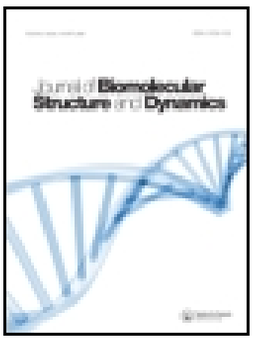

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In silico targeting breast cancer biomarkers by applying rambutan (*Nephelium lappaceum*) phytocompounds

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ABSTRACT

Worldwide, breast cancer is the leading type of cancer among women. Overexpression of various prognostic indicators, including nuclear receptors, is linked to breast cancer features. To date, no effective drug has been discovered to block the proliferation of breast cancer cells. This study has been designed to discover target-based small molecular-like natural drug candidates that have anti-cancer potential without causing any serious side effects. A comprehensive substrate-based drug design was carried out to discover the potential plant compounds against the target breast cancer biomarkers including phytochemicals screening, active site identification, molecular docking, pharmacokinetic (PK) properties prediction, toxicity prediction, and molecular dynamics (MD) simulation approaches. Twenty plant compounds extracted from the rambutan (*Nephelium lappaceum*) were obtained from PubChem Database; and screened against the breast cancer biomarkers including estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR). The best docking interaction was chosen based on the higher binding affinity. Analyzing the pharmacokinetic properties and toxicity prediction results indicated that the fifteen selected plant compounds have good potency without toxicity and are safe for humans. Four phytochemicals with a higher binding affinity were chosen for each breast cancer biomarker to study their stability in interaction with the target proteins using MD simulation. Among the above compounds, Ellagic acid showed the high binding affinity against all three breast cancer biomarkers.

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KEYWORDS
Breast cancer; virtual screening; pharmacokinetics; ADME; MD simulation

1. Introduction

Breast cancer is the most prevalent type of malignancy leading to death among women. The World Health Organization (WHO) report demonstrated that 2.3 million breast cancer cases are diagnosed worldwide in 2020 (GLOBOCAN, 2018; Supramaniam & Elengoe, 2020; World Health Organization, 2018). One woman out of nineteen is at risk of breast cancer while almost 50 percent of people diagnosed with breast cancer are under 50 years of age (Nordqvist, 2017). Knowledge of risk factors is insufficient, but strong evidence has been discovered about the significance of family history and diet as risk factors. As for therapy, options for surgery and chemotherapy, in particular, are uncertain to the public, the public is vaguely unknowing about quitting smoking to avoid early breast cancer occurrence. With improved understanding, early diagnosis can be done, as it results in a better prognosis and decreased risk (Verma et al., 2012).

Breast cancer is essentially a chronic hormone-dependent disorder. Estrogen and progesterone are closely connected with breast cancer pathogenesis. Their nuclear receptors such as estrogen receptor (ER), progesterone receptor (PR), and androgen receptor (AR) are the most vital biomarkers for breast cancer (Higa & Fell, 2013; Omoto & Iwase, 2015; Sever & Glass, 2013). These three biomarkers are the prognostic factors that help in the detection and diagnosis of breast cancer.

While tremendous strides have been made in diagnosing and managing breast cancer growth, there are still major gaps and scope for the development site. Perhaps, there are a variety of unwanted harmful consequences during chemotherapy. Natural treatments can eliminate harmful negative impacts, such as the use of plant-derived products in cancer care. A few herbal drugs have been used to cure cancer. Acetogenin is one of the chemical constituents found in the fruit of *Annona muricata*. It is a type of polyketide natural product. Based on Rady et al.'s study (2018), it has been reported that it reduced the size and weight of the breast tumor while exhibiting anti-metastatic properties and apoptosis induction activities *in vitro* and *in vivo* (Rady et al., 2018). Beta-sitosterol is a phytosterol found in plants such as

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carrots, soybeans, corn oils, peanuts, etc. Jordan et al. (2013) study showed that beta-sitosterol decreased the cell viability of breast cancer cell line (MCF-7) (Jordan et al., 2013). Beta-sitosterol was extracted from ethyl acetate of Daucus *carota*. It exhibited cytotoxicity at an IC_{50} value (107) of 112 μg/ml. According to Wattanathorn et al. (2018) study, *Pandanus amaryllifolius* leaves decreased viability by inhibiting proliferation in MCF-7 and MDA-MB-231 cells (Wattanathorn et al., 2018). Pandan leaves consist of propylene glycol which is a phytochemical constitute.

