	*Nellikkai*	Euphorbiaceae	Betulonic acid [11] Ellagic acid [12]

2D and 3D Structure of Phytocomponents:

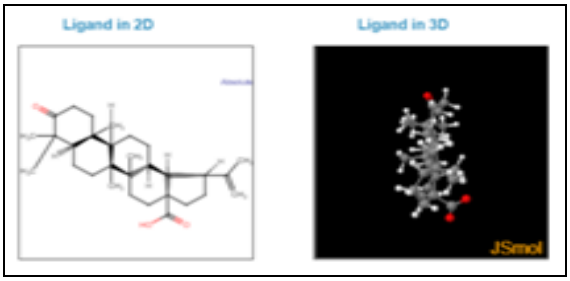


FIG. 1: BETULONIC ACID

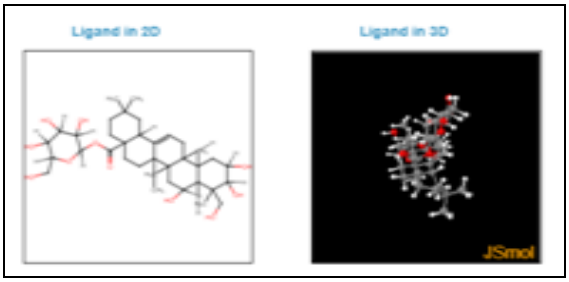


FIG. 2: CHEBULOSIDE

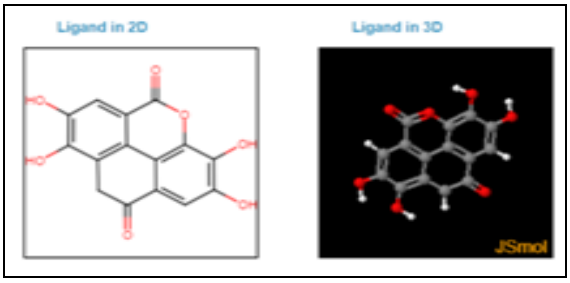


FIG. 3: ELLAGIUC ACID

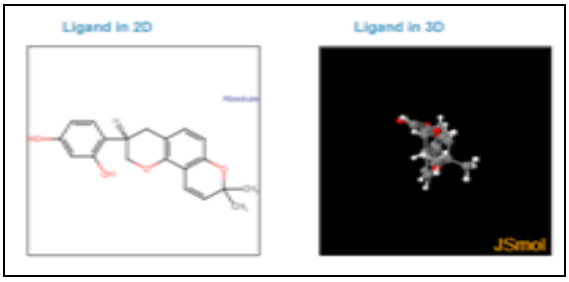


FIG 4: GLABRIDIN

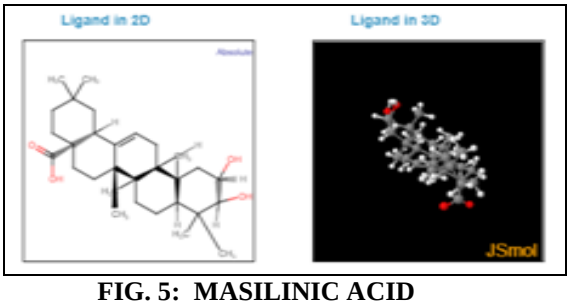


FIG. 5: MASILINIC ACID

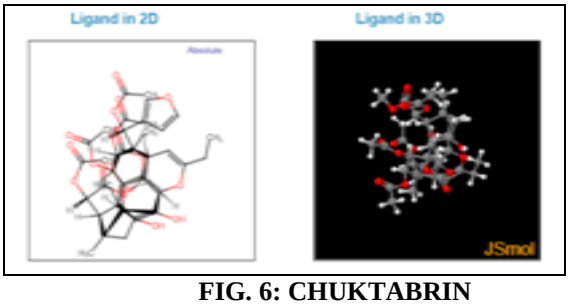


FIG. 6: CHUKTABRIN

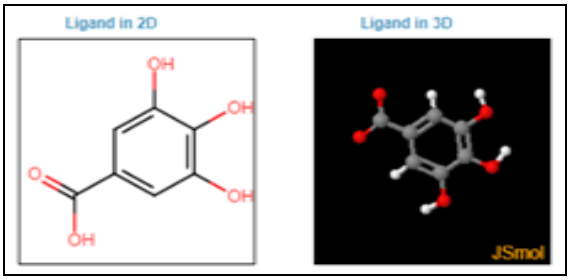


FIG. 7: GALLIC ACID

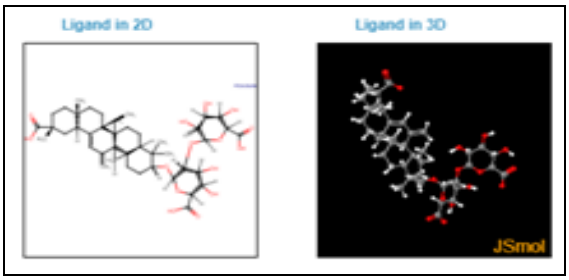
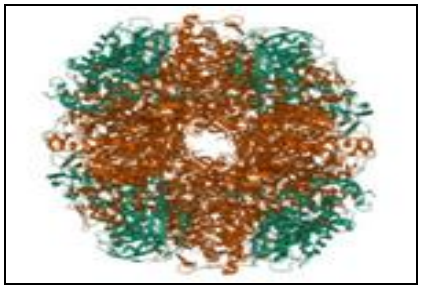


FIG. 8: GLYCYRRHIZIN

TABLE 2: PROPERTIES OF SELECTED LIGANDS FOR DOCKING ANALYSIS

Compound	Molecular weight g/mol	Chemical Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Betulonic acid	454.7 g/mol	$C_{30}H_46O_3$	1	3	2
Chebuloside	666.8 g/mol	$C_{36}H_{58}O_{11}$	8	11	5
Ellagic acid	302. 194 g/mol	$C_{14}H_6O_8$	4	8	0
Glabridin	324.4 g/mol	$C_{20}H_{20}O_4$	2	4	1
Maslinic acid	472.7 g/mol	$C_{30}H_{48}O_4$	3	4	1
Chuktabrin	790.8 g/mol	$C_{38}H_{46}O_{18}$	3	18	14
Gallic acid	170.12g/mol	$C_7H_6O_5$	4	5	1
Glycyrrhizin	822.9 g/mol	$C_{42}H_{62}O_{16}$	8	16	7

Target Protein: The crystalline structure of the target enzyme *H. pylori* Urease (PDB - 1E9Y) was obtained from the protein data bank. A thorough protein clean-up process was performed, and any missing hydrogen atoms were added as necessary. Using the Auto dock program, various orientations of the lead molecules in relation to the targeted protein were assessed. The best docking pose was selected based on an analysis of the interaction study [13].



