

In silico estrogen receptor alpha antagonist studies and toxicity prediction of Melia azedarach leaves bioactive ethyl acetate fraction

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ORIGINAL ARTICLE

In silico estrogen receptor alpha antagonist studies and toxicity prediction of *Melia azedarach* leaves bioactive ethyl acetate fraction

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ABSTRACT

The estrogen hormone dependent accounts for a major cause in the incidence of women breast cancer. Thus, their receptor, especially the estrogen receptor α (ER- α), is becoming a target in endocrine treatment. These ligand-inducible nuclear functions are regulated by an array of phytochemical and synthetic compounds, such as 17 β -estradiol and tamoxifen (4-hydroxytamoxifen [4OHT]). The Chinaberry (*Melia azedarach*) leaves are known naturally for relieving internal and external diseases. Previous studies revealed the potency of *Melia*'s ethanolic extract and ethyl acetate fractions as anticancer; furthermore, this study aimed to resolve possible ER- α antagonist's mechanism and safety from *M. azedarach* leaves ethyl acetate fraction contents. *Melia*'s phytochemical content was analyzed with electrospray ionization liquid chromatography-mass spectrometry, while its ER- α antagonist's potency was investigated by *in silico*. The computational docking was used to 3ERT (a human ER- α -4OHT binding domain complex) with Autodock Vina and related programs. The results presented Energy binding (ΔG) of *Melia*'s quercetin 3-O-(2',6"-digalloyl)- β -D-galactopyranoside was similar to 4OHT, and lower than its agonist 17 β -estradiol. Furthermore, the toxicity prediction of these compounds were revealed safer than 4OHT. The *Melia*'s leaves ethyl acetate fraction, therefore, is a potential pharmacological material for further studies.

Key words: Estrogen receptor α , flavonoids, limonoids, *Melia azedarach*, steroids

INTRODUCTION

Estrogen, an ovarian steroid hormone, performs by binding to estrogen receptor (ER). The ER antagonists and inhibitors are the most targeted ligands and widely used in breast cancer endocrine therapies, including for

ER-positive metastatic breast cancer patients.^[1] The ERs are ligand-inducible receptors, such as biosynthesized agonist 17 β -estradiol (EST); in addition to antagonist synthetic selective ER modulators, tamoxifen. The latter is often given as an adjuvant treatment for postmenopausal tumor for ER- α -positive individuals and has proved decreasing breast cancer relapse. The ER-tamoxifen complex inhibits the proliferation of the cancer cell, though accompanied with mild-to-severe side effects.^[2] Furthermore, many ER-positive patients develop intrinsic resistance to hormonal therapies, and this calls for superior alternative treatments.

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The selective estrogen ligands were reported to be highly promising targets. These included some analog and phytoestrogen compounds, which bind ERs and exhibit estrogenic or antiestrogenic activities.^[3] Moreover, their antiproliferative properties could be useful for breast cancer therapies. Genistein isoflavones have similar structures as 17 β -estradiol (EST) [Figure 1], which may be responsible for the same active binding sites to ER, thereby producing estrogenic activity. The *Eugenia aqua* leaves dimethylchalcone derivate is reported to not only inhibit MCF-7 human breast cell proliferation but also yield apoptosis through poly-adenosine diphosphate ribose polymerase activations.^[4]

Some *in silico*, semi-to-synthetic research studies have been reported on phytoconstituents in antiestrogen by, among others, modifying their structures to improve activities and selectivities or to reduce the side effects. Powers and Setzer^[5] presented the molecular docking of a wide variety of secondary estrogenic metabolites (e.g., flavonoids, isoflavonoids, phenolics, steroids, and triterpenoids) from dietary herbal supplements. They found the phenolics as the strongest to bind ER, while triterpenoids were the weakest among the metabolites.

Chinaberry, *Melia azedarach*, has been used for traditional medicine in some parts of Asia. Various *in vitro* to *in vivo* studies showed *Melia* leaves with ranges of activities.^[6] The *Melia's* leaves extract has the highest yield compared to other plant parts and classified as safe to use as IC₅₀ >1000 μ g/mL against Vero cells.^[7] Thus, some flavonol glycosides, steroids, and limonoids have been isolated from its extract.^[8] Moreover, previous researches resulted in highest antioxidant and cytotoxic activities of ethyl acetate fraction. The leaves had potency to some hormonal-dependent cell lines, such as breast cancer MCF7 and T47D.^[8,9] The wide uses of *Melia* leaves depend, among others, on understanding the possibility mechanisms of the leaves' secondary metabolites to bind with ER- α . Thus, the research will give a scientific base to traditionally uses of the leaves

and supporting to further work on their safety to efficacy as breast cancer adjuvant/co-chemotherapy alternatives. The present study reports findings along these lines.

