Research Article

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ANTI CANCER EFFECT OF THE SIDDHA POLYHERBAL FORMULATION ASWATHY CHOORANAM AGAINST BRCA 1 GENE USING *IN SILICO* MODEL

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ABSTRACT

India is one among the countries with enormous antiquity of health care practices. Siddha system is the pride of South India with alchemy and enormous spiritual insight in its root. Traditional medicines support primary health care needs of about 70% of Indian population. Cancer is the leading non communicable diseases with high mortality rates in which the abnormal cells divide uncontrollably with a potential to invade other parts of the body. Aswathy chooranam is a polyherbal drug with a potency to cure a wide range of diseases. The constituents are of hot potency and hence can be an effective choice to mitigate Kapha humour which is the main cause for formation of vippuruthi (tumour). The present study is performed *in silico* to evaluate the anti-cancerous potential of Siddha herbal formulation Aswathy chooranam through inhibition of BRCA 1 genes. Withaferin A, Glycyrrhizin, Gingerenone- A, Piperic acid, Piperine, Grandisin, Myristicin, Picroside I, β-caryophyllene and Vitexin in the formulation are found to have remarkable action against BRCA 1 genes. Compounds such as Withaferin A and Glycyrrhizin are well known for inducing apoptosis of cancer *in vitro* and *in vivo* and also in management of post chemotherapy management. Myristicin has potent inhibitory activities on cytokines, chemokines and nitric oxide. Piperine and piperic acid have been reported to have effective chemo preventive activities. The computational analysis shows effective binding against target Breast Cancer gene BRCA1. Hence Aswathy chooranam can be a promising choice for management and treatment of Breast Cancer.

Keywords: Breast Cancer, Siddha drug, Aswathy chooranam, Polyherbal drug, *In silico*

INTRODUCTION

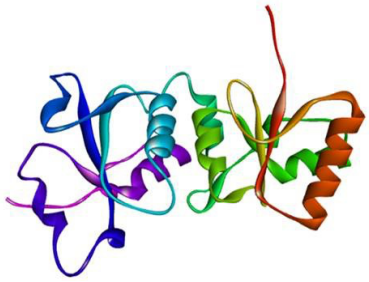
Breast Cancer is of a rising concern globally with the global estimate revealing 2.3 million women diagnosed in 2022. It is the second most common cancer in women after skin cancer. Female genders are prone to higher risk for Breast Cancer. The risk factors have been recognized as age, obesity, alcoholic addiction, strong genetic predisposition, history of radiation, reproductive history, post-menopausal theory. High penetrance gene mutations such as mutations of BRCA1, BRCA2, PALB2. With the advancement of science and technology, chemo preventive strategies and surgical procedures have become available globally, yet traditional medicines are an excellent option for localized cancer. Herbal interventions are the need of the hour owing to their inhibitory and preventive potential. They are cost effective and safe mode of treatment especially in the elderly and are a perfect option for prophylaxis. Aswathy chooranam is a polyherbal Siddha drug obtained from "Agasthya vaithiya kaviyam 1500"[1]. The ingredients are Amukkara, Chukku, Milagu, Thippili, Athimathuram, Omam, Kurosani omam, Kirambu, Saathikai, Saathipathiri, Katukurohani and white sugar. It is indicated for various conditions PCOD, menorrhagia, anaemia, infertility, arthritis, scabies, cancer (Vippuruthi) cough and various toxins like scorpion bite. The docking study throws light upon the anti-cancer potential of the drug *in silico* through BRCA 1 gene inhibition and promises to be a potential therapeutic agent for the management of Breast Cancer.

The Breast Cancer gene (BRCA) interacts with several proteins involved in cell cycle progression, cellular pathways, ubiquitination, gene transcription control, and DNA damage response. BRCA1 is a multifunctional protein involved in the DNA Damage Response (DDR), playing roles in both DNA activation and checkpoint repair. The majority, around 80-90%, of instances of Breast Cancer that are passed down through generations are caused by genetic abnormalities in the BRCA1 and BRCA2 genes[2]. Molecular docking simulation is an effective method employed to test possible active drugs against specific target proteins, such as BRCA1 and BRCA2. Thus, these factors are crucial focal points for the development of putative anti-cancer properties. Phytochemicals can serve as inhibitors of the Breast Cancer susceptibility gene 1, offering an alternative therapy for Breast Cancer in addition to conventional pharmaceuticals. Binding of phytocomponents with the core amino acids (1654 – 1659, 1662,1677- 1679, 1689,1690,1698, 1700-1702) of the target by forming hydrogen bond will hinder the function of Breast Cancer gene 1 (BRCA1) with PDB – 4Y2G. These amino acid residues are functionally responsible for binding inhibitors. These phytocomponents through the inhibition of the target BRCA1 possess potential therapeutic

potency in the management of Breast CANCER condition[3].