RECEPTOR STRUCTURE

FIG. 9: 3D- STRUCTURE OF H PYLORI UREASE (PDB) - 1E9Y

Molecular Docking Methodology: Docking calculations were conducted for the retrieved phytocomponents against the target enzyme *H. pylori* urease. AutoDock tools were employed to add essential hydrogen atoms, Kollman united atom type charges, and solvation parameters. The Autogrid program generated affinity (grid) maps with specific grid points and spacing. The van der Waals and electrostatic terms were calculated using AutoDock parameter set- and distance-dependent dielectric functions. Docking simulations utilized the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. The ligand molecules initial position, orientation, and torsions were randomly set, with all rotatable torsions released during docking. Two runs of each docking experiment were performed, terminating after a maximum of 250,000 energy evaluations. A population size of 150 was employed, with a translational step of 0.2 Å and quaternion and torsion steps of 5 during the search process [14, 15, 16].

RESULT AND DISCUSSION: A total of eight bioactive lead compounds were extracted from the herbs used in the siddha formulation PMKC. The phytochemicals, including Betulonic acid, Chebuloside, Ellagic acid, Glabridin, Maslinic acid, Chuktabrin, Gallic acid, and Glycyrrhizin demonstrated a maximum of 2 interactions, accounting for 75% of the occupancy, with the core active amino acid residues on the target protein enzyme *H. pylori* urease.

