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RESEARCH ARTICLE

NATURAL COMPOUNDS IN BREAST DRUG DISCOVERY: IN SILICO FILTERING OF PLANT COMPOUNDS AS ANTICANCER AGENT WITH A SYSTEMATIC COMPUTATIONAL BACKGROUND.

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ABSTRACT

Breast cancer is the most frequent cancer type and severe physical illness causing untold suffering to women. The inherited cases of breast cancer are due the occurrence of germ line mutation and rearrangement of the genome in the human BRCA gene, especially spontaneous change encountered with BRCA 1 gene.BRCA 1 protein has colossal therapeutic significance governed by the fact that its uncontrolled expression can be interrupted by small molecular candidates to negate the progression of cancer. In the modern drug discovery, molecules from herbal plants are of high therapeutic significance. In this present study, small molecules of plant origin with anticancer traits were chosen from the Duke's database and are virtually screened against the target protein using glide. Four top-ranked promising hit molecules were later tested by means of QikProp to analyze pharmacokinetics potential. Cross validation study against available commercial drugs also yielded advantageous results. We underline the fact that group of molecules from Duke's database could be a lead candidate to enter further *in vitro* studies which would help in breast cancer management.

INTRODUCTION:

Breast cancer in women is a major public health problem. It is the widespread cancer among women both in developed and developing countries and few reports also exist with male population. Cancer of the female breast is one in ten of all new cancers diagnosed worldwide each year[1]. It is estimated worldwide that over 508 000 women died in 2011 due to breast cancer. Although breast cancer is thought to be a disease of the developed world, almost 50% of breast cancer cases and 58% of deaths occur in less developed countries[2].Breast cancer is the second leading cause of cancer death in American women. In the year 2011 nearly 232,620 (230,480 Female, 2,140 Male) new cases are diagnosed with breast cancer and 39,970 (39,520 Female, 450 Male) cases succumb to death due to the severity of the disease. On the whole, approximately 182,000 new women are reported yearly with breast cancer and 46,000 cases continued to die in the USA[3]. Breast cancer is also the most common cause of cancer death among women (522 000 deaths in 2012) and the most frequently diagnosed cancer among women in 140 of 184 countries worldwide. Projections based on the GLOBOCAN 2012 estimates predict a substantive increase to 19.3 million new cancer cases per year by 2025, due to growth and ageing of the global population. More than half of all cancers (56.8%) and cancer deaths (64.9%) in 2012 occurred in less developed regions of the world, and these proportions will increase further by 2025[4]. About 5-10% of the carcinoma of the breast occurs by hereditary predisposition and mutations in the genes viz; BRCA1 and BRCA2. In the presence of a BRCA 1 mutation, women have 70-80% lifetime risk of developing breast cancer.Fundamentally, germ line mutations in the human BRCA1 gene accounts for the 80% of inherited breast cancer cases[5]. Human BRCA1 is characterized by two main features firstly amino terminal RING finger domain and secondly C-terminal BRCT domain. Both domains

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possess tumor suppressor activity. In fact germ line mutation occurring in this binding region alters the structural conformation and amounts to the increased expression of the protein product [6,7,8,9,10]. Germ line mutation in human wild type BRCA1 results in the deletion of normal residue and inserts new residue which increases the probability of unexpected functionality of the protein and more over germ line mutation observed in the RING finger domain of human BRCA1 residues such as Cys64Gly, Ser988Ala and Pro1749Arg amino acid in the BRCT domain and Ser1423Ala leads to imbalanced cell cycle mechanisms inside cell's biochemical environment and eventually causes breast cancer disease condition[11,12,13].Blocking of the BRCA 1 protein amounts to greater therapeutic value and down regulates the excessive protein accumulation in breast tissues.

