IN-SILICO ANTI-INFLAMMATORY ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF MOOLATHUKKU CHOORANAM –A SIDDHA MONOHERBAL FORMULATION

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Abstract-
Background- Siddha system of medicine is the indigenous system of medicine practiced in south India especially in Tamilnadu. Moolam (Hemorrhoids) is one of the disease out of 4448 diseases mentioned in Siddha literatures.Yugi Muni has described about Moolam in "Yugi Vaithiya Chinthamani'. Moolathukku Chooranam is a monoherbal drug indicated for Moolam which is mentioned in Gunapadam Mooligai Vakuppu.
Aim: The aim of the study is to analyze the in-silico anti inflammatory potential and biochemical analysis of Moolathukku chooranam.
Methodology: Binding of phytocomponents with the core amino acids (His70,Asp71, Ser72, Val91,Pro117, Ser119, Thr120, Pro121, Ser122, Thr124, Thr125) of the target by forming hydrogen bond will hinder the function of the inflammatory cytokine IL6 (Interleukin 6) with PDB – 1N26. These amino acid residues are functionally responsible for binding of substrate and inhibitors. Thereby phytocomponents which inhibit the target IL6 (Interleukin 6) may act as a potential therapeutic agent for management of inflammation
Result: The study result concludes that Moolathukku chooranam has significant Anti- inflammatory activity.

Keywords: **Moolam, Hemarrhoids, Moolathukku chooranam, Anti-inflammatory activity.**

INTRODUCTION:
Haemorrhoids are a very common anorectal disorder defined as symptomatic enlargement and distal displacement of the normal anal cushions. They affect millions of people worldwide and are a major medical and socioeconomic problem. Several factors have been suggested to cause haemorrhoids, including constipation and prolonged exertion. Abnormal dilation and deformation of the vascular canal and destructive changes in the supporting tissue of the anal pad are the main findings of hemorrhoidal disease. Inflammatory reactions and vascular hyperplasia can occur in haemorrhoids.Hence this study is carried out to study the Anti-inflammatory activity of Moolathukku chooranam by in-silico assays and biochemical analysis.

MATERIALS AND METHODS:
DRUG SELECTION:
The Siddha formulation Moolathukku chooranam is mentioned in Siddha literature Gunapadam mooligai vakuppu

INGREDIENTS OF MOOLATHUKKU CHOORANAM:
1.Marul (*Sansevieria roxburghiana*)

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- INFLAMMATORY ACTIVITY EVALUATION OF MOOLATHUKKU CHOORANAM:
Docking calculations were carried out for retrieved phytocomponents against target protein. 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*). 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 (*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.

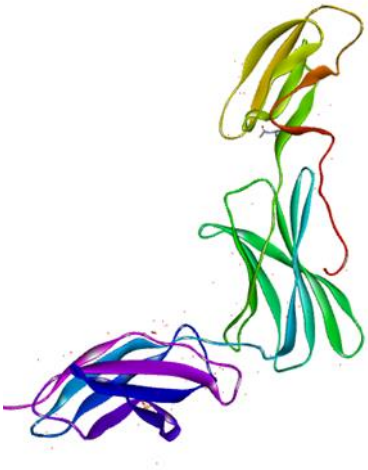
Table 1

Herbs	Phytochemicals
Sansevieria roxburghiana	• Gallic acid • Palmitic acid, • Neoruscogenin • Beta-Sitosterol

PDB	Name of the Target
1N26	IL6 (Interleukin 6)

Table 2 List of Phytocomponents Selected for docking

Figure 1 IL6 (Interleukin 6) (1N26)



RECEPTOR STRUCTURE

Crystalline structure of the target protein IL6 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 MOOLATHUKKU CHOORANAM:

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 3Ligand Properties of the Compounds Selected for Docking Analysis

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Gallic acid	170.12g/mol	$C_7H_6O_5$	4	5	1
Palmitic acid	256.42 g/mol	$C_{16}H_{32}O_2$	1	2	14
Neoruscogenin	428.6 g/mol	$C_{27}H_{40}O_4$	2	4	0
Beta-Sitosterol	414.7g/mol	$C_{29}H_{50}O$	1	1	6

Table 4Summary of the molecular docking studies of compounds against IL6 (Interleukin 6) (1N26)

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Gallic acid	-5.88 kcal/mol	49.24 uM	-0.51 kcal/mol	-5.40 kcal/mol	394.838
Palmitic acid	-4.72 kcal/mol	347.75 uM	-0.83 kcal/mol	-8.64 kcal/mol	703.649
Neoruscogenin	-6.78 kcal/mol	10.68 uM	-0.06 kcal/mol	-7.38 kcal/mol	664.963
Beta-Sitosterol	-7.95 kcal/mol	1.48 uM	-0.05 kcal/mol	-9.32 kcal/mol	824.237

Table 5Amino acid Residue Interaction of Lead against IL6 (Interleukin 6) PDB- (1N26)

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

Compound	Interactions	Amino Acid Residues										
Gallic acid	3	46 PRO	69 LEU	72 SER	90 LEU	92 ASP	122 SER	123 LEU	124 THR			
Palmitic acid	5	69 LEU	93 VAL	95 PRO	96 GLU	115 TRP	117 PRO	119 SER	121 PRO	122 SER	125 THR	155 PHE
Neoruscogenin	4	69 LEU	72 SER	90 LEU	119 SER	120 THR	122 SER	123 LEU				
Beta-Sitosterol	4	46 PRO	69 LEU	72 SER	90 LEU	93 VAL	119 SER	122 SER	123 LEU	124 THR		

Table 6 : Biochemical Analysis

DISCUSSION:

Total of 4 bioactive lead compounds were retrieved from the herbs present in the siddha formulation. From reported data of the herb, the phytochemicals such as Palmitic acid, Neoruscogenin and Beta-Sitosterol possess maximum of three to four to five interactions with the core active amino acid residues present on the target IL6 (Interleukin 6).
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 all the bio-active compound's like Palmitic acid, Neoruscogenin and Beta-Sitosterol revels significant binding affinity against the target cytokine IL6 by interacting with active amino acid present on the active site thereby it was concluded that these compounds may exerts promising anti-inflammatory activity.

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