

**ANTICANCER POTENTIAL OF ISOLATED PHYTOCHEMICALS  
FROM *MACARANGA DENTICULATA* AGAINST BREAST CANCER: IN  
*SILICO* MOLECULAR DOCKING APPROACH**

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**ABSTRACT**

Cancer can be described as the uncontrolled growth of abnormal cells. Breast cancer is the second most common type of cancer after lung cancer. Normal breast cells and most breast cancer cells have receptors that attach to circulating estrogen. Estrogen bind to the receptors and may work with growth factors (e.g., oncogenes and mutated tumor suppressor genes) to cause cancer cell growth and proliferation. Over expression of estrogen receptor is seen in number of cases of breast cancer. The Protein- Ligand interaction plays a significant role in structural based drug designing. The aim of this study was to screen out the effective bioactive compounds from *Macaranga denticulata* namely 3-acetylauritic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol, which may be potential

inhibitors of estrogen receptor alpha (ER- $\alpha$ ) for searching a drug against the breast cancer. A wide range of docking score found during molecular docking by Schrodinger. 3-acetylauritic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol showed the docking score -4.482, -5.795, -6.647, -2.406, -6.569, -5.822 respectively. Among all the compounds macarangenin showed best docking score towards estrogen receptor alpha.

Article Received on  
19 Aug. 2016,

Revised on 09 Sept. 2016,  
Accepted on 29 Sept. 2016

DOI: 10.20959/wjpr201610-7183

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So, macarangin is the best compounds for selective inhibitors of estrogen receptor alpha, as it possessed best value in Molecular docking. Further *in vivo* investigation need to identify estrogen receptor alpha inhibitory activity of isolated compounds from *Macaranga denticulata*.

**KEYWORDS:** *Macaranga denticulata*, estrogen receptor alpha, Breast cancer, Caryophyllene oxide, Ampelopsin H.

## INTRODUCTION

Breast cancer is the most frequently occurring cancer in women.<sup>[1]</sup> Age is the strongest risk factor for breast cancer. Unlike many cancers that increase beginning at the end of the fifth decade of life, breast cancer begins to rise in the third decade of life, most likely due to the effects of ovarian hormones on breast tissue.<sup>[2-4]</sup> Up to now, many additional chance factors for breast cancer were identified. Some chance factors are non-modifiable, inclusive of age, BRCA1 and BRCA2 gene mutations; own family records, reproductive records, and excessive-dose radiation to the chest. Others are probably modifiable, together with excessive endogenous estrogens, hormone remedy, obesity (for postmenopausal breast cancer) and alcohol consumption.<sup>[3]</sup> There may be a few controversy concerning whether or not or not the hazard factor of excessive mammographic density is modifiable.<sup>[5-7]</sup> ER signaling is complicated. ER is known to associate with numerous cofactors that act at multiple levels, including transcription, translation and even posttranslation. The classical estrogen pathway is the direct binding of estrogen-responsive elements by using ligand-activated ER to alter gene expression. Estrogen may also act as a coactivator of other transcription elements to show on oncogenes in breast most cancers in the nonclassical pathway.<sup>[8-10]</sup> Moreover, estrogen can stimulate fast, extranuclear (nongenomic) signaling occasions, along with the activation of the Src/Ras/Erk signaling pathway.

Molecular docking methodologies are of terrific importance in the making plans and layout of new drugs. These strategies goal to expect the experimental binding mode and affinity of a small molecule within the binding site of the receptor target of interest. A successful docking methodology must be able to correctly predict the native ligand pose the receptor binding site (i.e. to find the experimental ligand geometry within a certain tolerance limit) and the associated physical-chemical molecular interactions.<sup>[11]</sup>

*Macaranga denticulata* (Bl.) Muell. Arg. is a tree with rich sources in Hainan and Xishuangbanna regions of China. Its roots had been used as traditional Chinese remedy in opposition to icteric hepatitis, eczema and epigastric ache. Previous phytochemical studies in this plant resulted within the isolation of isoprenylated flavonoids and diterpenylated flavonoids or stilbenes, some of which confirmed antioxidant, acetylcholinesterase-inhibiting, and antiangiogenic activities.<sup>[12]</sup>

The aim of this study was to screen out the effective bioactive compounds from *Macaranga denticulata*, which may be potential inhibitors of estrogen receptor alpha (ER- $\alpha$ ) in future and may act as a drug which may be effective in preventing the breast cancer.