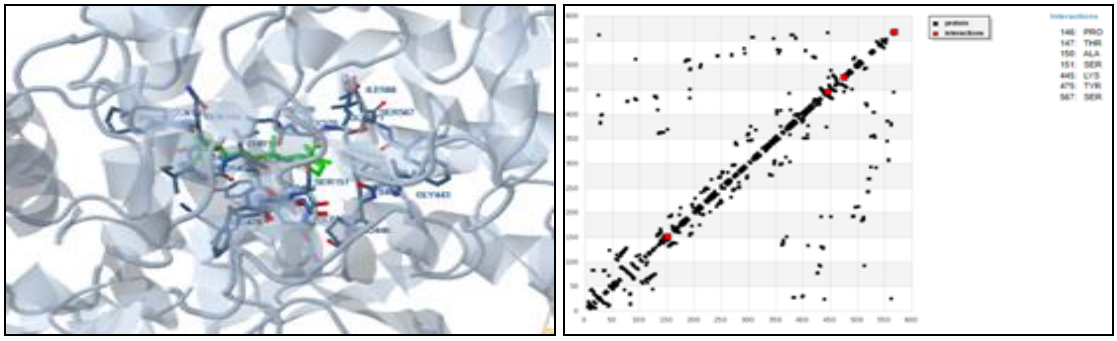


FIG. 10: INTERACTION ANALYSIS OF BETULONIC ACID WITH *H. PYLORI* UREASE WITH PDB 1E9Y

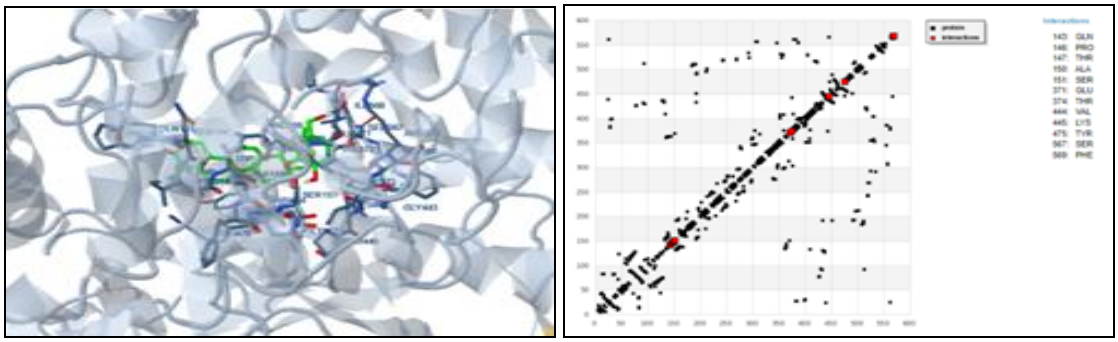


FIG. 11: INTERACTION ANALYSIS OF CHEBULOSIDE WITH *H. PYLORI* UREASE WITH PDB 1E9Y

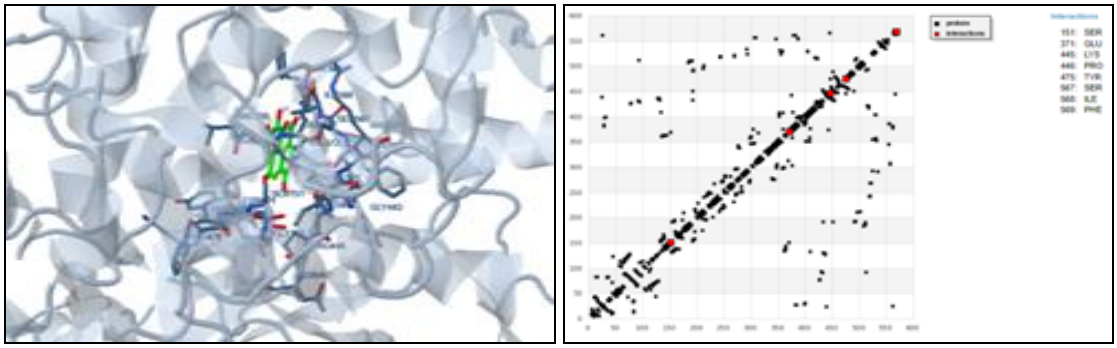


FIG. 12: INTERACTION ANALYSIS ELLAGIC ACID WITH *H. PYLORI* UREASE WITH PDB 1E9Y

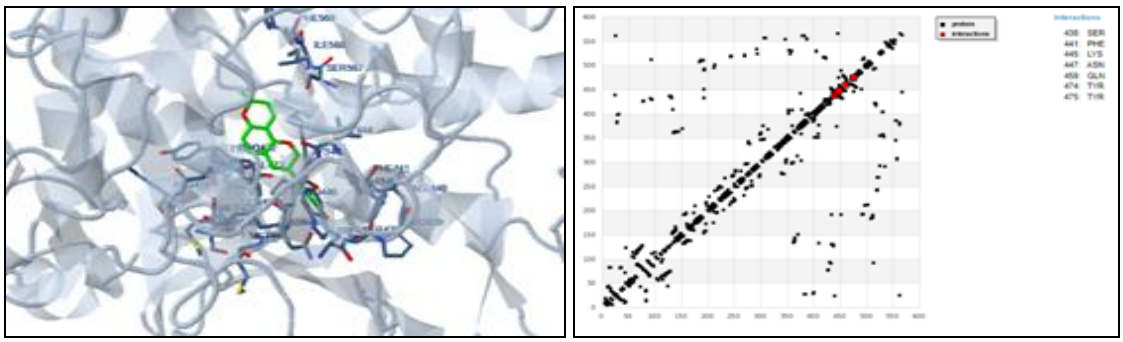


FIG. 13: INTERACTION ANALYSIS OF GLABRIDIN WITH *H. PYLORI* UREASE WITH PDB 1E9Y

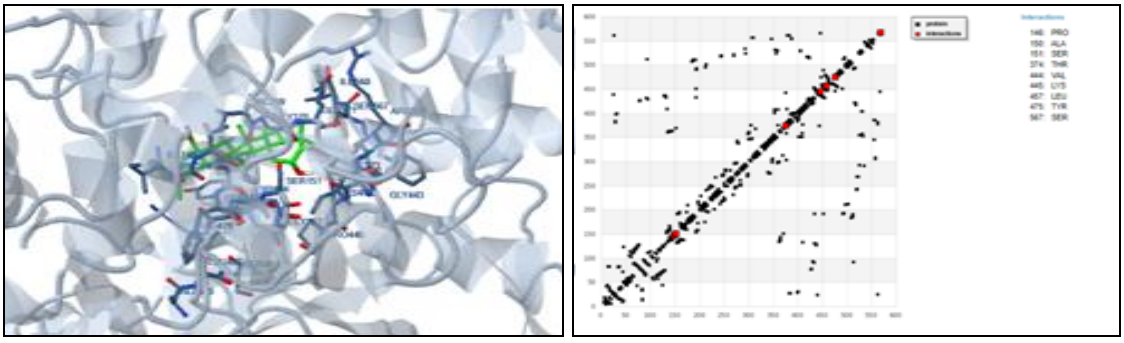


FIG. 14: INTERACTION ANALYSIS OF MASLINIC ACID WITH *H. PYLORI* UREASE WITH PDB 1E9Y

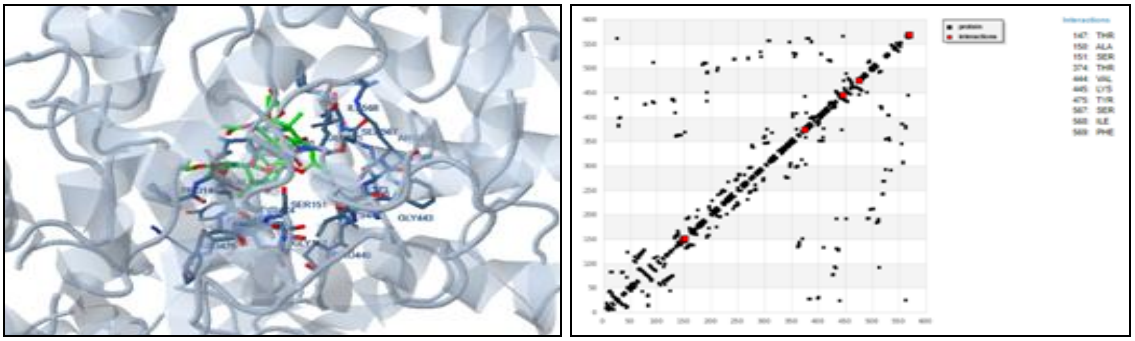


FIG. 15: INTERACTION ANALYSIS CHUKTABRIN WITH *H. PYLORI* UREASE WITH PDB 1E9Y