Current research in drug discovery from medicinal plants involves a comprehensive approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continued to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain [14].The drug discovery of molecules from plant source has been playing an important role in the treatment of cancer; especially their secondary metabolites and derivatives over the last half century have been applied towards combating cancer [15,16,17]. Conventional therapies cause serious side effects or even the therapeutic role of them just extends the life span for few years and they were devoid of offering complete cure. There is thus the need to carry out alternative concepts or approaches to the prevention of cancer have become mandatory. Molecules from plant origin have been recently reported with excellent medicinal traits with varied applications [18,19,20,21]. For our study we took 1,000 natural compounds from Duke's Database with anti cancer properties to check and experiment its efficacy as potential anti cancer agents for breast cancer[22]. The identification of novel lead structures is a central task at the beginning of a drug discovery campaign. There are many ways to identify hit molecules , which can then be used as starting points for hit-to lead optimization and then finally to the industrialization of the compounds. Structure-based screening integrates several molecular computational techniques for the identification and optimization of small-molecule modulators with desirable qualities working against a specific target [23]. In light of the immense potential of virtual screening methodologies in the identification of initial hits, various structure and ligand-based approaches were developed [24,25,26,27]. Structure-based methods depend on the structural information of the protein target and typically include methods such as molecular docking [28, 29] structure-based pharmacophores [30] and de novo design [31].

Molecular docking is one of the commonly used virtual Screening method and a central component in the majority of high throughput schemes. Knowledge about the active site of protein is predominantly required for three dimensional docking. Molecular docking algorithms predict the orientation and conformation of the ligands with respect to target receptor binding site. Hundreds of binding poses are generated that are evaluated by scoring functions [32]. In this approach, site map tool is employed for binding site prediction of the protein. A rapid and accurate, docking method glide adopted to predict the best conformation of the ligands with the target protein [33]. Best hit molecules are then later subjected to QikProp analysis for prediction of ADME (absorption, distribution, metabolism, and excretion and vital pharmacological properties[34]. Combining glide and QikProp, the anti-cancer potency of molecules from Duke's database is documented and also reflects the novelty of them which then accounts for rational inhibition of increased expression of BRCA 1 protein. And subsequent study is validation, by comparing with the docking performance of commercial drugs.

MATERIALS AND METHODS:

1)**Preparation of protein structure:**

The crystal structure of BRCA1 protein (PDB ID: 1T15), was retrieved from the Protein Data Bank [10]. After selecting the protein structure, protein preparation wizard of the Schrodinger suite [35] has been used to prepare the protein structure for docking analysis. All the water molecules were removed from the protein structure, metal was treated and hydrogen atoms were added, all atom force field (OPLS-2005) charges and atom types were assigned. Then, energy minimization of the protein structure was done. The prepared protein structure serves as the receptor/target structure and input file for docking study.

2)**Preparation of ligand:**

The 1000 small molecules presumed to have anticancer activity from Duke's database [22] and four commercially available compounds for breast cancer were retrieved from the pubchem database in the SDF file format [36]. A few of the Duke's database chemical compounds structures were not available in pubchem database, Hence we used chemsketch to draw the structure and all these ligands were prepared using LigPrep version 2.3 [37]. The ligand structure energy was minimized; partial atomic charges were computed using the OPLS-2005 force field by using Schrödinger suite.

3)**Protein binding site identification:**

Since a protein molecule is capable of binding to different type of molecules with varied binding sites, characterizing the protein binding site is crucial for protein-ligand based docking in small molecule drug design[38]. The target protein BRCA1 (PDB ID: 1T15) was retrieved from protein data bank. The binding site was predicted with the help of sitemap tool [39, 40]which revealed about ten different binding sites. However the best preferred binding residues in active site cleft were found to be TYR 1666, ALA 1669, ARG 1670, HIS 1673, ILE 1674, THR 1675, LEU 1676, SER 6, SEP 8.

4)**Molecular Docking study:**

The prepared ligands were then docked with prepared protein structure by means of glide tool embedded in Schrodinger suite. Each ligand goes and stereo chemically binds with the target, influenced by various factors. The resultant protein-ligand complexes were ranked orderly via scoring function and were recorded. Four commercially available breast cancer drugs were docked simultaneously with the same target protein to analyze the inhibiting potential. This paves way to understand the supremacy between the natural and synthetic drugs in perfectly masking and neutralizing the "Receptor".

