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Original Article

In Silico Computational Prediction of Anti-Breast Cancer Effect of Abruquinones from *Abrus precatorius* L.

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ABSTRACT: Knowledge based searching of phytochemicals with potential anti-breast cancer effect from *Abrus precatorius (*L) with prediction of mechanism of action using computational molecular docking approach was the aim of this investigation. Three abruquinones (A, B and C) were selected upon chemical association network analysis and literature search as candidate ligands, while estriol and genistein were, respectively, considered as positive and negative control. After structural investigation, the chain A of human estrogen receptor beta (ERβ; PDB: 2YLY) was selected as receptor for docking study. Docking was carried out by Molegro Virtual Docker (MVD) and ParDock. Results of the docking studies suggested the favorable binding of abruquinone B and abruquinone C to ERβ-receptor with respect to genistein. Ligand validation was confirmed by the drug-likeness characteristics of abruquinones without any violation of Lipinski's rule. Based on the docking studies it was proposed that anti-breast cancer effect abruquinones might be accomplished by their antagonistic effect on estrogen receptor beta.

KEYWORDS: breast cancer, Abruquinones, molecular docking.

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INTRODUCTION

Estrogen receptor beta (ERβ) is a nuclear receptor of the estrogen receptors family, encoded by the *ESR2* gene and activated by the sex hormone estrogen.[8,13] The ERβ follows a differential tissue distribution in ovary, uterus, breast, and brain.[16] It governs a variety of different physiological processes like reproductive organ development, bone modeling, cardiovascular functioning, metabolism.[10] The complex biological effect mediated by ERβ often involves communications between multiple proteins and signaling pathways. Modern cellular experiments suggest the formation of homodimers or heterodimers (with ERα) upon ligand binding which interact with specific DNA sequences to activate transcription of the responsive genes.[14] Accumulated experimental data clearly suggest the clinical significance of ERβ in breast cancer. The widespread expression of ERβ proteins in normal and neoplastic mammary tissues confirms positive association of ERβ expressions with breast cancer prognosis.[19] ERβ is considered as a potential therapeutic target for breast cancer with available synthetic estrogen receptor antagonists such as tamoxifen and toremifene.[15] Due to extensive side effect issues of conventional chemotherapeutic drugs, the search for a natural and innocuous therapeutic principle for ERβ is obviously an important concern in biological research.

Abrus precatorius L. is a member of 'Fabaceae' family and commonly known as 'Crab's eye'. It is a deciduous dextrose climber with slender flexible branches. *A. precatorius* enjoys a wide spread distribution in South Asia, including Bangladesh and locally known as 'Kunch'.[18] *A. precatorius* has traditional medicinal value with therapeutic use in leucoderma, fever, cough, abdominal pains, tumors, abortification, malaria, convulsion, hepatitis etc.[2] The plant has been reported to have anti-spermatogenic, anti-fertility, CNS depressant, analgesic, antiulcer, anti-diarrheal and anti-helminthic activities.[1] Different parts of the plant contain about 150 phytochemicals of flavonoid, glycoside, alkaloid, fixed oil, steroid, and terpenoid class. A recent study reports the growth inhibitory effect of *A. precatorius* against breast cancer cell line MDA-MB-231.[21] Though abrin-a and abrin-b have been claimed to possess anticancer

activity against Sarcoma 180 cells and Ehrlich ascites tumor cells but phytochemicals responsible for anticancer activity against breast cancer is yet to be resolved with specific candidate mechanism.[17] Therefore the present study was carried out to find knowledge based potential phytochemicals with anti-breast cancer activity from *A. precatorius* with prediction of mechanism of action using computational molecular docking approach.