## MATERIALS AND METHODS

### Protein Preparation

Three dimensional crystal Structure of estrogen receptor alpha (PDB id: 3ERT) was downloaded in pdb format from the protein data bank.<sup>[13]</sup> After that, structure was prepared and refined using the Protein Preparation Wizard of Schrödinger-Maestro v10.1. Charges and bond orders were assigned, hydrogens were added to the heavy atoms, selenomethionines were converted to methionines, and all waters were deleted. Using force field OPLS\_2005, minimization was carried out setting maximum heavy atom RMSD (root-mean-square-deviation) to 0.30 Å.

### Ligand Preparation

Compounds were retrieved from Pubchem databases, i.e. 3-acetylauritic acid (CID161616),  $\beta$ -Sitosterol (CID 222284), macarangenin (CID 10047854), oleanolic acid (CID 10494), scopoletin (CID 5280460) and stigmasterol (CID 5280794). The 3D structures for these were built by using Ligprep2.5 in Schrödinger Suite 2015 with an OPLS\_2005 force field. Their ionization states were generated at pH7.0 $\pm$ 2.0 using Epik2.2 in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

### Receptor grid generation

Receptor grids were calculated for prepared proteins such that various ligand poses bind within the predicted active site during docking. In Glide, grids were generated keeping the default parameters of van der Waals scaling factor 1.00 and charge cutoff 0.25 subjected to OPLS 2005 force field. A cubic box of specific dimensions centred around the centroid of the

active site residues (Reference ligand active site) was generated for receptor. The bounding box was set to  $14 \text{ \AA} \times 14 \text{ \AA} \times 14 \text{ \AA}$  for docking experiments.

### Glide Standard Precision (SP) ligand docking

SP flexible ligand docking was carried out in Glide of Schrödinger-Maestro v 10.1<sup>[14,15]</sup> within which penalties were applied to non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff was selected to be 0.80 and 0.15, respectively for ligand atoms. Final scoring was performed on energy-minimized poses and displayed as Glide score. The best docked pose with lowest Glide score value was recorded for each ligand.

