RESEARCH ARTICLE

Garlic Phytocompounds Possess Anticancer Activity by Specifically Targeting Breast Cancer Biomarkers - an in *Silico* Study

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Abstract

Background: Breast cancer (BC) is a serious lifestyle disease. There are several prognostic biomarkers like nuclear receptors whose over-expression is associated with BC characteristics. These biomarkers can be blocked by compounds with anti-cancer potential but selection must be made on the basis of no adverse side effects. This study is focused on finding of compounds from a plant source garlic. Materials and Methods: Twenty compounds from garlic and five targets considered involved in BC were retrieved from Pubchem database and Protein Data Bank respectively. They were docked using Accelrys Discovery Studio (DS) 4.0. The compounds which showed interaction were checked for drug likeliness. Results: Docking studies and ADMET evaluation revealed twelve compounds to be active against the targets. All the compounds displayed highly negative dock scores which indicated good interactions. Conclusions: The results of this study should help researchers and scientists in the pharmaceutical field to identify drugs based on garlic.

Keywords: Breast cancer - nuclear receptor - garlic - discovery studio - docking - ADMET

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Introduction

According to World Health Organization (WHO), Breast Cancer (BC) is the most leading cancer in women all over the world (Benson and Jatoi, 2012; Dubey et al., 2015). According to GLOBOCAN report (2012), around 1.67 million cases of BC were reported and it is the second most diagnosed after lung cancer (Ferlay, 2015). In India, BC is the most common type of cancer in women of the urban cities of Delhi, Mumbai, Ahmedabad, Kolkata, and Trivandrum, where over 100,000 new BC cases are diagnosed annually (Khokhar, 2012). The use of biomarkers in this field have relatively helped to understand disease biology and is currently used in clinical practice.

BC is generally a hormone dependent cancer, because of the involvement of sex steroid hormones such as estrogen and progesterone and also their respective nuclear receptors (NR) such as estrogen receptor (ER) and progesterone receptor (PR), which are the most important biomarkers for BC (Sever and Glass, 2013; Higa and Fell, 2013; Omoto and Iwase, 2015). There are other NRs which also lead to BC such as androgen receptor (AR), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) (Xu, 2015; Voutsadakis, 2016). AR is expressed in 89% of ER positive BC cases (Conzen, 2008; Garay and Park, 2012). GR is expressed in about 60% of BC and gets associated with 70% of ER positive tumors (Abduljabbar et al., 2015; Kach et al., 2015). Sometimes, MR may replace GR in terms of the laters' activity because of similar structures of both the two (Kingsley et al., 2002; Leo et al., 2004). These biomarkers in total are the prognostic factors which led to the detection and diagnosis of BC.

There is always a demand for herbal drugs in the market as they display low side effects, as compared to commercially available drugs. Garlic (Allium sativum L.) is a herbal crop containing compounds with medicinal properties, and is in discussion for decades for its usefulness in curing cancer, diabetes, blood pressure etc (Suleria et al., 2015). Huang et al. (2015) observed the antitumor effect of garlic compound on human triple-negative BC cells. In some other *in vitro* studies, garlic compounds and extract was found to inhibit human BC cell lines MCF-7 and MDA-MB-231 in a dose and time dependent manner by inducing cell cycle arrest and apoptosis (Ghazanfari et al., 2011; Tsubura et al., 2011; Modem et al., 2012; Zhang et al., 2014; Bagul et al., 2015). As the clinical study of all these compounds is time consuming, so in this study, an in *silico* tool 'molecular

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docking' was used to predict the binding mode and efficiency of garlic compounds with the targets of BC. Molecular docking is an efficient bioinformatics technique which can be used for finding out potent compounds acting against specific targets/ biomarkers of specific disease, without spending much time as like in a normal drug discovery pipeline (Ferreira et al., 2015).

Materials and Methods

Identification and retrieval of phytocompounds /ligands

Identification of phytocompounds from garlic was done from Dr. Duke's database (http://www.ars-grin.gov/duke/) containing facts on the medicinal activity of phytocompounds in humans (Barlow et al., 2012). Three dimensional structure of identified twenty compounds were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) as ligands in .sdf format (Kim et al., 2016).