MATERIALS AND METHODS

Selection of Receptor and Ligands

Chemicals either known or predicted to interact with the estrogen receptor beta (ERβ) were explored by STITCH 3.1[7] (Supplementary Figure S1). Literature search was taken as confirmatory step for the selection of candidate ligands. Canonical SMILES and 3D SDF file of abruquinone (32) A (CID: 9975772 and CID conformer count: 32), abruquinone (18) A (CID: 172847 and CID conformer count: 18), abruquinone B (CID: 44257521), abruquinone C (CID: 44257520), estriol (CID: 5756) and genistein (CID: 5280961) were downloaded from PubChem Compound server. Canonical SMILES files were feed to Corina online demo[20] to generate 3D Protein Data Bank (PDB) files. Human estrogen receptor ERβ was used as receptor in the present study. Protein Data Bank (PDB) file of human ERβ ligand binding domain (PDB id: 2YLY) was downloaded from RCSB PDB database.

Receptor Analyses

The functional information on 2YLY was gathered from UniProtKB. A detailed query on receptor (2YLY) structure was carried out by the Electron Density Server.[6] The receptor (2YLY) was screened for the potential ligand binding sites through Pocket-Finder[4] and GHECOM 1.0[5] server (Supplementary Figure S2).

Computer Simulated Molecular Docking Tools and Algorithm

Molegro Virtual Docker (MVD)[22] and online docking server ParDOCK[3] were used for computer simulated docking study. MVD performs flexible ligand docking with the optimization of ligand geometry during docking. MVD includes MolDock and PLANTS Score for evaluating docking solutions followed by ranking the best conformations. MVD uses a differential evolution algorithm. The solution to the function is the sum of intermolecular interaction energy between protein and ligand with the intra-molecular interaction energy of the ligand. The docking energy scoring function is based on a modified Piecewise linear potential (PLP) with new hydrogen bonding and electrostatics terms included. A lower score always indicates a higher affinity. ParDOCK is an all-atom energy based Monte Carlo, rigid protein ligand docking, implemented in a fully automated, parallel processing mode which predicts the binding mode of the ligand in receptor target site.

Molecular Docking Study

Before initiation of the docking operation, both protein and ligands were prepared by MVD by assigning bonds, bond orders, explicit hydrogens, charges, flexible torsions at the missing region only. Potential binding sites (cavities or active sites) were identified using the built-in cavity detection algorithm of MVD with a grid resolution (Å) of 0.30. Internal electrostatic interactions, internal hydrogen bonds, and Sp2-Sp2 torsions were selected as ligand evaluation terms. The "MolDock SE" was set as searching algorithm for 10 runs using a maximum of 1500 iterations with a total population size of 50 was used. Energy minimization and optimize H-bonds were enabled as post-docking steps for more accurate refinement of the docking results. Multiple pose were clustered based on the RMSD threshold of 1.0 while ignoring similar poses with RMSD threshold of 1.0. Docking with ParDOCK was run under default setting as instructed. The receptor was protonated with WHAT IF web servers.[23]