Nephelium lappaceum is a fruit that contains plant compounds with medicinal properties. It has significant pharmacological activities such as anti-cancer, anti-diabetic, anti-viral, anti-bacterial, anti-oxidant, anti-inflammation, anti-allergic, etc. It is also known as the rambutan. It can be found in Southeast Asia countries (Malaysia, Thailand, Indonesia, and the Philippines) and Central America regions (Costa Rica, Guatemala, Honduras, Mexico, and Panama).'The phenolic compound is the main secondary metabolite present in rambutan. Based on the studies by George et al. (2004) and Pande and Akoh (2009), they have been demonstrated that phenolic plant compounds are majorly found in the skin or peel than in the pulp and seed of fruits. Sun et al. (2011) study revealed that phenolic compounds such as phenolic acids, hydroxycinnamic acids, and flavan-3-ols such as gallic acid, p-coumaric acid, catechin, and rutin are present in the peel (Sun et al., 2011). According to Hervert-Hernández et al. (2009) study, rambutan inhibited cell proliferation and metastasis of breast tumors. It possessed 40% cytotoxicity and 100% inhibition at a concentration of 8 μg/mL (Hervert-Hernández et al., 2009). Khaizil Emylia et al. (2013) reported that methanol peel extract of yellow variety of rambutan showed anti-cancer activity against breast cancer cell line (MDA-MB 231) (Khaizil Emylia et al., 2013). The cytotoxicity assay results showed that the rambutan extract exhibited an IC_{50} value of 5.42 ± 1.67 μg/ml for MDA-MB 231. Chinnici et al. (2004) study demonstrated that rambutan peel extract exhibited an inhibitory effect on the proliferation of MCF-7 cells at an IC_{50} value of 130.7 μM (Chinnici et al., 2004).

The establishment of effective interaction between phytochemicals and the subsequent targets depends on the details of the established structure. Great strides have been made in this area with the advancement of biomolecular spectroscopic technology such as X-ray crystallography and nuclear magnetic resonance (NMR), contributing to substantial advances in our structural understanding of the drug goal. Unique ligands could be logically designed to provoke medicinal benefits, getting the benefit of the three-dimensional structure of the proteins. By identifying and improving the initial lead molecules, structure-based design (SBD) may also offer crucial research into potential drug design and production. The high-affinity ligand selectively controls approved drug targets to control particular cellular behaviors, which finally produces the desired pharmacological and therapeutic results (Cui et al., 2020).

This study was conducted to target breast cancer biomarkers by applying phytocompounds extracted from *Nephelium lappaceum* plant. In the next section, the study design has been described in detail. Moreover, the experimental results obtained by the in silico study are described in the results section.

2. Materials and methods

2.1. Search and obtain plant compounds

Plant compounds were selected through a literature search. The search was conducted using some databases including PubMed, Scopus, Elsevier, Frontiers, and the Malaysian Cancer National Registry Report. The twenty plant compounds from *Nephelium lappaceum* were selected based on the data about the medicinal activity of plant compounds in humans. The structures of the selected phytocompounds (ellagic acid, corilagin, geraniin, furfural, 2-phenylethanol, β-damascenone, cinnamic acid, vanillin, 3-phenylpropionic acid, phenylacetic acid, 9-Octadecenoic acid, 5-Methylfuran-2-carbaldehyde, 1,2-Benzenediol (Catechol), heptanoic acid, 3-Hydroxybenzoic acid, ethyl cinnamate, carvone, furaneol, (E)-2-nonenal and ethyl 2-methylbutyrate) were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in the SDF format (Kim et al., 2016).

2.2. Preparation and screening for ligands/ phytocompounds

The structure of phytocompounds was prepared for ligands using the 'Prepare ligand' action in Discovery Studio (DS) 4.0. In this step, removal of duplicates, enumeration of tautomers/isomers, the addition of hydrogen bonds, and energy minimization by CHARMM (Chemistry at Harvard Macromolecular Mechanics) force field were carried out (Brooks et al., 2009). Then, the prepared phytocompounds/ligands were screened using Lipinski's Rule of Five and Vebers' protocol (Ro5 & VP) (Lipinski, 2004; Veber et al., 2002). These protocols determine the benchmark for drug-like properties and are well-defined on drugs' bioavailability. further proceeded for molecular docking with breast cancer target proteins (They were used to filter the phytocompounds based on molecular weight (MW $\leq$ 500 Daltons), number of hydrogen bond donors (HBD $\leq$ 5), hydrogen bond acceptors (HBA $\leq$ 10), number of rotatable bonds (RB $\leq$10), log P value $\leq$5 and polar surface area (PSA $\leq$ 140 Å2).

2.3. Selection and retrieval of breast cancer target proteins

The most common molecular target proteins (ER, PR, and AR) which play important role in breast cancer metastasis were selected from the Therapeutic Target Database (TTD-http://bidd.nus.edu.sg/group/cjttd/) and Potential Drug Target Database (PDTD- http://www.dddc.ac.cn/pdtd/) for the aim of molecular docking analysis (Morshedian et al., 2019; Qin et al., 2014). The 3-D models of target proteins were retrieved from RCSB Protein Data Bank (PDB) (https://www.rcsb.org/) and downloaded in PDB format (Rose et al., 2015). The PDB-ID: 1ERR, 3D90, and 1E3G are for ER, PR, and AR respectively. The three 3-D x-ray crystallographic target

Table 1. Compounds from *Nephelium Lappaceum* selected for the study and their characteristics.