5)Prediction of the pharmacokinetics properties:

The best hit molecules were analyzed by QikProp [41]. It is very important to examine the properties such as QPPCaco2, QPlog MDCK, and Logp, octanol/water so that failure of molecules in early drug discovery pipeline could be detected in beforehand by this method.

RESULTS:

1. Docking analysis

The propensity of the ligand molecule to form interaction with the receptor molecule in the chemical space is of greater value in understanding the stability and conformational flexibility. Hydrogen bond interaction and docking score are the possible determining factor to fit the molecule into "lead like" class. The binding pose of aloin within the active site of the BRCA1 was assessed. The glide score and glide energy values for aloin were -6.559kcal/mol and -38.727kcal/mol, respectively. Upon the examination of docking features between aloin and BRCA1 two hydrogen bond interactions are noticed. Hydrogen atom of the aloin formed interaction with oxygen atom buried in the backbone of the SEP 8 with bond length of 1.70Å and same with polar residue ASN 1774 accompanied by a bond distance of 2.01Å, In addition to that residues mainly involved in hydrophobic interactions were identified to be LEU 1701, PRO1776, ILE 1680, PHE 11(figure1a). 1,2,8-Trihydroxy-6-methoxy-9H-xanthen-9-one formed two hydrogen bond interactions and the glide score was -5.446kcal/mol. Beyond that, residues mainly involved in hydrophobic interactions with 1,2,8-Trihydroxy-6-methoxy-9H-xanthen-9-one were PRO1659, VAL 1654, and PHE 1662 (figure 1b).

The docking analysis of Caffeic acid with BRCA 1 has become stable with three hydrogen bonds.Along with three hydrogen bonds, surprisingly only one π-cation interaction was formed between polar residue SER 8 with Caffeic acid, hydrophobic interactions was noticed among the residues namely MET 1689, PRO 9 and CYS 1697(figure 1c). With two hydrogen bond interactions with the target,(2S)-N-[2-[(3-methylbenzoyl)amino]ethyl]-2-(o-tolyl)pyrrolidine-1-carboxamide docked with a glide score of -2.611kcal/mol and a π-cation interaction was found between positive charged residue of LYS 1702 as depicted in figure 1d. The glide score and energy, interaction residues were illustrated in table1. And 2D representation of hit molecules was shown in figure 2.