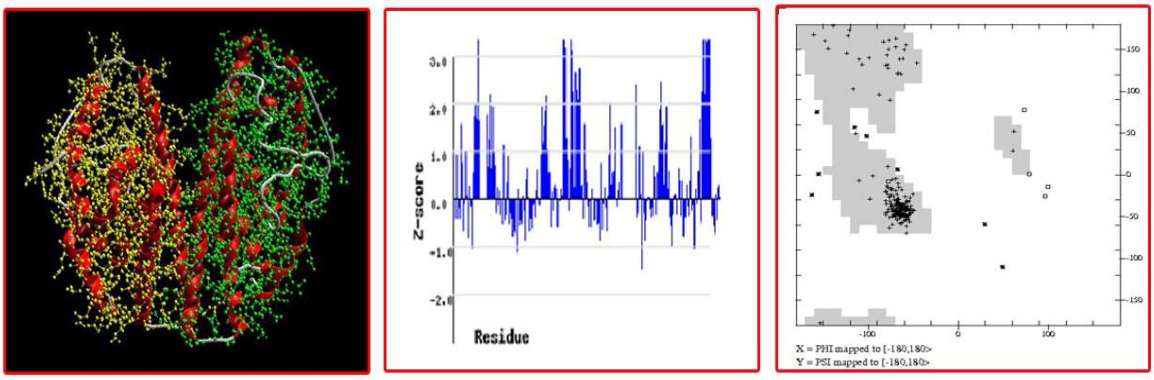

Figure 1. Selection and analysis of the receptor. (A) PDB structure of the 2YLY with resolution of 3.20 Å. There are two ERβ ligand binding domain, A (Green) and B (Yellow). (B) Z-score of the chain A. A large positive spike is indicative of a residue which has higher density than the average for that residue type in structures of similar resolutions. (C) Ramachandran plot of chain A.

Ligand Validation

Ligand molecules were validated using online validation tools, Molinspiration cheminformatics server. Molinspiration cheminformatics was exploited to calculate the molecular property associated with the drug-likeness and prediction of bioactivity including Nuclear receptor ligand, GPCR ligand and enzyme inhibitor.

RESULTS

Preparation of Receptor and Ligands

The action view of STITCH 3.1 exhibited the isoflavones like genistein and daidzein as the plant derived phytochemical interact with animal and human estrogen receptors. Isoflavones were also reported to interact significantly with estrogen receptor beta under breast cancer.[9,11,12] Thus, our focus was concentrated on the isoflavonoids and quinones of *A. precatorius.* The major isoflavonoids and quinones present in root of *A. precatorius* include abruquinone A (0.025–0.45%), abruquinone B (0.045–1.15%), abruquinone C (0.5%).[18]

Receptor Analyses

2YLY is the ligand binding domain (Residue: 260-500) of two ERβ (Homodimer: chain A & chain B). There are five regions in ligand binding domain: two NR C4-type zinc finger (149-169 & 185-209), DNA binding (149-214); modulating (1-148) and one steroid-binding (215-530) region. Result of the electron density server showed more compact structure (as indicated by comparative Z-score) of chain A with similar Ramachandran plot containing similar number of outlier residue. Therefore chain A was selected for further study.

Initial prediction of the probable ligand binding site was performed by Pocket-Finder and GHECOM 1.0. The prediction showed an active site of 645 cubic angstroms with a minimum coordination of –40 × –17 × –40 from the Pocket-Finder output (Figure 3B). GHECOM 1.0 was done with a 12 angstroms radius for the large probe. GHECOM 1.0 output showed pocketnes of five clusters. Output of Pocket-Finder was synchronized with GHECOM 1.0 output for the prediction with higher degree of accuracy.