No.	Bioactive compound	PubChem ID	MW (<500)	HBD (≤5)	HBA (≤10)	AlogP (≤5)	RB (≤10)	LR	Bio availability
1	Ellagic acid		302.194	4	6	2.32	0	YES	YES
2	Corilagin	73568	634.4528	11	15	1.1	3	NO	NO
3	Geraniin	3001497	952.65	14	27	−0.78	3	NO	NO
4	Furfural	7362	96.08	2	0	0.98	1	YES	YES
5	2-phenylethanol	6054	122.1644	1	1	1.49	2	YES	YES
6	β-damascenone	5366074	190.286	0	1	3.68	2	YES	YES
7	Cinnamic acid	444539	148.1586	1	2	2.14	2	YES	YES
8	Vanillin	1183	152.1473	1	3	1.22	2	YES	YES
9	3phenylpropionic acid	107	150.1745	1	2	2.06	3	YES	YES
10	Phenylacetic acid	999	136.1479	1	2	1.61	2	YES	YES
11	9-Octadecenoic acid	637517	282.468	1	2	6.78	15	NO	NO
12	5-Methylfuran-2-carbaldehyde	12097	110.1106	0	1	0.95	1	YES	YES
13	Catechol	289	110.1106	2	2	1.37	0	YES	YES
14	Heptanoic acid	8094	130.1849	1	2	2.26	5	YES	YES
15	3-Hydroxybenzoic acid	7420	138.122	2	3	1.33	1	YES	YES
16	Ethyl cinnamate	637758	176.2118	0	1	2.87	4	YES	YES
17	Carvone	7439	150.221	0	1	2.55	1	YES	YES
18	Furaneol	19309	128.1259	1	3	0.21	0	YES	YES
19	(E)-2-nonenal	5283335	140.2227	0	1	2.98	6	YES	YES
20	Ethyl2methylbutyrate	24020	130.1849	0	1	1.97	4	YES	YES

protein models were publicly available. All the protein models were chosen based on the presence of one or more active sites for docking with plant compounds/ligands.

2.4. Preparation of target proteins and identification of active sites on the target proteins

The active site (AS) of an enzyme can be defined as a region of an enzyme containing a specific shape that allows it to bind with a specific molecular substrate resulting in a chemical reaction of the enzyme. AS ensures the optimum and favorable catalytic microenvironments and helps chemical compounds to form enough contact points to generate strong binding with desired enzymes. Therefore, to obtain a strong binding affinity of our compound, the AS of the protein has been determined through BIOVIA Discovery Studio Visualizer v19.1.0.18287 (BIOVIA). The determined AS was considered as the interaction point for docking and utilized for generating the receptor grid by using the PyRx virtual screening tool AutoDock Vina. It also searched for the preview of molecular interactions between the crystal structure of the target protein and inhibitor which are displayed in PDB (Stierand & Rarey, 2010). A grid box was developed to cover the selected protein-binding site and to permit the ligand to move freely. It also included all the important functional residues.

2.5. Molecular docking

After filtering the phytomolecules on the basis of Lipinski's Rule of Five and Vebers' protocol, we left with 17 compounds. Molecular docking was carried out between the three breast cancer target proteins and the seventeen filtered plant compounds of *Nephelium lappaceum* (ligands) using the PyRx virtual screening tool AutoDock Vina (Dallakyan & Olson, 2015). Also, the comparative statements with known drugs for each docking were done (Majumder & Mukherjee, 2013; Mukherjee et al., 2016; Mukherjee & Majumder, 2009). The known drugs were chosen based on the previously reported inhibitory activity against the target protein. The control ligands are Raloxifene pubchem CID: 5035 for 1ERR (ER), Levonorgesteol pubchem CID: 13109 for 3D90 (PR), and Methyltrienolone pubchem CID: 261000 for 1E3G (AR). The docking approach was performed to identify the binding affinity between the target proteins and ligands. For docking, the default configuration options of the PyRx virtual screening tool were utilized. The best binding mode was chosen based on the lowest binding energy (kcal/mol) and negative value. Moreover, the number of hydrogen bonds that interact between the target protein and phytocompound was also recorded. With a tolerance of 0.5, the ideal distance between two atoms joined by a hydrogen bond is set to 1.9. Lastly, the binding interaction of the target protein-phytocompound complex has been visualized using the BIOVIA Discovery Studio Visualizer v19.1.0.18287 (BIOVIA).