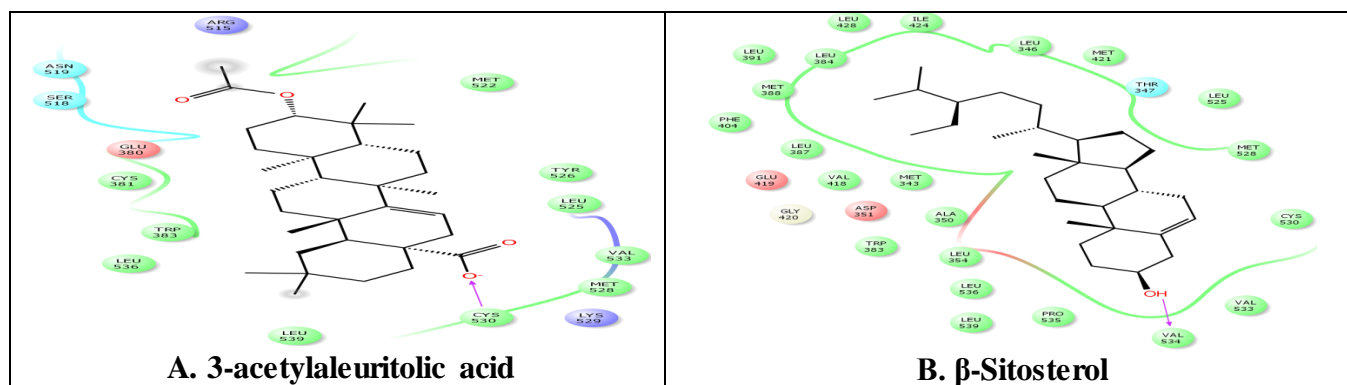
## RESULTS

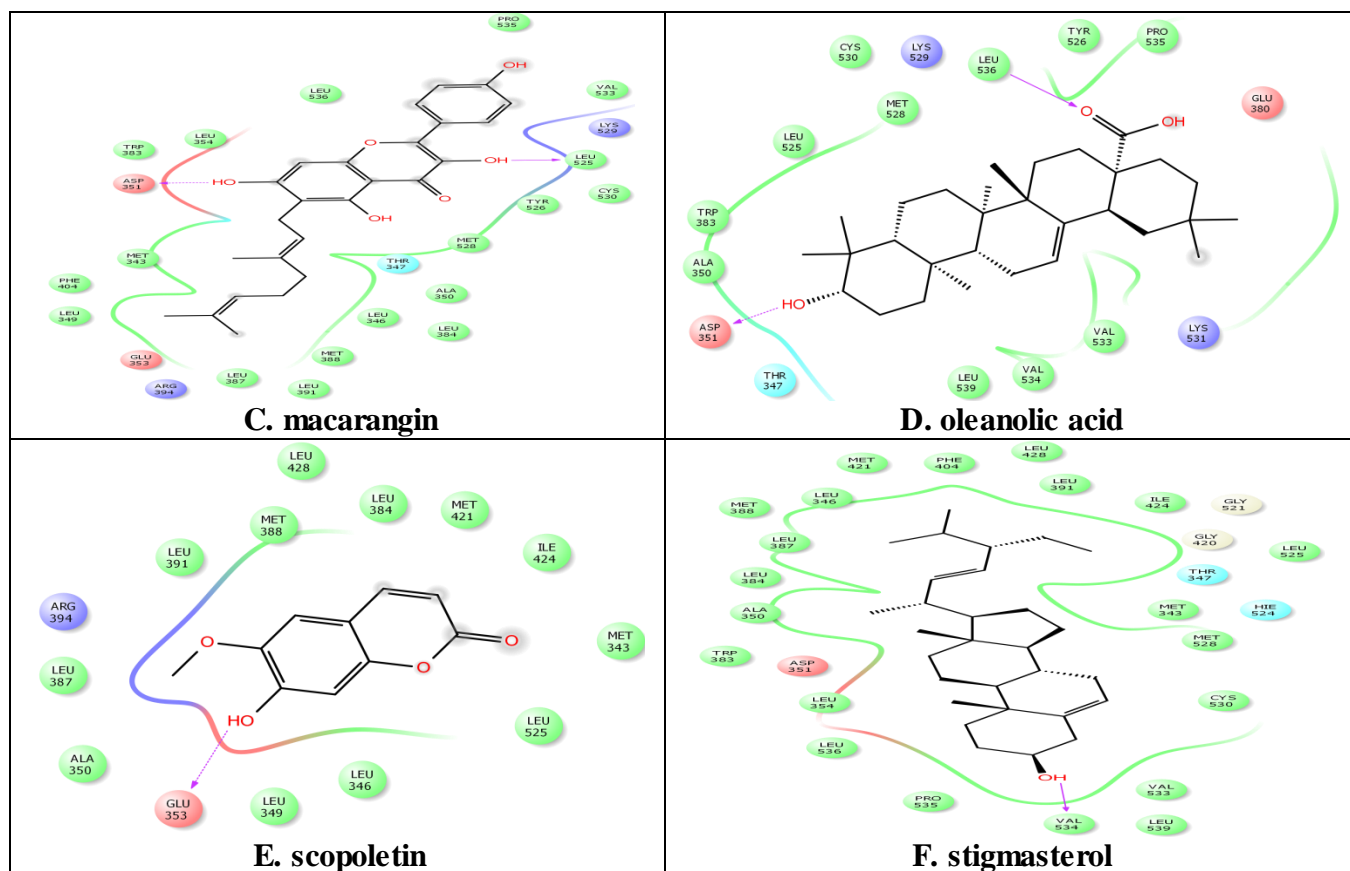
### *In silico* Molecular Docking analysis