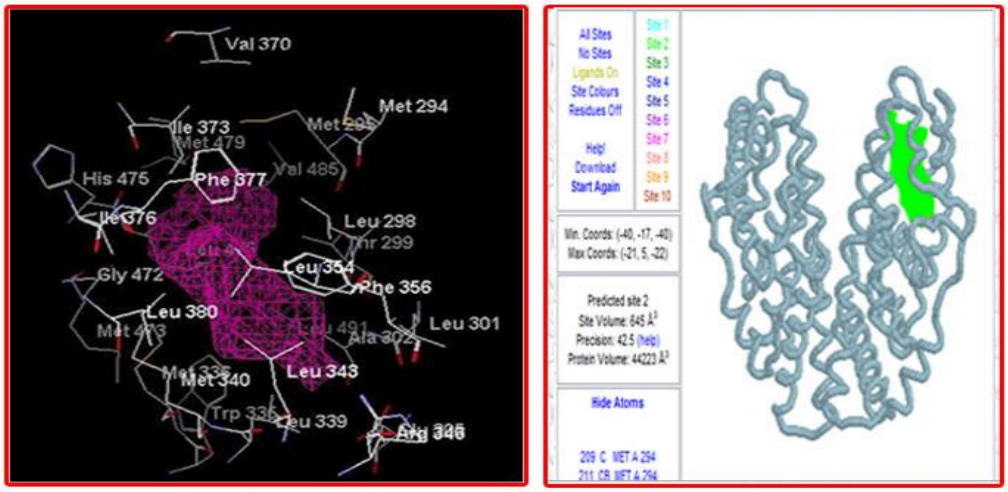

Figure 2. Prediction of binding site on the receptor. (A) MVD detected binding site (Cavity). The pink color indicates cavity with the amino acid residues located up to 4.0 Å. The MVD detected total five cavities with a grid resolution (Å) of 0.30 that matched well with both Pocket-finder and GHECOM 1.0 output. (B) Predicted binding site evaluated by Pocket-finder. Pocket-Finder works by scanning a probe radius 1.6 Å along all gridlines of grid resolution 0.9 Å surrounding the protein. The output of MVD cavity detection overlapped with the output of the Pocket-finder

Table 1. Molecular docking result of Molegro Vertual Docker and ParDOCK for the studied ligands bound to estrogen receptor beta.

Ligands	Molegro Vertual Docker			ParDOCK
	MolDock Score kcal/mol	H-bond kcal/mol	Steric energy kcal/mol	Score kcal/mol
Estratriol	- 105.56	- 7.27	- 105.62	- 4.98
Genistein	- 95.49	- 5.81	- 110.21	- 3.79
Abruquinone (32) A	- 97.60	- 2.18	- 130.15	- 4.96
Abruquinone (18) A	- 95.49	- 1.01	- 127.97	- 4.96
Abruquinone B	- 94.36	- 1.17	- 130.40	- 6.62
Abruquinone C	- 106.58	- 4.34	- 134.05	- 5.59

Relative binding affinities of estrogen (as positive control), geistein (as negative control) and abruquinones with the estrogen receptor.

Computational Molecular Docking

The result of the computational docking study of the ligand with the receptor (Coordinate: –29.91 × –7.67 × –30.33) by MVD was evolved in the terms of MolDock Score for the best pose. Positive control estriol exhibited the lowest docking score followed by the negative control genistein. Among the studied ligands, abruquinone C exhibited the most favorable binding to the receptor followed by the abruquinone A and abruquinone B. Most significantly, abruquinone C showed a similar binding pattern with respect to the estratriol while a much more potential binding pattern than that of the genistein. Abruquinone A and abruquinone B also exhibited a similar binding potential compared to genistein (Table 1). Result from the online docking tools ParDOCK was expressed as the binding energy of ligand to receptor in kcal/mol. The lower the binding energy the better is the pose. ParDOCK score suggested more favorable pose for abruquinones to the receptor than that of the negative control genistein (Table 1).

Ligand Validation

The ligand validation data from Molinspiration cheminformatics server was summarized in the table 2. All the studied ligands were found to have the potential of drug-like molecule as satisfied by all the terms of Lipinski's rule of five.

DISCUSSION

In the present study we evaluated three abraquinones from *A. precatorius* as potential phytochemicals with anti-breast cancer effect along with the prediction of the mechanism of action using computational molecular docking approaches. Initial selection of the candidate ligands from a large number of phytochemicals of *A. precatorius* was squeezed to only abruquinones (A, B & C) by the analysis of all known and predicted chemicals those interact with the ERβ. Estriol, a natural substrate for ERβ, was used as a positive control and genistein being a natural ERβ inhibitor of isoflavones class with anti-breast cancer effect was used as a negative control. From an array of