2.6. Prediction of pharmacokinetic (PK) properties

The computational biology tool 'ADME descriptors' aids in the determination of pharmacokinetic parameters and evaluate the quality of the molecule based on the drug absorption, distribution, metabolism, and excretion. The flow of medications into, through, and out of the body is mostly determined by the intensity and time course of PK (ADME) properties when taken simultaneously (Hsiao et al., 2021). This tool reduces the expenses and possibility of clinical failures for new drugs. The pharmacokinetic parameters assist and define the integrity and efficiency of plant compounds in the early stages of drug development. In this study, the SwissADME server[1] has been used to assess the early-stage pharmacokinetics properties of the seventeen screened plant compounds (Daina et al., 2017). It is a web-based free server tool that can determine the pharmacokinetics and drug-likeness properties of small molecules such as plant compounds.

2.7. Prediction of toxicity

Toxicity prediction is one of the major and important steps in the drug design process because it is vital to assess the

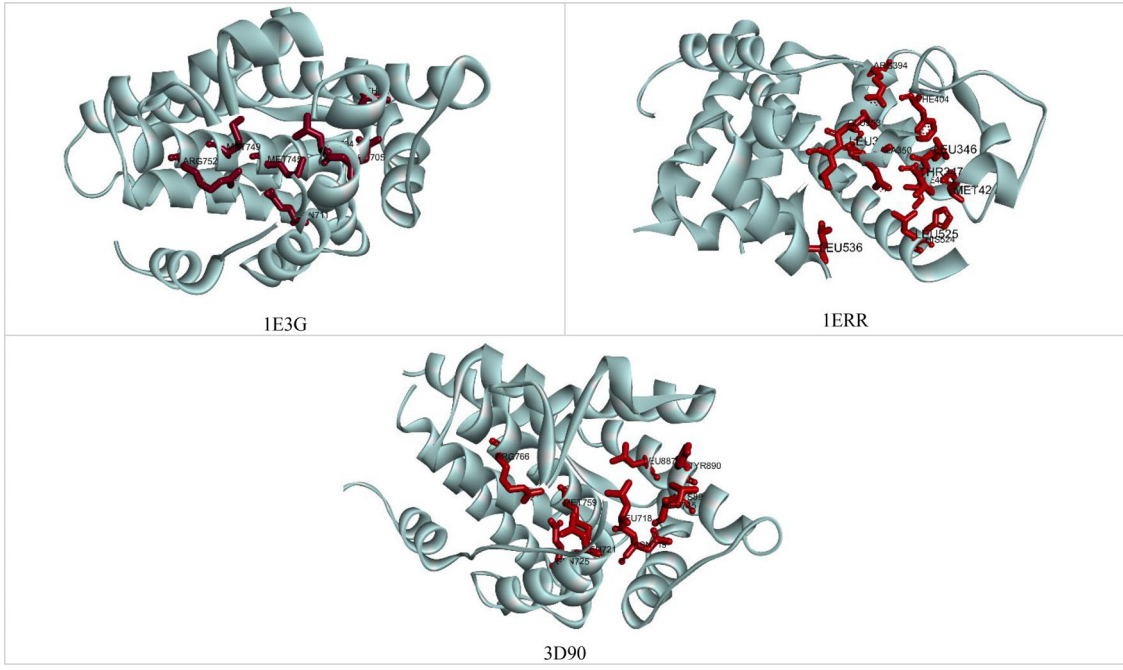


Figure 1. The active site of the 1E3G, 1ERR, and 3D90 proteins. The active sites are represented in a red stick.

harmful effect of chemical compounds before undergoing a drug trial. Toxicity evaluation refers to the calculation of the degree quality of a chemical compound being poisonous to organisms that cause severe effects on the organs. Thus, the toxicity of the seventeen filtered plant compounds has been determined using admetSAR 2.0[2]. AdmetSAR 2.0 (Yang et al., 2019) is a web-based server developed as a comprehensive source for the prediction of chemical properties including absorption, distribution, metabolism, excretion, and toxicity (ADMET properties) which play crucial roles in the discovery and development of drugs. The server provides several details related to the toxicity properties of a compound. These include carcinogenicity, hERG, AMES, P-glycoprotein inhibitor (PGI), and Rat (LD50) value.