In order to study the interaction of the compounds 3-acetylaleuritolic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol with estrogen receptor alpha (ER- $\alpha$ ), we performed Glide docking analysis by Schrodinger suite v10.1, where among of these compounds macarangenin shows highest docking score shown in Table 1. The negative and low value of free energy of binding demonstrates a strong favorable bond between 3ERT and macarangenin in most favourable conformations. The results of docking analysis were described in Table 1 and the docking figure showed in Figure 1.

**Table 1: Docking results of 3-acetylaleuritolic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol with estrogen receptor alpha (PDB: 3ERT).**

Compound Name	Docking Score	Glide emodel	Glide energy
3-acetylaleuritolic acid	-4.482	-46.727	-38.253
$\beta$ -Sitosterol	-5.795	-25.858	-26.883
macarangenin	-6.647	-62.161	-47.478
oleanolic acid	-2.406	-49.46	-37.3
scopoletin	-6.569	-42.165	-30.13
stigmasterol	-5.822	-26.014	-26.239





**Figure 1: Docking results of A. 3-acetylauritic acid, B.  $\beta$ -Sitosterol, C. macarangin, D. oleanolic acid, E. scopoletin, F. stigmasterol with estrogen receptor alpha (PDB: 3ERT).**

## DISCUSSION

Breast cancer is known as a death sentence and second major cause of death in world. Ratio of breast cancer in is one in nine in case of women.<sup>[16]</sup> Main cause of breast cancer is over expression of estrogen receptor alpha.<sup>[17]</sup> Therefore ER- $\alpha$  is used as a target for prevention of breast cancer. Tamoxifen is an antagonist of ER- $\alpha$  and commercially available as a drug to control the breast cancer.<sup>[18]</sup> It binds with Arg394 and blocks the function of estrogen receptor and inhibits the function of ER- $\alpha$ .<sup>[19]</sup>

Molecular docking discovers the binding geometry of two interacting molecules with known structures. It predicts the preferred orientation of receptor and ligand to each other to form a stable complex.<sup>[20]</sup> Currently, the use of computers to determine the binding of datasets of small molecules to known receptors is a major component of drug discovery. In recent research, computer aided drug designing (CADD) helps the researcher to decrease the time and money for drug designing projects.<sup>[21]</sup> Molecular docking is very helpful in studying the

interactions of ligand molecules with the target protein before its *in vitro* synthesis. Docking is performed through computer programs like Maestro.

The current investigation showed the behavior of protein ligand complex of Human estrogen receptor with phytochemicals. All the phytochemicals (Figure 1) included in this study were docked in the catalytic binding site.

To screen out the effective bioactive compounds from *Macaranga denticulata* namely 3-acetylauritic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol, which may be potential inhibitors of estrogen receptor alpha (ER- $\alpha$ ) for searching a drug against the breast cancer. We performed Glide docking analysis by Schrodinger suite v10.1. A wide range of docking score found during molecular docking. 3-acetylauritic acid,  $\beta$ -Sitosterol, macarangenin, oleanolic acid, scopoletin, stigmasterol showed the docking score -4.482, -5.795, -6.647, -2.406, -6.569, -5.822 respectively. Among of these compounds macarangenin shows highest docking score shown in Table 1. The negative and low value of free energy of binding demonstrates a strong favorable bond between 3ERT and macarangenin in most favourable conformations.

## CONCLUSION

In conclusion, we have simulated six designed compounds by molecular docking approach. Among all the compounds macarangenin showed best docking score towards estrogen receptor alpha. So, macarangenin is the best compounds for selective inhibitors of estrogen receptor alpha, as it possessed best value in Molecular docking. Further *in vivo* investigation need to identify estrogen receptor alpha inhibitory activity of isolated compounds from *Macaranga denticulata*.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## ACKNOWLEDGMENT

The authors thank to GUSTO A Research Group for providing the software and the financial support.

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