IN-VITRO ANTI-ULCER ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF GUNMATHUKKU KUDINEER –A SIDDHA POLYHERBAL FORMULATION

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Abstract-
Background: **Siddha system of medicine is the indigenous system of medicine practiced in south India especially in Tamilnadu. Gunmam (Peptic Ulcer) is one of the disease out of 4448 diseases mentioned in Siddha literatures.Yugi Muni has described about Gunmam in "Yugi Vaithiya Chinthamani'.Gunmam can be compared with Peptic Ulcer. Gunmathukku Kudineer is a polyherbal drug indicated for Gunmam which is mentioned in the Siddha Literature "Thanwanthiri Vaithiyakkummi-300.**
Aim: The aim of the study is to analyze the in-vitro anti ulcer potential and biochemical analysis of Gunmathukku Kudineer.
Methodology: The present study was undertaken to evaluate anti-ulcer activity of Gunmathukku Kudineer by binding of phytocomponents with the core amino acids (LYS-445 and VAL-473, TYR-475) of the target by forming hydrogen bond will hinder the function of the enzyme H pylori Urease with PDB – 1E9Y. The enzyme urease catalyses the hydrolysis of urea to ammonia. Urease has been reported to be a prominent virulence determinant of H pylori in the pathogenesis of ulcer. Thereby phytocomponents which inhibit the target viral enzyme H pylori urease may act as a potential therapeutic agent for management of ulcers caused by H Pylori.
Result: The study result concludes that Gunmathukku Kudineer has significant Anti-ulcer activity.

Keywords: **Gunmam, Peptic Ulcer, Gunmathukku kudineer, Anti-ulcer activity.**

INTRODUCTION:

Peptic ulcer is a break in the gastric or duodenal mucosa that penetrates through the muscularis mucosae. The most common causes of peptic ulcers are Helicobacter pylori (H. pylori) infection and nonsteroidal anti-inflammatory drugs (NSAIDs).The gram-negative bacteria H pylori was first linked to gastritis in 1983. Since then, further study of H pylori has revealed that it is a major part of the triad, which includes acid and pepsin, that contributes to primary peptic ulcer disease. The unique microbiologic characteristics of this organism, such as urease production, allows it to alkalinize its microenvironment and survive for years in the hostile acidic environment of the stomach, where it causes mucosal inflammation and, in some individuals, worsens the severity of peptic ulcer disease.When H pylori colonizes the gastric mucosa, inflammation usually results.Hence this study is carried out to study the Anti-ulcer activity of Gunmathukku Kudineer by in-vitro assays and biochemical analysis.

MATERIALS AND METHODS:

DRUG SELECTION:

The Siddha formulation Gunmathukku kudineer is mentioned in Siddha literature Thanwanthiri Vaithiyakkummi-300.

INGREDIENTS OF GUNMATHUKKU KUDINEER:

1.Pirandai (Cissus quadrangularis)
2.Omam (Trachyspermum ammi)
3.Moosambaram (Aloe vera)
4.Veppeerkku (Azadirachta indica)

AUTHENTICATION OF RAW MATERIALS:

The raw drug was identified and authenticated by Medicinal Botanist and faculties of Department of Gunapadam at Govt.Siddha Medical College,Palayamkottai,Tamilnadu.

PROCESS OF DRUG PREPARATION: The ingredients of the trial drug were purified according to the proper methods described in Siddha Classical literature. All the purified drugs were powdered separately and mixed togethrr and stored in a tight container.

ANTI-ULCER ACTIVITY EVALUATION OF GUNMATHUKKU KUDINEER:

Docking calculations were carried out for retrieved phytocomponents against target enzyme H pylori urease. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris, Goodsell

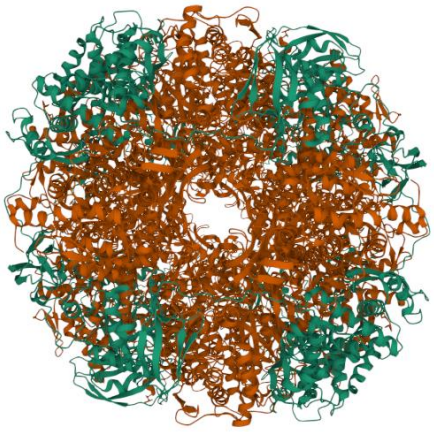
et al., 1998). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (Morris, Goodsell et al., 1998). Auto Dock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. 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.