2.8. MD simulation

To study the binding stability of the compounds to the target protein, the complexes were submitted to the MD simulation using the GROMACS 5.1.4 software. The GROMOS 96 43a1 was used as the appropriate force field to identify the intermolecular interactions during the simulation process (Shugg et al., 2020). The separate properly sized simulation cubic boxes were defined as the molecular environment with a pH of 7 as the corresponding pKa value. For both sides of the boxes, the orthorhombic periodic boundary box shape was defined with a distance value of 10 Å to maintain a specific volume. A proper number of counter ions was added to neutralize the charge of the complexes. The entire system was minimized using the steepest descent of 400 steps. The particle mesh Ewald method was used for electrostatic interactions (Parvizpour et al., 2017; Sharif et al., 2018). The simulations were run at 300 K for 100 ns. The stabilized structure from the trajectory of the system was used to identify the quality of the protein geometry and the structure folding reliability. The structural changes of the protein-ligand complexes were monitored based on the root-mean-square deviation (RMSD). Furthermore, the analysis of time-dependent H-bond formation was done during the simulation. The simulations were carried out in three independent runs for each complex under the same number of particles, pressure, and temperature. The Pymol graphical software was utilized for figure generation of ligand–protein conformational analysis.

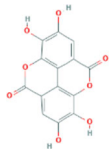
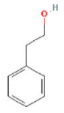
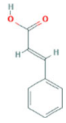
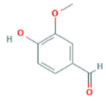
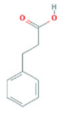
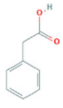
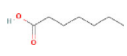
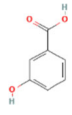
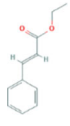
3. Results

3.1. Phytochemical retrieval and preparation

The available compounds of the desired plant were primarily searched within several databases including PubMed, Scopus, Elsevier, Frontiers, and the Malaysian Cancer National Registry Report. A set of twenty natural compounds from the *Nephelium lappaceum* were prepared as listed in Supplementary Table S1. The structure of phytochemical compounds was retrieved from the PubChem database and saved in the 2 D (SDF) file format.

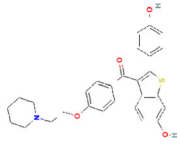
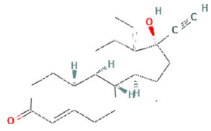
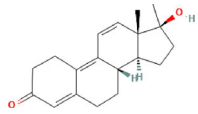
The ligand preparation step was carried out on the structure of phytocompounds using Discovery Studio (DS) 4.0. The preparation procedure deals with adding hydrogen bonds, removing duplicates and enumeration of tautomers/isomers, and minimizing energy by CHARMm force field. The drug-likeness and bioavailability of the prepared phytocompounds were evaluated using Lipinski's Rule of Five and Vebers' protocol. The phytocompounds were filtered based on different criteria including molecular weight (MW $\leq$ 500 Daltons), number of hydrogen bond donors (HBD $\leq$ 5), hydrogen bond acceptors (HBA $\leq$ 10), number of rotatable bonds (RB $\leq$ 10), logP value (logP $\leq$ 5), and polar surface area (PSA $\leq$ 140 Å2). According to the characteristics of phytocompounds in Table 1, three ligands (rows 2, 3, 11) were ignored for further investigation.

Table 2. Identity of each compound including the chemical name, and two-dimensional (2 D) structure of the selected best ligands for each target protein based on their binding affinity.

Compound ID	2D Structure	3D90(PR)	1ERR(ER)	1E3G(AR)
1-Ellagic acid PubChem CID: 5281855		**−8.7**	**−9.2**	**−8.0**
4-Furfural PubChem CID: 7362		−4.4	−4.2	−4.4
5-2-phenylethanol PubChem CID: 6054		−5.9	−5.5	−5.6
6-β-Damascenone PubChem CID: 5366074		**−7.7**	**−7.2**	−5.2
7-Cinnamic acid PubChem CID: 444539		**-7.3**	−5.9	−4.9
8-Vanillin PubChem CID:1183		−5.9	−5.7	−6.0
9-Hydrocinnamic acid PubChem CID: 107		**−6.9**	**−6.8**	**−6.5**
10-Phenylacetic acid PubChem CID: 999		−6.2	−4.9	**−6.7**
12-5-Methylfuran-2-carbaldehyde PubChem CID:12097		−4.9	−4.9	−5.0
13-Catechol PubChem CID: 289		−5.4	−5.2	−5.5
14-Heptanoic acid PubChem CID: 8049		−5.2	−4.8	−5.2
15-3-Hydroxybenzoic acid PubChem CID: 7420		−6.0	−5.9	−6.3
16-Ethyl cinnamate PubChem CID: 637758		−6.1	−5.8	−5.8

(*continued*)

Table 2. Continued.