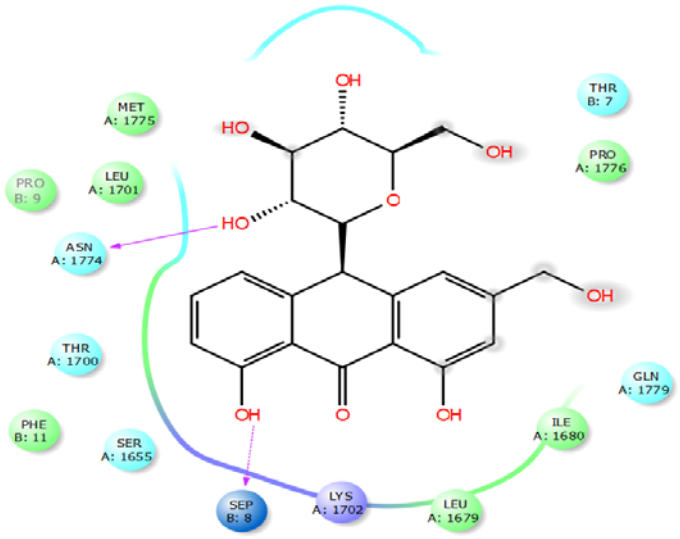

Figure 1a: Binding mode of Aloin

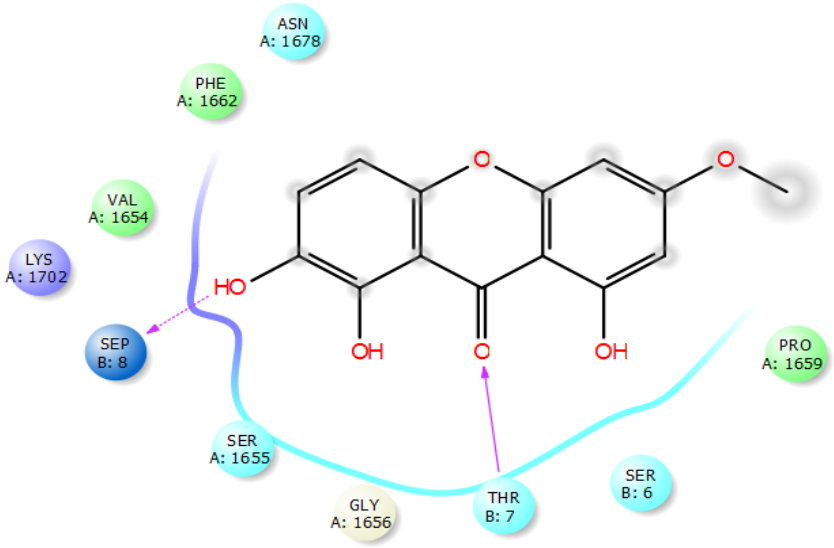

Figure 1b: Binding mode of 1,2,8-Trihydroxy-6-methoxy-9H-xanthen-9-one

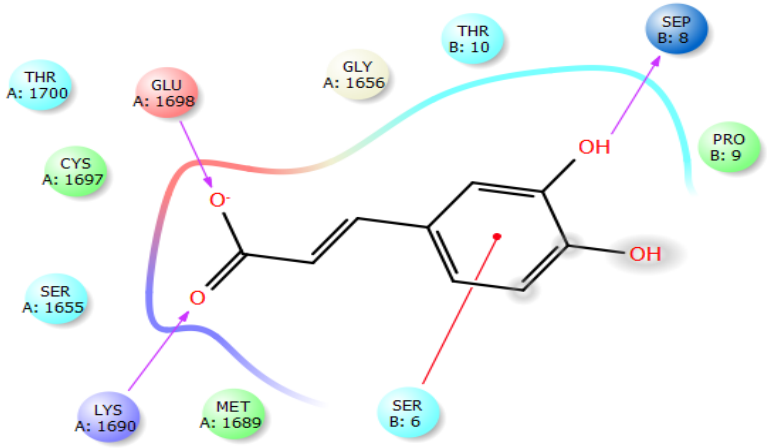

Figure 1c: Binding mode of Caffeic acid

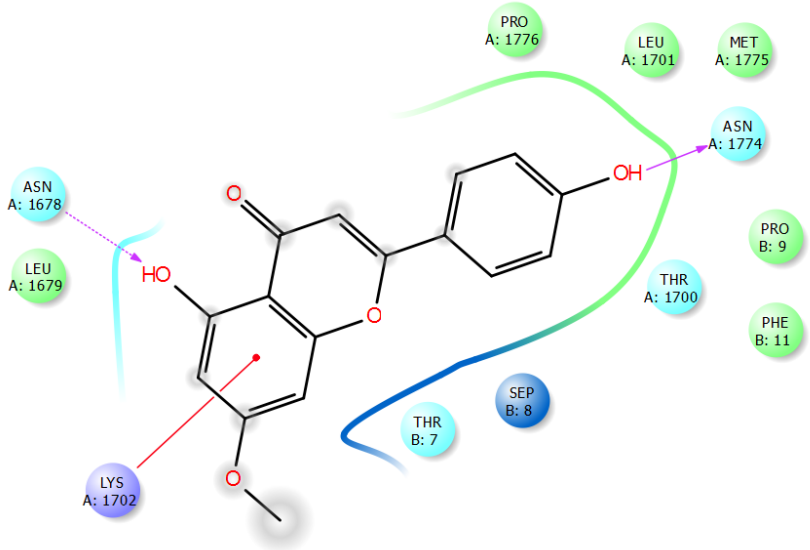

Figure 1d: Binding mode of (2S)-N-[2-[(3-methylbenzoyl)amino]ethyl]-2-(o-tolyl)pyrrolidine-1-carboxamide