PDB	Name of the Target
1E9Y	H pylori Urease

Table 1 List of Phytocomponents Selected for docking

Cissus quandrangularis	• β-amyrin • β-sitosterol • Resveratrol
Azadirachta indica	• Nimbolide • Nimbiol • Salannin
Trachyspermum ammi	• Carvone • β-pinene
Aloe vera	• Aloe-emodin • Aloin

Figure 1: 3D- Structure of H pylori Urease (PDB) - 1E9Y



RECEPTOR STRUCTURE

Crystalline structure of the target enzyme H pylori Urease (PDB) - 1E9Y was retrieved from protein data bank and protein clean-up process was done and essential missing hydrogen atom were being added. Different orientation of the lead molecules with respect to the target protein was evaluated by Autodock program and the best dock pose was selected based on the interaction study analysis.

BIOCHEMICAL ANALYSIS OF GUNMATHUKKU KUDINEER:

Preparation of the extract: 5gms of the drug was weighed accurately and placed in a 250ml clean beaker then 50ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100ml volumetric flask and then it is made to 100ml with distilled water. This fluid is taken for analysis.

RESULTS

Table 2- Summary of the molecular docking studies of compounds against H pylori Urease (PDB) - 1E9Y

Compound	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
β-amyrin	-8.11 kcal/mol	1.13 uM	-0.06 kcal/mol	-8.41 kcal/mol	760.483
β-sitosterol	-8.36 kcal/mol	745.54 nM	-0.04 kcal/mol	-9.77 kcal/mol	801.556
Resveratrol	-4.65 kcal/mol	388.58 uM	-0.19 kcal/mol	-6.15 kcal/mol	578.509
Nimbolide	-6.10 kcal/mol	33.65 uM	-0.16 kcal/mol	-6.93 kcal/mol	742.274

Nimbiol	-5.99 kcal/mol	40.71 uM	-0.18 kcal/mol	-6.29 kcal/mol	509.099
Salannin	-6.18 kcal/mol	29.58 uM	-0.29 kcal/mol	-7.91 kcal/mol	857.241
Carvone	-5.12 kcal/mol	176.84 uM	-0.01 kcal/mol	-5.42 kcal/mol	439.33
β-pinene	-5.11 kcal/mol	179.62 uM	-0.14 kcal/mol	-5.11 kcal/mol	423.171
Aloe-emodin	-4.37 kcal/mol	625.54 uM	-0.07 kcal/mol	-5.57 kcal/mol	478.771
Aloin	-7.97 kcal/mol	1.44 uM	-0.18 kcal/mol	-6.61 kcal/mol	685.302

Table 3:Amino acid Residue Interaction of Lead against H pylori Urease (PDB) - 1E9Y

Compounds	**Interactions**	**Amino Acid residual Interactions**										
β-amyrin	2	146 PRO	147 THR	150 ALA	151 SER	444 VAL	445 LYS	475 TYR	477 GLU	481 HIS		
β-sitosterol	2	146 PRO	147 THR	150 ALA	151 SER	374 THR	444 VAL	445 LYS	457 LYS	475 TYR	477 GLU	
Resveratrol	2	441 PHE	445 LYS	447 ASN	459 GLN	474 TYR	475 TYR					
Nimbolide	2	147 THR	150 ALA	151 SER	371 SER	374 THR	444 VAL	445 LYS	475 TYR			
Nimbiol	2	147 THR	150 ALA	151 SER	371 SER	374 THR	444 VAL	445 LYS	475 TYR	567 SER		
Salannin	2	147 THR	150 ALA	151 SER	374 THR	444 VAL	445 LYS	474 TYR	475 TYR	567 SER	568 ILE	569 PHE
Carvone	2	150 ALA	151 SER	371 GLU	374 THR	444 VAL	445 LYS	446 LYS	475 TYR	567 SER		
β-pinene	3	441 PHE	445 LYS	447ASN	459 GLN	473 VAL	475 TYR	474 TYR	475 TYR			
Aloin	2	147 THR	150 ALA	151 SER	445 LYS	474 TYR	475 TYR	567 SER				
Aloe-emodin	1	146 PRO	149 PHE	150 ALA	457 LEU	474 TYR	475 TYR	477 GLU	481 HIS			