MATERIALS AND METHODS

Extraction and liquid chromatography-mass spectrometry analysis of the extract

The extraction method and the liquid chromatography-mass spectrometry (LC-MS) analysis were followed as described by Erвина *et al.*^[9] The *M. azedarach* leaves extract was prepared with ethanol 95%, followed with n-hexane and ethyl acetate solvents fractionation. The ethyl acetate was purified with solid-phase extraction (Qasis® HLB solvents) and analyzed with gradient mobile phase step of water, ammonium formic, acetonitrile, and formic acid (0.1%) at 9.2 mL/min for 23 min on EI LC-MS system (Acquity UPLC®-H and Xevo G2-S QTof detector, Waters, Milford, USA), respectively. The chromatogram was processed with MassLynx 4.1 program and data in mass bank (Fiehnlab), Pubchem, or Chempidder.^[10-17]

Preparation of ligands

The *Melia's* ligands [Table 1] were prepared with a computer as described in Pratama *et al.*^[18-20] The HyperChem 7.5 (Hypercube Inc.), OpenBabel 2.4.1 (OpenBabel.org.), and AutoDockTools 1.5.6 and UCSF Chimera 1.13.1 (University of California, San Francisco) program were used.

Preparation of receptor

The pdb format of human ER- α (3ERT) in complex with 4-hydroxytamoxifen (4OHT) as original ligand was extracted from the protein data bank.^[21,22] It has 1.90Å resolution and prepared further with AutoDockTools 1.5.6. The prework orientation was done to obtain the lowest RMSD value below 2Å in the grid box parameter.^[23]

Validation of docking process

The PyMOL 2.3.1 (Schrodinger LLC.) program was used for proofing the docking processes.^[18]

Molecular docking

The docking was proceeded with Autodock Vina 1.1.2.^[18] and resulted free energies (ΔG in a.pdb). The highest ligand affinity was compared to the co-crystal ligand validation result.^[21] Another parameter was the similarities of amino acid residues and bonding type of their interactions. The Discovery Studio Visualizer v. 19.1.0.18287 was used to visualize them.

The toxicity prediction of the most potential substances

The SMILES format of the three most potential, quantified, and reference compounds were submitted to GUSAR servers.^[18]

RESULTS AND DISCUSSION

As previously obtained, the LC-MS peaks spectra were analyzed

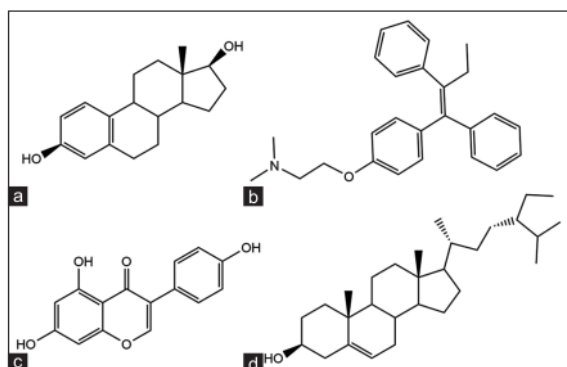


Figure 1: Typical estrogen receptor alpha antagonist ligands (a) estrogen, (b) tamoxifen, (c) genistein, and (d) β -sitosterol