Compound ID	2D Structure	3D90(PR)	1ERR(ER)	1E3G(AR)
17-Carvonhydrat PubChem CID: 7439		−5.9	**−7.1**	**−6.4**
18-Furaneol PubChem CID: 19309		−5.2	−5.1	−5.3
19-(E)-2-nonenal PubChem CID: 5283335		−5.3	−4.7	−5.2
20-Ethyl2-methylbutyrate PubChem CID: 24020		−4.8	−4.0	−4.5
Raloxifene control for 1ERR(ER) pubchem CID: 5035		–	**−10.0**	–
Levonorgesteol control for 3D90 (PR) pubchem CID: 13109		**−9.0**	–	–
Methyltrienolone control for 1E3G (AR) pubchem CID: 261000		–	–	**−8.9**

3.2. Identification and retrieval of breast cancer target proteins

The three most important target proteins (ER, PR, and AR) in breast cancer metastasis were identified from the PDTD and TTD databases. The 3 D-structure of target proteins were retrieved from the RCSB PDB web server (Barbezan et al., 2017) including PDB-ID:1ERR (ER) (Brzozowski et al., 1997), PDB-ID:3D90 (PR) (Petit-Topin et al., 2009), PDB-ID:1E3G (AR) (Matias et al., 2000). The server provides the x-ray crystallographic structure of the retrieved proteins. In addition, the presence of one or more active sites with an appropriate number of residues for each retrieved protein was considered for molecular docking with selected phytocompounds.

3.3. Identification of active site

The active site amino acid residues involve catalysis and substrate binding and stabilize the intermediates of the reaction or the structure of the binding cleft. They are suitable for the catalysis microenvironments and enable substrates to form enough contact points for strong binding. In this study, the position of active site residues was identified by using the Discovery Studio software. These include LUE 715, LUE 718, ASN 719, LUE 721, GLN 725, MET 759, ARG 766, LUE 887, TYR 890, CYS891 for PDB-ID: 3D90 (PR), LUE 346, THR 347, ALA 350, ASP 351, GLU 353, LUE 354, TRP 383, LEU 387, ARG 394, PHE 404, MET 421, ILE 424, HIS 524, LEU 536 for PDB-ID: 1ERR (ER), and LUE 704, ASN 705, GLN 711, MET 745, MET 749, ARG 752, THR 877 for PDB-ID: 1E3G (AR). The predicted active site residues for all target proteins are shown in Figure 1.

3.4. Molecular docking analysis

The best intermolecular interaction between the target proteins and phytocompounds was screened and determined through the molecular docking study. The AutoDock Vina wizard from PyRx was employed to carry out the molecular docking between the selected phytochemical compounds and each target protein. For each docking, a control ligand was used based on the previously reported inhibitory activity against the target protein. The control ligands are Raloxifene pubchem CID: 5035 for 1ERR (ER), Levonorgesteol pubchem CID: 13109 for 3D90 (PR), Methyltrienolone pubchem CID: 261000 for 1E3G (AR). The grid box for 1ERR with a dimension $x = 30.1$, $y = 22.0$, and $z = 27.1$, for 1E3G with a dimension $x = 51.6$, $y = 44.6$, and $z = 25.0$, and for 3D90 with a dimension $x = 31.5$, $y = 30.2$, and $z = 25.0$ in angstrom (Å) were identified and used for molecular docking simulation.

Four phytochemical compounds were chosen for each protein based on the best binding affinities as shown in Table 2. The selected compounds for ER are Ellagic acid (PubChem CID: 5281855), Hydrocinnamic acid (PubChem CID:

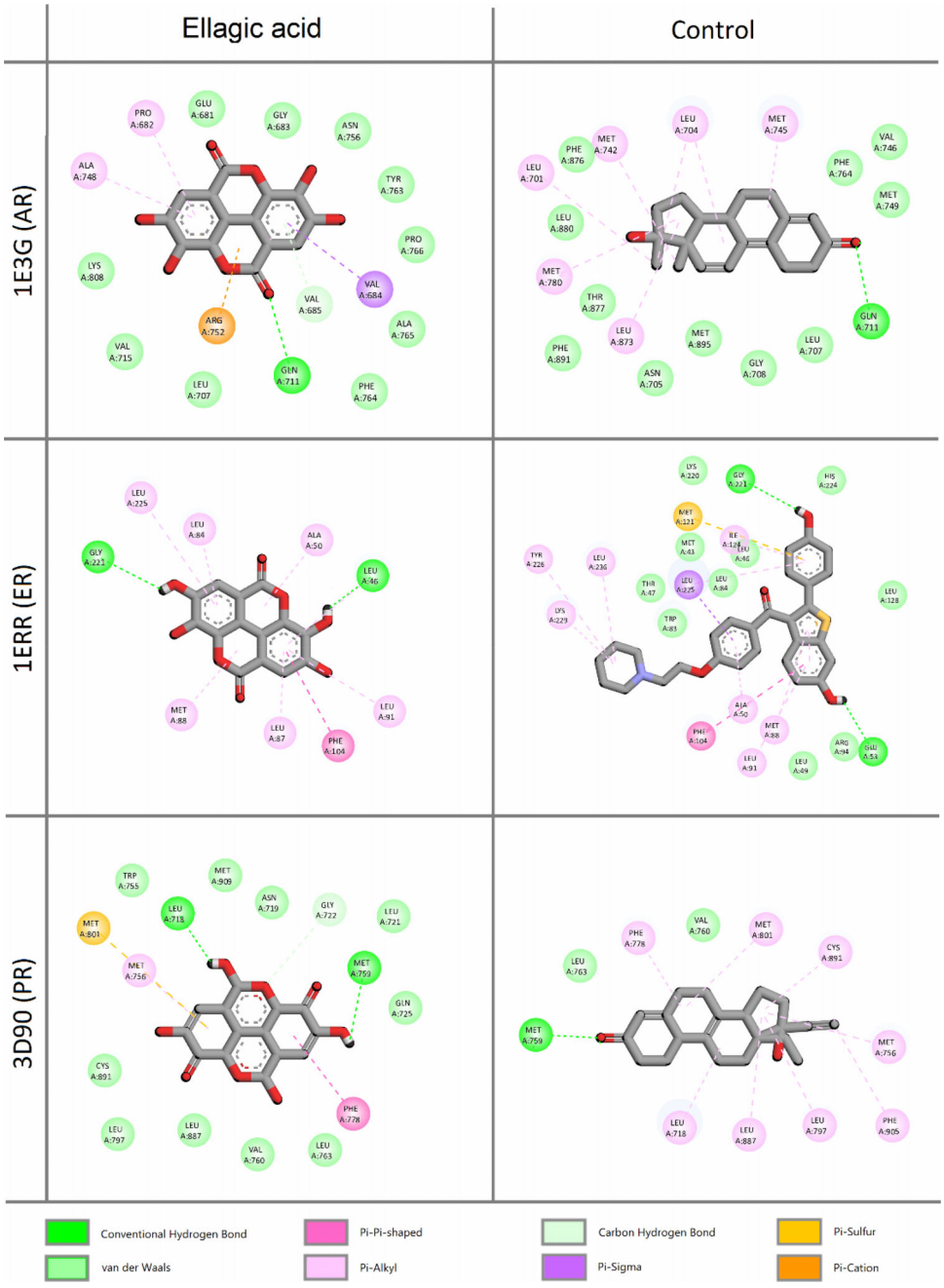


Figure 2. The 2 D visualization of protein-ligand interaction of the target proteins 1ERR (ER), 3D90 (PR), 1E3G (AR), with Ellagic acid (CID: 5281855) (left) and the control compounds (right).

5281855), Carvonhydrat (PubChem CID: 7439), and β-Damascenone PubChemC 5366074 B). Moreover, Ellagic acid (PubChem CID: 5281855), Cinnamic acid (PubChem CID: 444539), Hydrocinnamic acid (PubChem CID: 5281855), and β-Damascenone (PubChem CID: 5366074) were selected for PR, and phenylacetic acid (PubChem CID:999), Ellagic acid (PubChem CID: 5281855), Hydrocinnamic acid (PubChem CID: 5281855), and Carvonhydrat (PubChem CID: 7439) were chosen for AR.

The interaction between each target protein and four selected ligands was further investigated by using BIOVIA Discovery Studio Visualizer. The interaction of three target proteins with Ellagic acid (CID: 5281855) is depicted visually in Figure 2.

3.5. Prediction of pharmacokinetic (PK) properties

The selected compounds were evaluated based on the ADME properties to understand their pharmacokinetic characteristics in interaction with the target proteins. Through the study, favorable and unfavorable pharmacological features of a drug candidate are determined. These include absorption, distribution, metabolism, and excretion. The results of this study in identifying the unfavorable features may lead drug designers to reject a drug candidate before further continuing the drug development. The SwissADME online server was employed to evaluate the ADME properties of the phytochemicals. Table 3 represents the ADME properties of the selected compounds generated by SwissADME.

Table 3. Pharmacokinetics properties of all 20 compounds include physicochemical properties, Bioactivity (BA), lipophilicity, Lipinski 5′ Rules (LR), water-solubility, drug-likeness, and medicinal chemistry.