S.NO	EXPREMENTS	OBSERVATION	INFERANCE
1	**Test for calcium:** 2ml of the above prepared extract taken in a clean test tube.to this add 2ml of 4% ammonium oxalate solution.	A white precipitate is formed	**Indicates the presence of calcium**
2	**Test for sulphate:** 2ml of the extract is added to 5%barrium chloride solution.	A white precipitate is formed	**Indicates the presence of sulphate**
3	**Test for chloride:** The extract is treated with silver nitrate solution.	A white precipitate is formed	**Indicates the presence of chloride**
4	**Test for carbonate:** The substance is treated with concentrated HCL.	No brisk effervescence is formed	Indicates the absence of carbonate
5	**Test for starch:** The extract is added with weak iodine solution.	Blue colour is formed	**Indicates the presence of starch**
6	**Test for ferric iron:** The extract is acidified with glacial acetic acid and potassium Ferro cyanide.	Blue colour is not formed	Indicates the absence of ferric iron
7	**Test for ferrous iron:** The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blood red colour is formed	**Indicates the presence of ferrous iron**
8	**Test for phosphate:** The extract is treated with ammonium molybdate and concentrated nitric acid**.**	Yellow precipitate is not formed	Indicates the absence of phosphate
9	**Test for albumin:** The extract is treated with esbach's reagent**.**	Yellow precipitate is not formed	Indicates the absence of albumin
10	**Test for tannic acid:** The extract is treated with ferric chloride.	Blue black precipitate is not formed	Indicates the absence of tannic acid
11	**Test for unsaturation:** Potassium permanganate solution is added to the extract.	It gets decolorised	**Indicates the presence of unsaturated compounds**
12	**Test for the reducing sugar:**5ml of benedict'qualidative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minute	Colour change occurs	**Indicates the presence of reducing sugar**
13	**Test for amino acid:**One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	**Indicates the presence of amino acid**
14	**Test for zinc:** The extract is treated with potassium Ferro cyanide.	White precipitate is not formed	Indicates the absence of zinc

Table 4 : Biochemical Analysis

DISCUSSION:

Total of 10 bioactive lead compounds were retrieved from the herbal ingredients. From the reported data of the herb, the phytochemical β-pinene reveals maximum of 3 interactions (100%) with the core active amino acid residues present on the target protein enzyme H pylori urease. Followed by this other compounds such as β-amyrin, β-sitosterol, Resveratrol, Nimbolide, Nimbiol, Salannin, Carvone and Aloin reveals maximum of 2 interactions (75%) with the core active amino acid residues present on the target protein enzyme H pylori urease.

Biochemical analysis reveal the presence of Calcium, Sulphate, Chloride, Starch, Ferrous iron, Unsaturated compounds, Reducing sugar And Amino acids.

CONCLUSION:

Based on the results of the computational analysis it was concluded that the bio-active compound's like β-pinene, β-amyrin, β-sitosterol, Resveratrol, Nimbolide, Nimbiol, Salannin, Carvone and Aloin present in the herb possess significant binding against the target H pylori urease by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exerts promising anti-ulcer activity by inhibiting the enzyme H pylori urease that catalyze the hydrolysis of urea to ammonia. Urease has been reported to be a prominent virulence determinant of H pylori in the pathogenesis of ulcer. Thereby phytocomponents which inhibit the target H pylori urease may act as a potential therapeutic agent for management of ulcer. It was concluded that the phytochemicals present in the herb reveals significant anti-ulcer activity.From this it can conclude that GUNMATHUKKU KUDINEER possess promising anti ulcer activity. This result can be further analyzed in-vivo and more promising results may be expected.

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