Crystalline structure of the target protein Breast Cancer gene 1 (BRCA1) 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.
Breast Cancer gene 1 (BRCA1) (4Y2G).

Receptor Structure



MATERIALS AND METHODS

Preparation of Aswathy chooranam

Table 1: Ingredients

Traditional name	Botanical name	Quantity
Chukku	*Zingiber officinale* Rosc.	15 gm
Milagu	*Piper nigrum* Linn.	15 gm
Thippili	*Piper longum* Linn	15 gm
Saathipathiri	*Myristica fragrans* Houtt.	15 gm
Saathikaai	*Myristica fragrans* Houtt	15 gm
Omam	*Carum copticum* Benth&Hook	15 gm
Kurosani omam	*Hyoscyamus niger* Linn	15 gm
Katukurohani	*Picrorhiza kurroa* Royle ex Benth	15 gm
Athimathuram	*Glycyrrhiza glabra* Linn	15 gm
Kirambu	*Syzygium aromaticum* Linn.	15 gm
Amukkara	*Withania somnifera* Linn	300 gm
Sarkarai	*Saccharum officinarum* Linn	150 gm

The raw drugs were purified separately through de weeding, drying, removing the outer skin, and washing in clean water. They were further dried in the sunlight until there was no moisture content. Then they were fried in a pan individually until they were dry. Further the medicinal drugs were powdered in required amounts and mixed along with powdered fine sugar.

Protein preparation

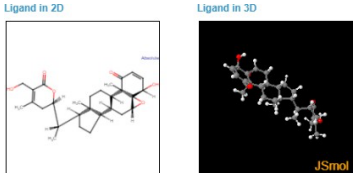
Three-dimensional protein structure of the target protein Breast Cancer gene 1 (BRCA1) with PDB – 4Y2G were retrieved from the online repository of Protein Data Bank and subjected to protein clean prior to docking simulation.

Ligand Preparation

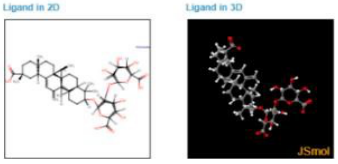
Phytochemical subjected to the investigation were retrieved from the herbs listed in the table based on the literature survey and 3D structure of the same were built using Chem Draw prof online tool version 12.0. Ligands prepared through geometry optimization method (MMFF94).[4]