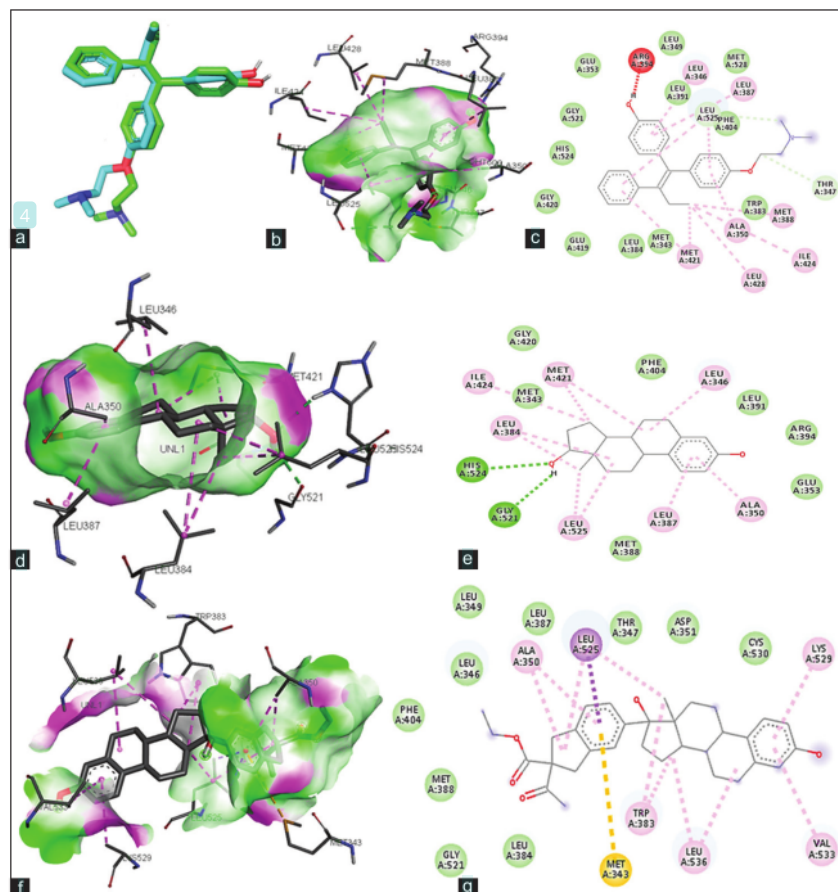


Figure 2: The molecular structure of (a) 4-hydroxytamoxifen redocking, (b and c) 3ERT-4OHT binding interaction, (d and e) 3ERT-17 β -estradiol (agonist) and (f and g) 3ERT-quercetin-3-*o*-digalloyl)- β -*d*-galactopyranoside

similar to its co-crystal ligand, 4OHT; while lower than its agonist EST (-9.9 and -9.4 kcal/mol). The phenolic glycosides have a variety of ΔG results, such as 3-*O*- and 7-*O*-substituents. The QOG observed has a better potency to bind ER- α than its 7's (9.9 compared to 7.7). The potency of QOG was also better than *Melia*'s steroid or limonoids. As highlighted above, the binding exothermic energy indicates a higher affinity of ligand binding to 3ERT active sites.

The three dimensions of 4OHT structures [Figure 2a] show interactions to 3ERT with 22 bonds, which consist of hydrogen (H) bonds on 347-Thr and 525-Leu [Figure 2b and c]. The rest bindings consist mostly of Van der Waals (VdW) forces, Alkyl/Pi-Alkyl (A/P) bonds, and unfavorable bump/donor-donor (Ubd) on 394-Arg. Meanwhile, the reference EST-3ERT complex interactions had 16 total bindings with 36.6% and 72.73% similarities on interactions and amino acid residues binding to its original ligand. They are hydrogen bonds (521-Gly and 524-His) [Figure 2d and e]. This phenomenon was interesting since this bind was originally

VdW bonds. There were also some changes from H to VdW bonds and to weaker A/P bonds and vice versa, while Ubd changed to VdW bond.

The potency of QOG had 17 total bindings with 40.91% and 18.18% similarities of amino acid residues and binding types [Figure 2f and g]. Most of them were A/zA bonds. Interestingly, when compared to 4OHT, there were also converting type of binding from H to VdW (347-Thr), VdW to $\pi\delta$ (383-Trp), A/ π A (343-Met and 384-Leu), from VdW to H (525-Met), and vice versa from π -alkyl to VdW. The disappearance of VdW bond was observed on many sites such as 353-Glu, 391-Leu, 404-Phe, 419-Glu, 420-Gly, 521-Gly, and 524-His; though some new bindings were on amino acid residues above 529. Moreover resulted, limonoids (1-CHM, TSN) had lesser % similarities, respectively.

The ER as nuclear receptor (NR) superfamily is a ligand-activated transcription factor. Its two dimer isoforms have distinctive regions of the DNA and ligand-binding domain. The difference region of the hormone-receptor

complex binding can trigger receptor conformational changes. The ER combines with specific DNA-response element and transcription cellular components, such as co-regulators with derived activation or repression of the lead genes. There are some parts on the ER regions protein named activation functions (AF1 and AF2), which are related to the co-regulators production. Varieties of agonist or antagonist ligands would bind ER. Among the ER residues, the position of helix 12 (H12) will determine agonist or antagonist effects. In the ER-EST complex, H12 lies across the ligand-binding cavity,^[24] while in the ER-4OHT complex, H12 hinders co-activator recognition grooves by imitating interaction NR box peptides, while the LBD hinders the co-activator. These authors found different LBD ER-4OHT secondary and tertiary conformations compared to ER-DES (diethylstilbestrol, a pure agonist ligand). In 4OHT complexes, the main bonds adopt extended conformations and are different in the formation and position of H12. In 4OHT complexes, H12 is composed of residues 536–544. Furthermore, the complexes occupy the part of the coactivator-binding groove formed by residues 3, 4, and 5 and the turn connecting helices 3 and 4. The ER-ligand bindings on His-524 determine agonist or antagonist of the ligand. His-524 H bonds were observed on EST, but VdW bond on 4OHT and no interactions on the ligands tested. The high affinities in ER- α -QOG complexes indicated the highest similarities amino acid residue to 4OHT as describe in Figure 2b-c and 2f-g.