Preparation of ligands and their filtration

Preparation of the retrieved compounds/ ligands was done using 'Prepare ligand' protocol in DS 4.0, which removed duplicates, enumerated tautomers/isomers, added hydrogen bonds and minimized energy using CHARMm (Chemistry at Harvard Macromolecular Mechanics) force field (Brooks et al., 2009). The prepared ligands were filtered by Lipinski's Rule of five and Vebers' protocol (Ro5 & VP) that sets the criteria for drug like properties and focuses on drugs' bioavailability (Veber et al., 2002; Lipinski, 2004). Ro5 & VP was used to screen the compounds on the basis of molecular weight (MW, ≤500 daltons), no. of hydrogen bond donors (HBD, ≤5) and hydrogen bond acceptors (HBA, ≤10), no. of rotatable bonds (RB, ≤10), logP value (≤5) and polar surface area (PSA, ≤140 $Å^2$). The filtered ligands were then forwarded for molecular docking with BC target proteins.

Target protein identification and retrieval

Successful molecular target proteins involved in BC metastasis, such as ER, PR, AR, GR and MR were selected from Therapeutic Target Database (TTD- http://bidd.nus.edu.sg/group/cjttd/) and Potential Drug Target Database (PDTD- http://www.dddc.ac.cn/pdtd/) for purpose of molecular docking studies (Qin et al., 2014; Chen et al., 2015). Their three dimensional X ray crystallographic structures were downloaded from Protein Data Bank (PDB- http://www.rcsb.org/pdb/home/home.do) and saved in .pdb format (Rose et al., 2015). All the structures were selected on the basis of the presence of one or more active site for binding with ligands and high active side residue count. The PDB IDs of the retrieved targets were 1ERR (ER), 3D90 (PR), 1E3G (AR), 1M2Z (GR) and 2AB2 (MR).

Preparation of protein molecules and active site identification

'Prepare protein' protocol of DS 4.0 corrected the protein structures by inserting missing atoms, adding hydrogen atoms, modelling loop regions and side chains, removing water molecules, natural ligands and hetero atoms and minimizing energy to avail a stable conformation by using CHARMm forcefield. The energy minimized structure was used as the template for molecular docking.

'PDB site records' of DS 4.0 were used to identify the active sites. This method look for poseview software molecular interactions of protein target crystal structure and inhibitor displayed in PDB (Stierand and Rarey, 2010). A grid receptor sphere was generated, including the selected binding active site and incorporating all the critical functional residues.

Molecular interaction and binding

Molecular docking was performed between the prepared BC target proteins and identified garlic phytocompounds by 'CDOCKER' protocol of DS 4.0. (Wu et al., 2003). The pose which contained the least difference between CDOCKER energy and CDOCKER interaction energy was considered as best interaction, along with the lowest binding energy calculation as the scoring function (Oda et al., 2007). Number of hydrogen bonds between the targets and the ligands were also recorded. The optimal distance between two atoms connected by a hydrogen bond is set to 1.9 Å with a tolerance of 0.5 Å (Stierand and Rarey, 2010).

Pharmacokinetic evaluation

In *silico* tool 'ADMET descriptors' provided by DS 4.0 can help in the evaluation of pharmacokinetic parameters and assess the quality of the molecule in terms of absorption, distribution, metabolism, excretion and toxicity after human ingestion (Tian et al., 2015). This technique reduces the cost and chance of clinical failures of new drugs. The parameters calculated by this descriptor included human intestinal absorption, aqueous solubility, blood brain barrier (BBB), hepatotoxity, CYP2D6 inhibition and plasma protein binding (PPB) (Usha et al., 2014).

Results and Discussion

Preparation of garlic compounds and BC target proteins

The garlic compounds (allyl compounds and flavonoids) which were used for the in *silico* study are enlisted in Table 1. After preparation, all the ligands were subjected to filtation by Ro5 & VP, where none of them violated the rule. However, compounds such as L-γ-Glutamyl-S-allyl-L-cysteine (γGSAC) and myricetin were found to possess a PSA higher than 140 $Å^2$, which indicated low absorption and poor permeability of the molecule across cell membranes.

Preparation of the target proteins led to removal of all the non-standard residues from the structures. The potential energy, Vander-Waals energy and electrostatic energy were reduced, when the protein structures were subjected to energy minimization. Many active sites were found present in the crystallographic structures, out of which the best active site was selected looking at the PDB site records for preparing the receptor grid. The energy minimized stable (prepared) structure of PR along with its unprepared 3D structure is shown in Figure 1a and 1b.