Table 2: 2D and 3D Structure of Phytocomponents

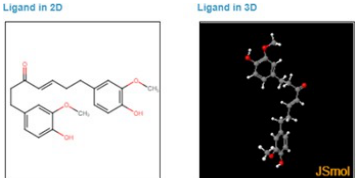
Withaferin A



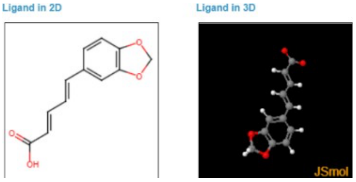
Glycyrrhizin



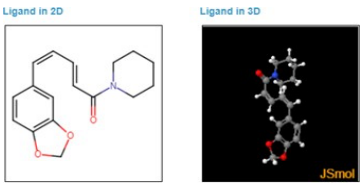
Gingerenone-A



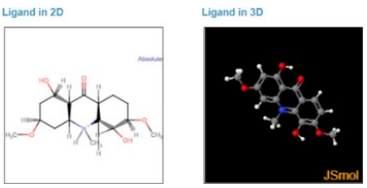
Piperic acid



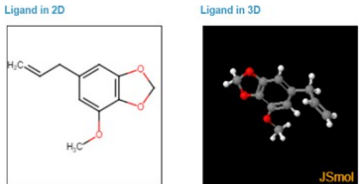
Piperine



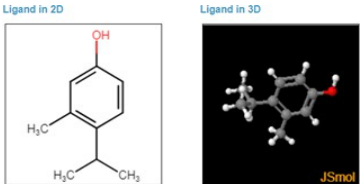
Grandisin



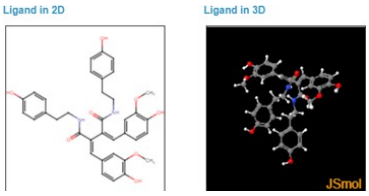
Myristicin



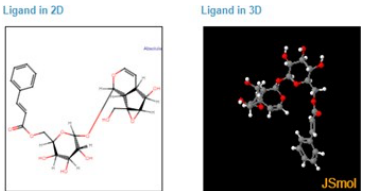
Thymol



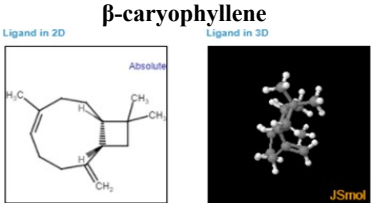
Hyoscyamide



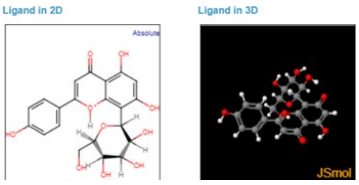
Picroside I



β-caryophyllene



Vitexin



Methodology for Docking

The retrieved phytocomponents were used to conduct docking calculations against the target protein. AutoDock tools were employed to add essential hydrogen atoms, Kollman united atom type charges, and solvation parameters. Utilizing the Autogrid program (Morris, Goodsell *et al.,* 1998), affinity (grid) maps with ×× Å grid points and 0.375 Å spacing were generated.[5] The calculation of the van der Waals and the electrostatic terms utilized AutoDock parameter set- and distance-dependent dielectric functions.[6] Docking simulations utilized the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method. [7] The initial position, orientation, and torsions of the ligand molecules were randomly set. All rotatable torsions were available during docking. Each docking experiment originated from 2 separate runs, scheduled to stop after a maximum of 250000 energy evaluations. The population size was 150. Throughout the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were employed. The study was carried out at Noble research solutions, Perambur, Chennai, India.

OBSERVATIONS AND RESULTS

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
Withaferin A	470.6 g/mol	C28H38O6	2	6	3
Glycyrrhizin	822.9 g/mol	C42H62O16	8	16	7
Gingerenone-A	356.4 g/mol	C21H24O5	2	5	9
Piperic acid	218.2 g/mol	C12H10O4	1	4	3
Piperine	285.34 g/mol	C17H19NO3	0	3	3
Grandisin	276.37 g/mol	C16H24N2O2	1	4	0
Myristicin	192.21 g/mol	C11H12O3	0	3	3
Thymol	150.221 g/mol	C10H14O	1	1	1
Hyoscyamide	624.7 g/mol	C36H36N2O8	6	8	13
Picroside I	492.5 g/mol	C24H28O11	5	11	8
β-caryophyllene	204.35 g/mol	C15H24	0	0	0
Vitexin	432.4 g/mol	C21H20O10	7	10	3

Table 4: Summary of the molecular docking studies of compounds against Breast Cancer gene 1 (BRCA1) with PDB – 4Y2G

Compounds	Est. Free Energy of Binding	Est. Inhibition Constant, Ki	Electrostatic Energy	Total Intermolec. Energy	Interact. Surface
Withaferin A	-7.52 kcal/mol	3.08 uM	-0.01 kcal/mol	-7.98 kcal/mol	676.603
Glycyrrhizin	-9.64 kcal/mol	85.15 nM	-0.38 kcal/mol	-9.42 kcal/mol	1012.658
Gingerenone-A	-6.08 kcal/mol	34.70 uM	-0.15 kcal/mol	-5.07 kcal/mol	548.065
Piperic acid	-6.06 kcal/mol	35.90 uM	-0.01 kcal/mol	-5.88 kcal/mol	464.96
Piperine	-6.23 kcal/mol	27.25 uM	-0.03 kcal/mol	-6.14 kcal/mol	482.267
Grandisin	-4.50 kcal/mol	500.37 uM	-0.04 kcal/mol	-6.88 kcal/mol	686.36
Myristicin	-3.66 kcal/mol	2.07 mM	-0.06 kcal/mol	-4.48 kcal/mol	434.098
Thymol	-3.69 kcal/mol	1.96 mM	-0.09 kcal/mol	-4.31 kcal/mol	393.37
Hyoscyamide	-7.34 kcal/mol	4.20 uM	-0.03 kcal/mol	-6.86 kcal/mol	759.277
Picroside I	-3.44 kcal/mol	3.02 mM	-0.20 kcal/mol	-4.28 kcal/mol	438.046
β-caryophyllene	-5.54 kcal/mol	86.36 uM	-0.01 kcal/mol	-5.54 kcal/mol	446.821
Vitexin	-7.17 kcal/mol	5.55 uM	-1.11 kcal/mol	-6.88 kcal/mol	556.257