Table 1: Molecular Docking Studies of HIT Molecules and Commercial Drugs:

Compound Name	Glide Score	Glide Energy	No. of. Hydrogen Bonds	Interacting Residues
Aloin	-6.559	-38.727	2	ASN 1774, SEP 8
1,2,8-Trihydroxy-6-menthoxy-9H-xanthen-9-one	-5.446	-34.150	2	SEP 8, THR 7
Caffeic acid	-3.864	-31.257	3	SEP 8, LYS 1690
2S)-N-[2-[(3- methylbenzoyl)amino]ethyl]-2-(o-tolyl)pyrrolidine-1-carboxamide	-2.611	-31.257	2	ASN 1678, ASN 1774
Gemcitabine	-4.47	-31.64	3	LEU 1676, TYR 1666, HIS 1673
Doxorubicin	-4.15	-51.14	2	HIS 1672(2)
Anastrozole	-2.58	-26.19	1	ARG 1670
Toremifene	-2.07	-11.00	1	ILE 1676

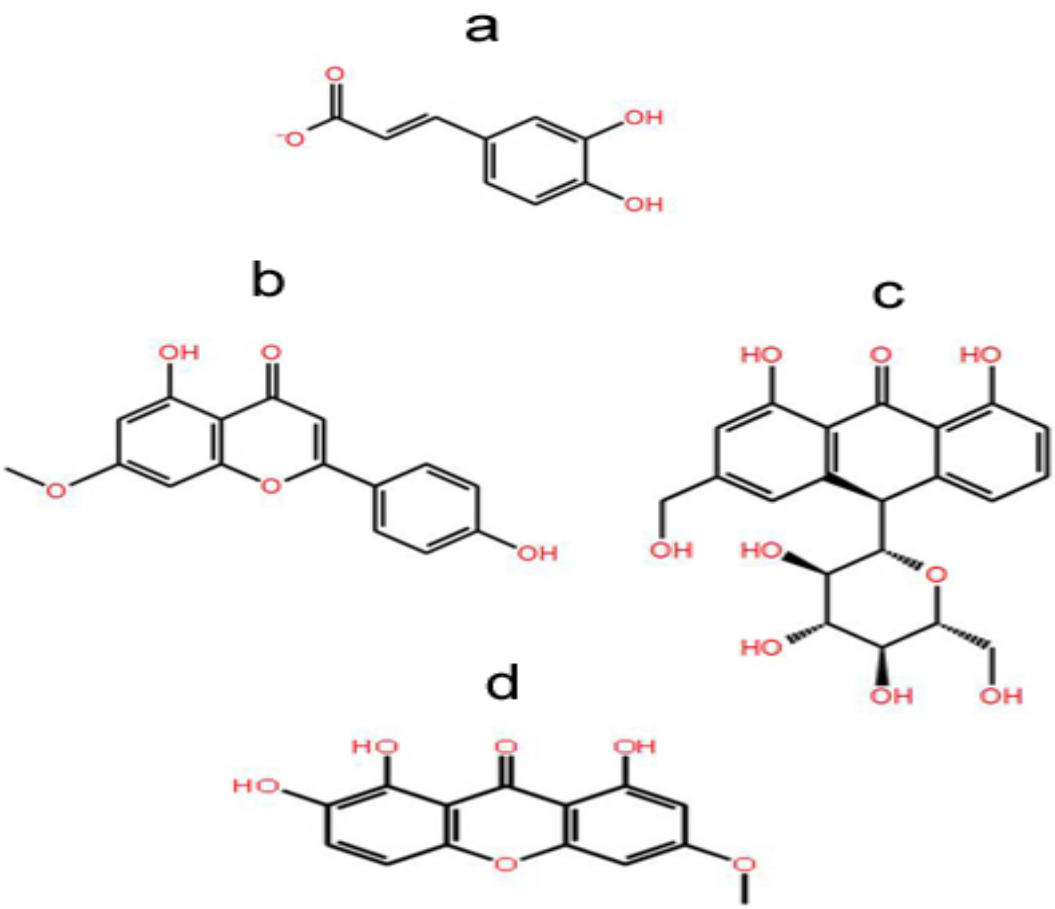

Figure 2: 2D reprsentataion of hit molecules a)Aloin, b)1,2,8-Trihydroxy-6-methoxy-9H-xanthen-9-one, c)Caffeic acid and d)(2S)-N-[2-[(3-methylbenzoyl)amino]ethyl]-2-(o-tolyl)pyrrolidine-1-carboxamide.

2. Prediction of the Pharmacokinetics properties

The promising four ligands were taken from docking studies and checked for its pharmacokinetics properties such as QPPCaco2, QPlog MDCK, and Logpoctanol/water and quantify its efficacy and specificity in reaching and inhibiting the target receptor. All these compounds were found to have acceptable/permissible value as depicted in table 2.

Table2: ADME properties of hit molecules using Qikprop

Compound Name	LogP (octanol/water)	MW	HBD	HBA	Serum Protein Binding	QP Log BB
Aloin	-0.421	418.399	5.000	11.700	-0.628	-2.857
1,2,8-Trihydroxy-6-menthoxy-9H-xanthen-9-one	1.834	274.229	1.000	3.500	-0.059	-1.139
Caffeic acid	0.560	180.160	3.00	3.500	-0.799	-1.561
2S)-N-[2-[(3-methylbenzoyl)a mino]ethyl]-2-(o-tolyl)pyrrolidine-1-carboxamide	2.779	284.268	1.000	3.750	0.249	-1.119

Normal ranges:

- Logp (octanol /water) :(-2.0/6.5.0)
- QP log K hsa Serum Protein Binding : (-1.5 / 1.5)
- Solute Molecular Weight : (130.0 / 725.0)
- Solute as Donor - Hydrogen Bonds : (0.0 / 6.0)
- Solute as Acceptor - Hydrogen Bonds : (2.0 / 20.0)
- QP log BB for brain/blood : (-3.0 / 1.2)

DISCUSSION:

Computational approaches that 'dock ' the small molecules into the structures of macromolecular targets and 'score' their potential complementarity to binding sites are widely used in hit identification and lead optimization. The majority of the efficeint drugs are the isolates from plants. This study concerned with molecular docking analysis of plant compounds. Aloin has become the significant out come of the study since it's glide score and stability of the moleucle is exceptional.In addition to that, less toxicity is observbed with Aloin. The energy based docking scores of molecules from Duke's proven to have much efficacy when compared with the commercial drugs. In addition to that, *in silico* ADME prediction for all hit molecules were found to be promising. On top of the results, natural compounds have a very less toxic behavior inside the human system and it magnifies the importance of this study**.**

CONCLUSION:

The risk of getting breast cancer is now days increasing in trend but the challenge of devising a drug molecule remain unexplored due to the complexity of the disease. Realizing the crucial importance of the BRCA 1 gene and its potentiality as the therapeutic target for breast cancer, we invested this short research to find out a novel anti-cancer molecule using *insilico* approaches. As a result of germ line mutations, the BRCA1 gene tends to be expressed more or over expressed beyond its normal pattern in cell mechanics and thereby resulting in the abundant protein product increases that increases the incidence of the malignancy of the breast. Recently, natural products or molecules of plant source origin have a huge impact in treating cancer or in other words they possess immense properties to become an effective anti-cancer agent. Molecules from the herbal plant are reported to have good medicinal value. In this connection, to find the "drug-like compounds", structure based virtual screening of 1000 compounds against the target were carried out. Among that four molecules possessed with a well- suited docking score and significant denominations for pharmacokinetics values. This increases the "lead like pattern" for them. When compared with the potency of the four available commercial drugs, the molecules from Duke's database gave promising results. We believe that the molecules from Duke's database can act as a versatile anticancer agent provided positive correlation with *in vitro* results.

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