Binding ability of garlic compounds with BC target proteins

Twelve compounds from garlic showed good interaction with the BC targets (Table 2). A very less difference was found between CDOCKER and CDOCKER interaction energy (around 2 Kcal/mol). The binding energies of the compounds from garlic with the BC targets was found to be ranging from -66.84 Kcal/mol to -168.57 Kcal/mol. The lowest score (-168.57 Kcal/mol) was shown by kaempferol while interacting with PR at the residues GLN725, ARG766 and CYS891 of active site 1 with hydrogen bond lenghts 2.2 Å, 1.7 Å and 2.4 Å respectively (Figure 2). PR is an NR, the alteration of functions of which lead to co-expression with ER and lead to dangerous forms of BC (Diep et al., 2015).

ER is the most important prognostic biomarker in BC

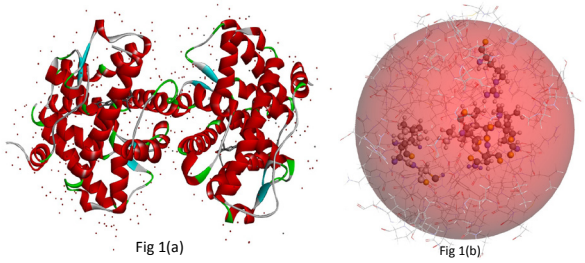

Figure 1. a): 3D Structure of Progesterone Receptor (b): Prepared Structure of Progesterone Receptor Protein Ready for Docking

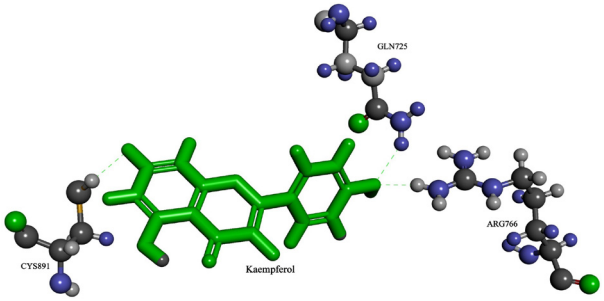

Figure 2. Interaction of Garlic Compound Kaempferol with Active Site Residues of Progesterone Receptor

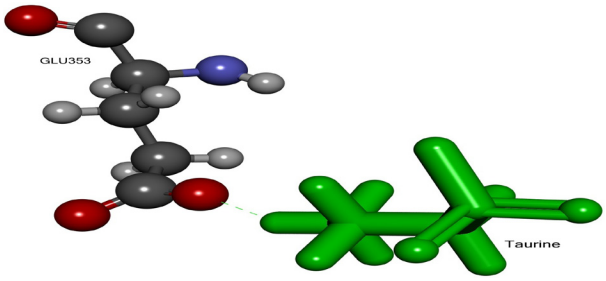

Figure 3. Interaction of Garlic Compound Taurine with Active Site Residues of Estrogen Receptor

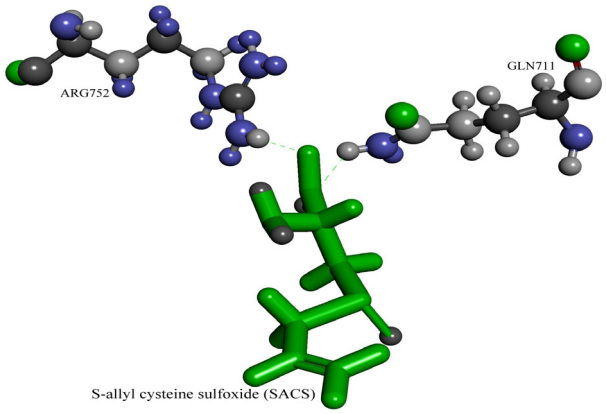

Figure 4. Interaction of Garlic compound SACS with Active Site Residues of Androgen Receptor