Table 5: Amino acid Residue Interaction of Lead against Breast Cancer gene 1 (BRCA1) with PDB – 4Y2G

Compounds	Interactions	Amino acid Residues Interaction								
Withaferin A	8	1654	1655	1657	1659	1662	1700	1701	1702	
		VAL	SER	LEU	PR O	PH E	TH R	LE U	LY S	
Glycyrrhizin	6	1654	1655	1662	1679	1680	1700	1701	1774	1779
		VAL	SER	PHE	LE U	ILE	TH R	LE U	AS N	GL N
Gingerenone-A	5	1654	1657	1662	1678	1702				
		VAL	LEU	PHE	AS N	LY S				
Piperic acid	5	1654	1659	1662	1678	1702				
		VAL	PRO	PHE	AS N	LY S				
Piperine	6	1654	1657	1659	1662	1678	1702			
		VAL	LEU	PRO	PH E	AS N	LY S			
Grandisin	6	1654	1655	1657	1662	1678	1702			
		VAL	SER	LEU	PH E	AS N	LY S			
Myristicin	5	1654	1657	1659	1662	1678				
		VAL	LEU	PRO	PH E	AS N				
Thymol	4	1654	1662	1678	1702					
		VAL	PHE	ASN	LY S					
Hyoscyamide	4	1655	1680	1700	1701	1702	1776			
		SER	ILE	THR	LE U	LY S	PR O			
Picroside I	6	1654	1659	1662	1678	1679	1702			
		VAL	PRO	PHE	AS N	LE U	LY S			
β- caryophyllene	5	1654	1657	1662	1678	1702				
		VAL	LEU	PHE	AS N	LY S				
Vitexin	8	1654	1656	1657	1659	1662	1678			
		VAL	GLY	LEU	PR O	PH E	AS N			

DISCUSSION

The siddha polyherbal formulation Aswathy chooranam is an effective choice for the management of Breast cancer which is a rising concern. Total of 10 bioactive lead compounds were retrieved from the herbs present in the siddha formulation. From reported data of the herb, the phytochemicals such as Withaferin A,[8] Glycyrrhizin, [9] Gingerenone-A, Piperic acid, Piperine, Grandisin, Myristicin[10,11], Picroside I, β-caryophyllene and Vitexin possess maximum of five to eight interactions with the core active amino acid residues present on the target Breast Cancer gene 1 (BRCA1). Also, most of the phytochemicals of Aswathy chooranam binds with BRCA1 gene with an effective docking score. The phytochemicals not only help in combating Breast Cancer but also aids in the management of associated symptoms of cancer such as fatigue, myalgia, anemia and improves the immunity. The phytochemicals are also well known for their immunomodulatory, anti-inflammatory and potent antioxidant free radical scavenging activity that makes the drug a suitable option for Breast Cancer management.

CONCLUSION

Based on the results of the computational analysis it was concluded that all the bio-active compound's like Withaferin A, Glycyrrhizin, Gingerenone-A, Piperic acid, Piperine, Grandisin, Myristicin, Picroside I, β-caryophyllene and Vitexin present in the siddha formulation aswathy chooranam revealed significant binding affinity against the target Breast Cancer gene 1 (BRCA1) by interacting with active amino acid present on the active site thereby it was concluded that these compounds which has a tendency to inhibit the target BRCA1 may act as a potential therapeutic agent for the management of Breast Cancer condition.

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