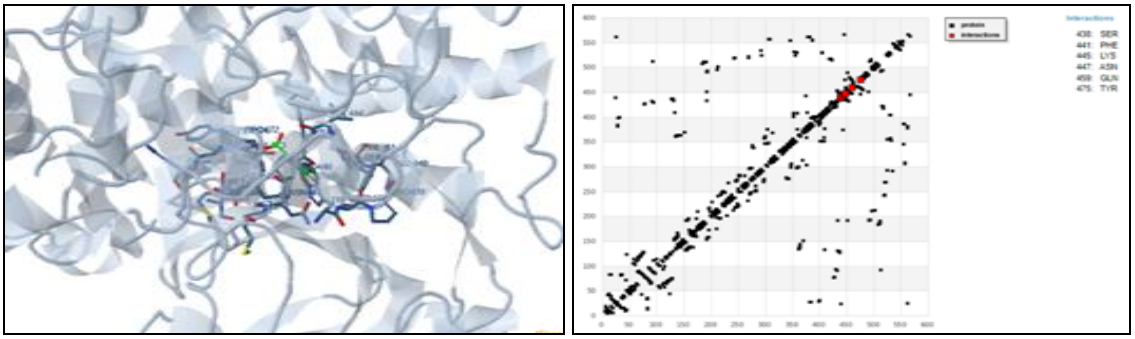


FIG. 16: INTERACTION ANALYSIS OF GALLIC ACID WITH *H. PYLORI* UREASE WITH PDB 1E9Y

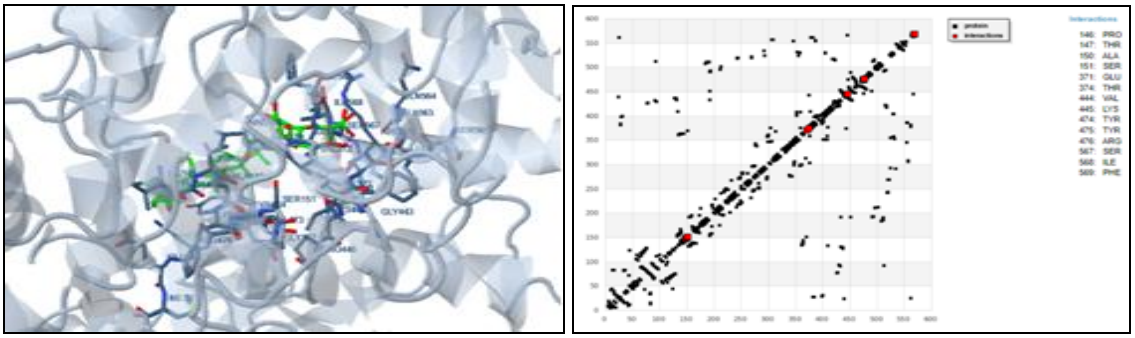


FIG. 17: INTERACTION ANALYSIS OF GLYCYRRHIZIN WITH *H. PYLORI* UREASE WITH PDB 1E9Y

TABLE 3: SUMMARY OF DOCKING STUDIES FOR COMPOUNDS AGAINST *H. PYLORI* UREASE (PDB: 1E9Y)

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Betulonic acid	-7.15 kcal/mol	5.72 uM	-0.42 kcal/mol	-7.71 kcal/mol	698.376
Chebuloside	-9.48 kcal/mol	112.27 nM	-0.30 kcal/mol	-9.45 kcal/mol	899.123
Ellagic acid	-5.94 kcal/mol	43.95 uM	-0.05 kcal/mol	-4.95 kcal/mol	535.935
Glabridin	-6.05 kcal/mol	36.47 uM	-0.27 kcal/mol	-6.95 kcal/mol	664.471
Maslinic acid	-7.65 kcal/mol	2.45 uM	-0.20 kcal/mol	-7.44 kcal/mol	795.79
Chuktabrin	-7.65 kcal/mol	2.45 uM	-0.20 kcal/mol	-7.44 kcal/mol	795.79
Gallic acid	-5.37 kcal/mol	116.30 uM	-0.33 kcal/mol	-4.90 kcal/mol	412.083
Glycyrrhizin	-8.54 kcal/mol	549.94 nM	-0.88 kcal/mol	-9.58 kcal/mol	1154.508

TABLE 4: AMINO ACID RESIDUE INTERACTIONS OF LEAD COMPOUNDS AGAINST H. PYLORI UREASE (PDB: 1E9Y)

Compounds	Interactions	Amino acid residues											
Betulonic acid	2	146 PRO	147 THR	150 ALA	151 SER	445 LYS	475 TYR	567 SER					
Chebuloside	2	143 GLN	146 PRO	147 THR	150 ALA	151 SER	371 GLU	374 THR	444 VAL	445 LYS	475 TYR	567 SER	569 PHE
Ellagic acid	2	151 SER	371 GLU	445 LYS	446 PRO	475 TYR	567 SER	568 ILE	569 PHE				