Table 1. Compounds from Garlic Selected for the Study and their Characteristics

Sl. No.	Compound name	Pubchem ID	MW (≤500 daltons)	HBD (≤5)	HBA (≤10)	AlogP (≤5)	RB (≤10)	PSA (≤140 Å^2)
1	Allicin	65036	162.27	0	1	2.01	5	61.58
2	SACS	87310	177.22	3	4	-3.39	5	104.05
3	Allyl mercaptan	13367	74.14	0	0	1.23	1	38.79
4	Apigenin	5280443	270.23	2	5	1.71	1	89.82
5	E-Ajoene	5386591	234.4	0	1	2.06	8	86.88
6	Ferulic acid (FA)	445858	194.18	1	4	0.19	3	69.59
7	Isobutyl isothiocyanate	68960	115.19	1	1	2.1	2	46.06
8	Kaempferol	5280863	286.23	2	6	0.47	1	112.88
9	γGSAC	91820320	290.33	4	7	-4.51	10	162.3
10	Methyl Propyl Disulfide	16592	122.25	0	0	2.26	3	50.6
11	Myricetin	5281672	318.23	4	8	-0.02	1	153.34
12	pCA	637542	164.15	1	3	0.21	2	60.36
13	Phloroglucinol	359	126.11	3	3	1.1	0	60.69
14	Quercetin	5280343	302.23	2	7	-0.48	1	135.94
15	S-allyl cysteine (SAC)	98280	161.22	3	3	-2.28	5	93.07
16	S-allyl mercapto cysteine (SAMC)	9794159	193.28	0	3	-0.48	5	120.97
17	Sinapinic acid	637775	224.2	1	5	0.18	4	78.81
18	Taurine	1123	125.14	3	4	-4.49	2	93.22
19	Thiacremonone	539170	160.19	1	4	-0.703	0	85.66
20	2-Vinyl-4H-1,3-Dithiin	133337	144.25	0	0	1.98	1	50.6

Table 2. Dock scores of Garlic Phytocompounds with the BC Target Proteins

Sl. No.	Garlic phytocompound	Target Proteins	(-) CDOCKER energy (Kcal/mol)	(-)CDOCKER Interaction energy (Kcal/mol)	Binding Energy (Kcal/mol)	Interacting residues
1	SACS	ER	23.66	25.89	-78.41	GLU353, ARG394
		PR	32.8	32.85	-85.07	LEU718, GLN725
		AR	37.22	37.79	-142.12	ARG752, GLN711
		GR	27.55	28.23	-91.24	ASN564
		MR	28.57	30.36	-87.73	ARG817, GLN776, LEU810
2	SAC	PR	28.82	29.05	-83.84	GLN725 (2), ARG766
		AR	32.58	33.38	-101.26	MET745, GLN711 (2), ARG752
		GR	24.89	26.27	-93.06	ASN564
3	p CA	ER	32.77	35.95	-83.71	GLU353
		PR	27.96	28.77	-104.83	ARG766, GLN725
		MR	28.05	30.89	-97.13	ARG817 (2), GLN776
4	Phloroglucinol	PR	36.24	37.03	-149.31	ARG766, LEU718, GLN725
		AR	39.25	40.16	-157.08	ARG752
5	Kaempferol	PR	41.24	41.51	-168.57	GLN725, ARG766, CYS891
		AR	40.85	42.85	-88.97	THR877
6	Isobutyl isothiocyanate	ER	20.53	21.12	-84.73	GLU353, ARG394
		AR	20.84	21.46	-117.64	ASN705
7	Quercetin	PR	46.31	48.68	-154.66	GLN725, CYS891
8	γGSAC	ER	40.72	42.81	-69.37	GLU353
		MR	49.19	51.53	-91.56	ARG817 (2), GLN776, LEU810
9	SAMC	AR	35.47	35.56	-114.88	ARG752 (2), MET745, GLN711
		GR	22.79	24.11	-100.84	ASN564
10	FA	GR	26.54	28.01	-66.84	ASN564
		MR	30.61	31.31	-96.95	ARG817, GLN776
11	Taurine	ER	28.52	29.79	-88.12	GLU353
12	Apigenin	PR	50.51	51.99	-114.45	GLN725, ARG766, CYS891
		GR	19.42	20.01	-73.81	ASN564