The GUSAR servers were made to obtain the QSAR/QSPR model based on the ligands data. The results were most in applicable in the model use. The peroral (po) data were observed have higher dose than others [Figure 3a], this means the po route has wider safety margin than others. The ligands have a wide range of LD₅₀ prediction parameter for compound toxicity [Figure 3a]. All compounds have LD₅₀ lower than 5000 mg/kg/day. Therefore, they were classified into 4–5 class. This means that the substances may be slightly toxic if swallowed.^[25]

Moreover, this study provided the potential for adverse effects of most test molecules. The graph describes their IC₅₀ of the potential adverse effect of the compounds [Figure 3b]. The GUSAR antitarget scheme (in applied model) not only predicts the adverse effect but also provides the potential activity of the compounds. Thirteen receptors were hydroxytryptamine (HT) 1B and 2A, 2C, adrenergic α 1A, 1B and 2A (ADR), androgen (AND), dopamine (D) D1A, D3, opioid (O) Δ , μ , κ and estrogen (ER). The 5-H2ARA was an example of receptor related to the central nervous system; thus, its antagonist interactions would affect on sickness, emesis, diarrhea, sleeplessness, and anxiety. The ADRA (α -blockers) may cause disturbances on heart rate, blood tension, nasal congestion, to sleep disturbances and so on. Many adverse effects related to the ER antagonist action are noted such as depression, headache, obesity,

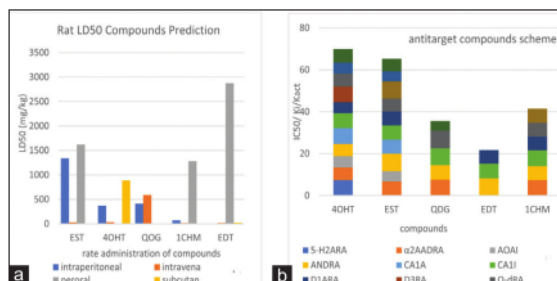


Figure 3: Toxicity and some adverse effect predictions of the *Melia azedarach* potential compounds (a) LD₅₀ parameter, (b) antitarget scheme of the compounds, 5 H2ARA: 5 hydroxytryptamine 2A receptor antagonist, α 1ADRA: Alpha 1a adrenergic receptor antagonist, AOA: Amine oxidase A inhibitor, AND: Androgen receptor antagonist, CA1A: Carbonic anhydrase I activator, D1ARA: D(1A) dopamine receptor antagonist, O dRA: Delta type opioid receptor antagonist, ER: Estrogen receptor alpha antagonist, Na Cl GABA1A: Sodium and chloride dependent GABA transporter 1 antagonist, Na dRA: Sodium dependent serotonin transporter antagonist

and hot flashes. Other compounds' interactions were to enzymes (AOIA) and transporters (serotonin, GABA, and dopamine) [Figure 3b, respectively]. The main adverse effects of AOIA inhibitors are blood pressure instability to hepatotoxicity, while severe anaphylaxis to bone marrow suppression would have been caused by CA1A. The GABA and dopamine transporter blockers were caused acute respiratory distress syndrome. Among the tested ligands, it was 4OHT showed 17 cell antitargets, while the sequences from the least were the EDT (5), QOG (8), and 1CHM (13).

These findings implied that the EDT is relatively safer compare to others. The side effects of the QOG flavonoid glycoside may occur on hormone and neurotransmitter signaling disorders due to binding with ADR, while 4OHT and EST may result in nervous system and fertility disorder because they interfere with AOA, AND and ER [Figure 3b].^[26,27] These findings highlighted the molecular potency, interaction mechanisms, and safety prediction of *M. azedarach* phytochemicals.

CONCLUSION

The *Melia's* quercetin-3-O-digalloyl- β -D-galactopyranoside, limonoid, and steroid possessed low binding energy and measured inhibitory effects (antagonists) interactions on 3ERT. These compounds were found safer than 4OHT.

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Conflicts of interest

There are no conflicts of interest.

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