		R	U	S	O	R	R		E						
Glabridin	2	438 SER	441 PHE	445 LYS	447 ASN	459 GLN	474 TYR	475 TYR							
Maslinic acid	2	146 PRO	150 ALA	151 SER	374 THR	444 VAL	445 LYS	457 LEU	475 TYR	567 SER					
Chuktabrin	2	147 THR	150 ALA	151 SER	374 THR	444 VAL	445 LYS	475 TYR	567 SER	568 ILE	569 PHE				
Gallic acid	2	438 SER	441 PHE	445 LYS	447 ASN	459 GLN	475 TYR								
Glycyrrhizin	2	146 PRO	147 THR	150 ALA	151 SER	371 GLU	374 THR	444 VAL	445 LYS	474 TYR	475 TYR	476 ARG	567 SER	568 ILE	569 PHE

Molecular docking is one of the efficient methods for pharmacological research in the recent days. It gives a strong perception on the reaction between the drug and the pathogen at the atomic level. Since most of the siddha medicine formulation consist of multiple herbs it is difficult to analyse each and every phytoconstituent specifically with the pathogen however molecular docking aids in this obstacle. From the molecular docking of PMKC against *H. pylori* the phytoconstituent glycyrrhizin with the molecular weight of 822.9 g/mol exhibits highest inhibition of 549.94nM. The phytoconstituent gallic acid with the molecular weight of 170.12g/mol showed subsequent inhibition of 116.30 Um.

The phytoconstituents masilinic acid with molecular weight 472.7g/mol showed minimal inhibition of 2.45uM and the chuktabrin with the molecular weight 790.8 g/mol showed the same inhibition against *H. pylori* urease. There is considerable amount of prior research work done on the individual constituent of the siddha formulation PMKC but almost all the research work explains about the phytoconstituents extracted from the fruits and seeds such as, a study on the extract from the fruits of *Terminalia chebula* shows wound healing properties [17] and another study showed the growth inhibition and anti-bacterial activity of ellagic acid from the fruits of the same siddha herb [18]. Certain studies on the fruits of *Phyllanthus emblica* showed that it possesses anticancer activity [19] and provides cytoprotecting activity against gastric ulcer formation with the help of its anti-oxidant property [20]. Fruits of *Terminalia bellirica* shows anti-oxidant and anti-microbial activity [21]. On the other hand, there was a study which portrayed that the glycyrrhizin extracted from the roots of *Glycyrrhiza glabra* showed that, it is a potent antioxidant and inhibits urease enzyme [22] and barks of *Chukrasia tabularis* has also been found to have anti-inflammatory activity [23]. However not many research has been done on the root extracts of the above-mentioned siddha herbal drugs, this study scientifically proves that the root extracts of *Glycyrrhiza glabra*, *Chukrasia tabularis*, *Terminalia chebula*, *Terminalia bellirica*, *Phyllanthus emblica* possess anti-ulcer activity.

CONCLUSION: The computational analysis conducted in this study provides robust evidence of the therapeutic potential of bioactive compounds from the Siddha formulation PMKC against *H. pylori* urease, a key enzyme involved in ulcer development. The results demonstrate that compounds such as Betulonic acid, Chebuloside, Ellagic acid, Glabridin, Maslinic acid, Chuktabrin, Gallic acid, and Glycyrrhizin exhibit strong binding affinity to *H. pylori* urease by interacting with specific amino acids on its active site. This interaction suggests their potential as inhibitors of the enzyme, which plays a crucial role in the hydrolysis of urea to ammonia. Considering the significant role of this enzyme in ulcer pathogenesis, these results underscore the promising therapeutic properties of these phytochemicals in ulcer management. Furthermore, the study reinforces the anti-ulcer activity of the phytochemicals present in PMKC, emphasizing the necessity for further investigation to fully uncover their therapeutic potential in the treatment of ulcers.

ACKNOWLEDEGEMENTS: The author would like to thank the Head of Department, Department of Gunapadam, The Director, National Institute of Siddha for the support provided.

CONFLICT OF INTEREST: The authors declared no conflict of interest.

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How to cite this article:
Yasiga S and Visweswaran S: Potential anti-ulcer activity of Siddha poly herbal formulation *Pancha moola Kudineer chooranam*: insights from molecular docking. Int J Pharm Sci & Res 2024; 15(3): 979-85. doi: 10.13040/IJPSR.0975-8232.15(3).979-85.