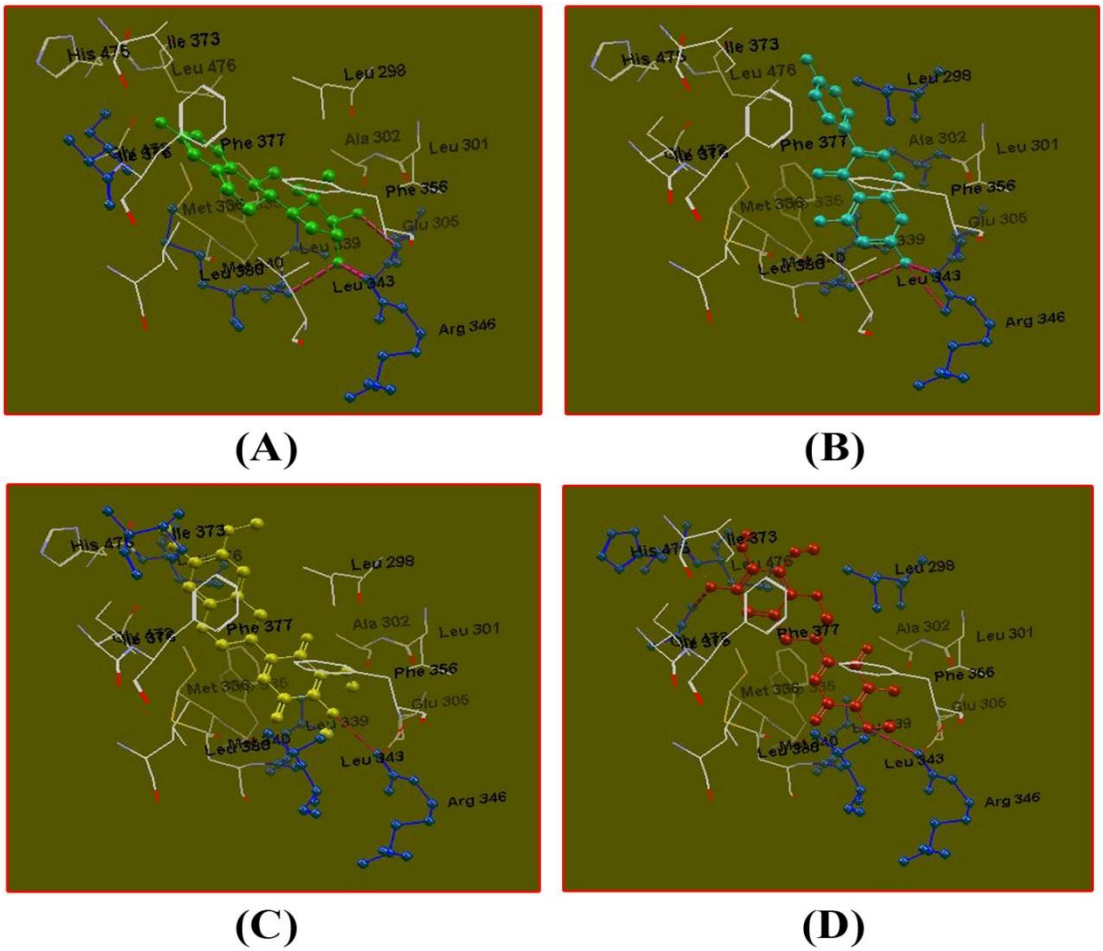

Figure 3. Docking poses of the ligands and receptor with the residues located within 4 A° around the pose ligand and interaction. Here, residue of receptor participate in hydrogen bonding with corresponding ligand are shown in blue color. **(A).** Best docking pose of estriol with receptor. The interaction includes hydrogen bond with Glu 305, Arg 346 and steric interaction with Leu 339, Met 340, Ile 376. (B). Best docking pose of genistein with receptor. The interaction includes hydrogen bond with Glu 305, Leu 339, Arg 346 and steric interaction with Leu 298, Thr 299 and Ala 302. (C). Best docking pose of abruquinone A with receptor. The interaction includes hydrogen bond with Arg 346 and steric interaction with Ile 373, Leu 339, Leu 343 and Leu 476. (D). Best docking pose of abruquinone C with receptor. The interaction includes hydrogen bond with Gly 472, Arg 346 and steric interaction with Leu 298, Leu 339, Leu 343, Arg 346, Gly 472, His 475 and Leu 476.

Table 2. Molecular property and predicted bioactivity of the ligands.