Table 3. ADMET Properties of Docked Compounds from Garlic

Sl. No.	Ligand	Solubility level (2-4)	BBB level (2-4)	CYP2D6 Prediction (False-non inhibitor)	Hepatotoxic Prediction (False-non toxic)	Absorption level (0-1)	PPB Prediction (False-poorly bound)
1	SACS	5	4	FALSE	FALSE	3	FALSE
2	SAC	5	4	FALSE	FALSE	3	FALSE
3	p CA	4	3	FALSE	FALSE	0	FALSE
4	Phloroglucinol	4	3	FALSE	TRUE	0	FALSE
5	Kaempferol	4	3	FALSE	TRUE	0	FALSE
6	Isobutyl isothiocyanate	4	2	FALSE	TRUE	0	FALSE
7	Quercetin	4	4	FALSE	TRUE	1	TRUE
8	γGSAC	5	4	FALSE	FALSE	3	FALSE
9	SAMC	4	3	FALSE	FALSE	0	FALSE
10	FA	4	3	FALSE	FALSE	0	TRUE
11	Taurine	5	4	FALSE	TRUE	3	FALSE
12	Apigenin	4	3	FALSE	TRUE	0	FALSE

diagnosis, the alteration of the function of which lead to over-expression in around 80% of BC cases (Contro et al., 2015; Lumachi et al, 2015). This target also showed good interaction with garlic phytocompounds such as taurine, (SACS) p-coumaric acid (pCA), isobutyl isothiocyanate. It was seen that the critical residues such as GLU353 and ARG394 of active site 1 interacted very properly with the compounds of garlic. The lowest binding score was shown by taurine (Table 2; Figure 3).

AR is expressed in BC more frequently than ER and PR (Park et al., 2010; Fioretti et al., 2014). It was found that garlic compounds responded well in interacting with this target while binding with critical residues ARG752, GLN711, MET745, THR877 and ASN705 from active site 1. The interaction of SACS with AR is shown in Figure 4. Garlic compounds also interacted with GR and MR with good binding scores. The residues such as ASN564 of active site 4 from GR and ARG817, GLN776, LEU810 of active site 2 from MR were found to be the most active to bind with the garlic phytocompounds.

Evaluation of drug likeliness

ADMET properties of the garlic compounds which interacted with all the BC targets are enlisted in Table 3. The ADMET parameters were found supported by the Ro5 & VP. 4 compounds are having very poor intestinal absorption i.e. SACS, SAC, γGSAC and taurine, because of presence of more no. of hydrogen bond donors and

hydrogen bond acceptors (Honorio et al., 2013). The same compounds were also found to be highly soluble in water, due to the lower logP value of the compounds (Table 1), which depicted lower lipophilicity (Kujawski et al., 2012). Some compounds of the flavonoid group showed hepatotoxic activity like phloroglucinol, kaempferol, isobutyl isothiocyanate, quercetin, taurine and apigenin. Already many studies on the medicinal benefits of these phyto-compounds have been reported. It can be said that these compounds can be taken into consideration for making a drug with a low dosage, so that there is less toxic effect in the liver. All the compounds are non-inhibitor of CYP450 enzyme (an enzyme helping in the metabolism of drugs for easy absorption and excretion), which can be known from the CYP2D6 reading. PPB binding of all the compounds was found to be poor except quercetin and FA, which depicts that all the compounds are pharmacologically active and can get unbound from plasma protein easily (Smith et al., 2010). The BBB level of all the compounds was low, which means there is less chance of side effects of the central nervous system (Cecchelli et al., 2007).

In conclusion, BC alone accounts for 29% of all cancers diagnosed in women and around 61,000 cases are expected to be diagnosed in 2016 (Siegel et al., 2016). By 2020, BC cases are expected to increase by 26% and most of these will be seen in developing countries, especially in the global south (Confortini and Krong, 2015). So, it is an alarming situation to find out drugs which can be suitable for curing BC in women with least side effects. Garlic is a potent herb which yield compounds with plenty of medicinal properties. All the compounds being taken for the study passed Ro5 & VP, and the interaction experiment revealed only 12 compounds to bind with the BC targets. The ADMET profiling of the compounds revealed that almost all the compounds are perfect for proceeding into drug pipeline, if the dosage is considered. The study forms a strong base for researchers and scientists of the pharmaceutical industry, who can perform wet lab (*in vitro* and *in vivo*) analysis of these garlic compounds for developing into drugs.

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