Ligands	Log P	TPSA	MW	NA	NV	NR ligand	GPCR ligand	EI
Abruquinone (32) A	1.035	80.312	360.362	26.0	0	0.05	-0.20	0.20
Abruquinone (18) A	1.035	80.312	360.362	26.0	0	0.05	-0.20	0.20
Abruquinone B	1.217	89.546	390.388	28.0	0	-0.06	- 0.21	0.18
Abruquinone C	0.91	100.54	376.361	27.0	0	0.05	-0.16	0.25

Here, MV= Molecular Volume; Log P = Octanol/water partition coefficient; MW= Molecular Weight; NA = Number of atoms; TPSA= Molecular Polar Surface Area; Nrotb = Number of Rotatable Bonds; NV= Number of violation of rule of five; NR= Nuclear receptor; EI= Enzyme inhibitor.

available functional information and literature search ligand binding domain of human ERβ was selected as the receptor. Before the docking study, a cavity (Coordinate: –29.91 × –7.67 × –30.33) was selected as probable ligand binding site from the synchronization of the result found from three different tools namely Pocket finder, GHECOM 1.0 and MVD cavity detection algorithm. The docking was performed by MVD software and ParDOCK online server. The docking result suggested abruquinone A, abruquinone B, abruquinone C as potential ligands as that of the natural substrate estriole for the ERβ. MolDock score clearly suggests abruquinone C has the best ligand potency with ample hydrogen bonding energy among the studied ligands, positive control estriole and negative control genistein. But ParDOCK score suggests abruquinone B has the best ligand potency. Thus, docking results suggested us to conclude that abruquinone B and/or abruquinone C might have stronger anticancer effect than that of the inhibitor genistein. In case of estriol interaction with receptor, the amino acid residue Glu 305 and Arg 346 were involved in hydrogen bonding while Leu 339, Met 340 and Ile 376 were involved in steric interaction. The amino acid residue Arg 346 was involved in the hydrogen bonding with the abruquinone's (C & A) interaction receptor. Abruquinone optimized Gly 472 at the binding site to the hydrogen bonding during interaction with receptor. The amino acid residue Leu 339 was a common residue involved in the steric interaction for abruquinone (C & A) and estriol. The overall hydrogen bonding and steric interaction of abruquinone and estriol also suggested a nearly similar ligand-receptor interaction pattern. Thus, the anticancer effect of abruquinones could be exerted by the antagonistic effect on the receptor. The candidate ligands were also validated by the calculation of the Drug-likeness property using online molecular property calculation. Ligand validation exhibited that all the studied ligands have a potency of drug like molecule with zero violation of Lipinski's rule of five or drug likeness rule. But interestingly biological property search provided us an extra filtering for the most efficient ligand molecule with abruquinone C being more potent ligand than that of the abruquinone B.

CONCLUSION

Our study demonstrates that abruquinones from *A. precatorius* have anticancer potential against breast cancer. We propose that antagonistic effect of abruquinone is imparted to the ligand (estrogen)-binding domain of the estrogen receptor as possible mechanism of action. This study also provides a scientific basis of traditional use of *A. precatorius* in cancer treatment. However, more detailed experiments involving the breast cancer specific animal and human study are required for the precise and evidence based therapeutic evaluation.

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REFERENCES

1. Acharya, B., Roy, S. 2013. A review on therapeutic utilities and purificatory procedure of gunja (Abrus precatorius Linn.) as described in ayurveda. *Research & Reviews: J. Agric.l Sci. Technol.* **2**, 2278 – 2206
2. Bhatia, M., Siddiqui, N. A., Gupta, S. 2013. Abrus Precatorius (*L.*): An evaluation of traditional Herb. *Indo Am. J. Pharmaceu. Res.* **3**, 3295-3315.
3. Gupta, A., Gandhimathi, A., Sharma, P., Jayaram, B. 2007. ParDOCK: An All Atom Energy Based Monte Carlo Docking Protocol for Protein-Ligand Complexes. *Protein and Peptide Letts.* **14**, 632-646.
4. Huang, S. Y., Zou, X. 2006. An iterative knowledge-based scoring function to predict protein-ligand interactions: I. Derivation of interaction potentials. *J. Comput. Chem.* **27**, 1866-1875.
5. Kawabata, T. 2010. Detection of multi-scale pockets on protein surfaces using mathematical morphology. *Proteins* **78**, 1195-1121
6. Kleywegt, G. J., Harris, M. R., Zou, J. Y., Taylor, T. C., Wählby, A., Jones, T. A. 2004. The Uppsala Electron-Density Server. *Cta. Cryst.* **D60**, 2240-2249.
7. Kuhn, M., Szklarczyk, D., Franceschini, A., Mering, C. V., Jensen, L. J., Bork, P. 2012. STITCH 3: zooming in on protein–chemical interactions. *Nucl. Acids Res.* **40**(D1), D876-D880.
8. Kuiper, G. G., Enmark, E., Pelto-Huikko, M., Nilsson, S., Gustafsson, J. A. 1996. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci U.S.A.* **93**, 5925–5930.
9. Lamartiniere, C. A. 2000. Protection against breast cancer with genistein: a component of soy. *Am. J. Clin. Nutr.* **71**, 1705s-1707s.
10. Lee, H. R., Kim, T. H., Choi, K. C. 2012. Functions and physiological roles of two types of estrogen receptors, ERα and ERβ, identified by estrogen receptor knockout mouse. *Lab Anim Res* **28**, 71-76.
11. Liu, X., Suzuki, N., Laxmi, Y. R., Okamot, Y., Shibutani, S. 2012. Anti-breast cancer potential of daidzein in rodents. *Life Sci.* **9**, 415-419.
12. Marik, R., Allu, M., Anchoori, R., Stearns, V., Umbricht, C. B., Khan, S. 2011. Potent genistein derivatives as inhibitors of

estrogen receptor alpha-positive breast cancer. *Cancer Biol Ther* **11**, 883-892.
13. Mosselman, S., Polman, J., Dijkema, R. 1996. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* **392** (1), 49–53.
14. Murray, R. K., Granner, D. K., Mayes, P. A, Rodwell, V. W. 2000. Harper's Biochemistry. 25th ed. Appleton & Lange, Connecticut, pp. 534-549.
15. Navolanic, P. M., McCubrey, J. A. 2005. Pharmacological breast cancer therapy (review). *Int J Oncol* **27**, 1341-1344.
16. Pettersson, K., Gustafsson, J. A. 2001. Role of estrogen receptor beta in estrogen action. *Annu Rev Physiol* **63**, 165-192.
17. Rahman, M. M., Khan, M. A. 2013. Anti-cancer potential of South Asian plants. *Nat Prod Bioprospect* **3**, 74-88.
18. Ross, I. A. 2003. Medicinal Plant of the world. Volume 1: Chemical Constituents, Traditional and Modern Medicinal Use. 2nd ed. Humana Press, China, pp. 15-31.
19. Saji, S., Hirose, M., Toi, M. 2005. Clinical significance of estrogen receptor beta in breast cancer. *Cancer Chemother Pharmacol* **56**, 21-26.
20. Schwab, C. H. 2010. Conformations and 3D pharmacophore searching. *Drug Discovery Today: Technologies* **7**, e245-e253.
21. Sofi, M. S., Sateesh, M. K., Bashir, M., Harish, G., Lakshmeesha, T. R., Vedashree, S., Vedamurthy, A. B. 2013. Cytotoxic and pro-apoptotic effects of *Abrus precatorius* L. on human metastatic breast cancer cell line, MDA-MB-23. *Cytotechnology* **65**, 407-417.
22. Thomsen, R., Christensen, M. H. 2006. MolDock: A New Technique for High-Accuracy Molecular Docking. *J Med Chem* **49**, 3315-3321.
23. Vriend, G. 1990. WHAT IF: a molecular modeling and drug design program. *J Mol Graph* **8**(1), 52-56.