Bioactive compound	PubChem ID	MW (<500)	HBD (≤5)	HBA (≤10)	AlogP (≤5)	Log S	RB (≤10)	PSA (≤140 Å2)	SA	GI	LR	BA
Ellagic acid	5281855	302.194	4	6	2.32	S	0	26.34	E	H	YES	YES
Furfural	7362	96.080	2	0	0.98	S	1	30.21	E	H	YES	YES
2-phenylethanol	6054	122.164	1	1	1.49	S	2	13.87	E	H	YES	YES
β-damascenone	348291621	190.286	0	1	3.68	S	2	17.07	E	H	YES	YES
Cinnamic acid	444539	148.158	1	2	2.14	S	2	15.03	E	H	YES	YES
Vanillin	1183	152.147	1	3	1.22	S	2	46.53	E	H	YES	YES
3phenylpropionicacid	107	150.174	1	2	2.06	S	3	15.94	E	H	YES	YES
Phenylacetic acid	999	136.147	1	2	1.61	S	2	37.3	E	H	YES	YES
5-Methylfuran-2-carbaldehyde	12097	110.110	0	1	0.95	S	1	30.21	E	H	YES	YES
Catechol	289	110.110	2	2	1.37	S	0	10.69	E	H	YES	YES
Heptanoic acid	8094	130.184	1	2	2.26	S	5	15.33	E	H	YES	YES
3-Hydroxybenzoic acid	7420	138.122	2	3	1.33	S	1	12.92	E	H	YES	YES
Ethyl cinnamate	637758	176.211	0	1	2.87	S	4	19.67	E	H	YES	YES
Carvone	7439	150.221	0	1	2.55	S	1	17.66	E	H	YES	YES
Furaneol	19309	128.125	1	3	0.21	S	0	12.48	E	H	YES	YES
(E)-2-nonenal	5283335	140.222	0	1	2.98	S	6	17.07	E	H	YES	YES
Ethyl2-methylbutyrate	24020	130.184	0	1	1.97	S	4	15.09	E	H	YES	YES

These include Pharmacokinetics and physicochemical properties, lipophilicity, water-solubility, drug-likeness, and medicinal chemistry. It can be observed from the results in Table 3 that all compounds satisfy the requirements for a drug candidate in terms of pharmacokinetic characteristics.

3.6. Toxicity prediction

The toxicity of the selected phytocompounds was evaluated for identifying their potential to be toxic and damage an organism. According to the previous reports, toxicity is the reason for 20% of failures in late drug development. The experimental methods for toxicity evaluation of a drug candidate is a complex, costly, and time-consuming procedure and need animal trials (Ahammad et al., 2021). This is while toxicity analysis through *in silico* approaches is fast and inexpensive and free from animal experiments (Zhou et al., 2016). Toxicity analysis of the phytochemicals indicates a noncarcinogenic property of the compounds except for (E)-2-nonenal and Ethyl 2-methylbutyrate. Genotoxicity evaluation of the compounds by using the AMES test revealed that the compounds have not capable of reverse mutations (Barbezan et al., 2017). The potential toxicity of the compounds for the heart through producing lethal cardiac arrhythmia was assessed based on inhibition of human Ether-à-go-go-Related Gene (hERG) potassium ion channels. Thus, it is essential to identify putative hERG inhibitors or non-inhibitors in an early stage to reduce the cardiotoxicity of the compounds. The result of the tests reveals that the phytochemicals are non-inhibitor of hERG (Shugg et al., 2020). Furthermore, the immediate or acute toxicity test of the compounds based on the LD50 measure denotes the compounds are in a normal range. Regarding the carcinogenicity of (E)-2-nonenal and Ethyl 2-methylbutyrate, they were ignored for further evaluations.

3.7. MD Simulation analysis

The stability of the protein-ligand complexes was examined through MD simulation. The stability of a protein-ligand complex indicates that the binding mode of the complex is well-established. The simulations were run for 100 ns, and then, the steady nature and conformations stability of the complexes was analyzed with one reference antagonist that binds with every target protein. The results of the MD simulation have been shown in the form of the RMSD plot (Figure 3). The RMSD plot of the complexes is represented based on the average of the triplicate MD simulation runs. The RMSD plot depicts the stability of the complexes obtained from the molecular docking. The plot represents the deviation of the backbone structure movement during the simulation. An RMSD with an arbitrary threshold of 2 Å confirms the success in molecular docking as shown by Morris and Lim-Wilby (Parvizpour et al., 2019). The total number of intermolecular and intramolecular hydrogen bonds was counted (Figure 4). As depicted in the figure, it can be seen that the ER, AR, and PR proteins with the Ellagic acid complex showed the maximum number of intermolecular hydrogen bonds. For gaining more insight regarding the newly adopted ligand–protein conformations within the MD simulation runs, frames of 0 and 100 ns of each system were extracted. Figure 5 illustrates the comparative conformations of the ellargic acid-protein complex at 0 and 100 ns. There is no significant orientation change for the ligand within the binding site of ER, PR, and AR proteins between the time frames.

4. Discussion

Despite the proposition of several preventive and therapeutic strategies against breast cancer, the statistics published by National Cancer Institute (NCI) of the United States indicate that the number of individuals with the risk of breast cancer is expected to rise up to around 19 million by 2024 (Weikum et al., 2018). The complexity of the breast tumors that arises from genetic heterogeneity is the main obstacle to developing effective therapies for breast cancer. Accordingly, understanding the molecular basis of this heterogeneity can efficiently give insight into the way for prevention and treatment of malignancy.

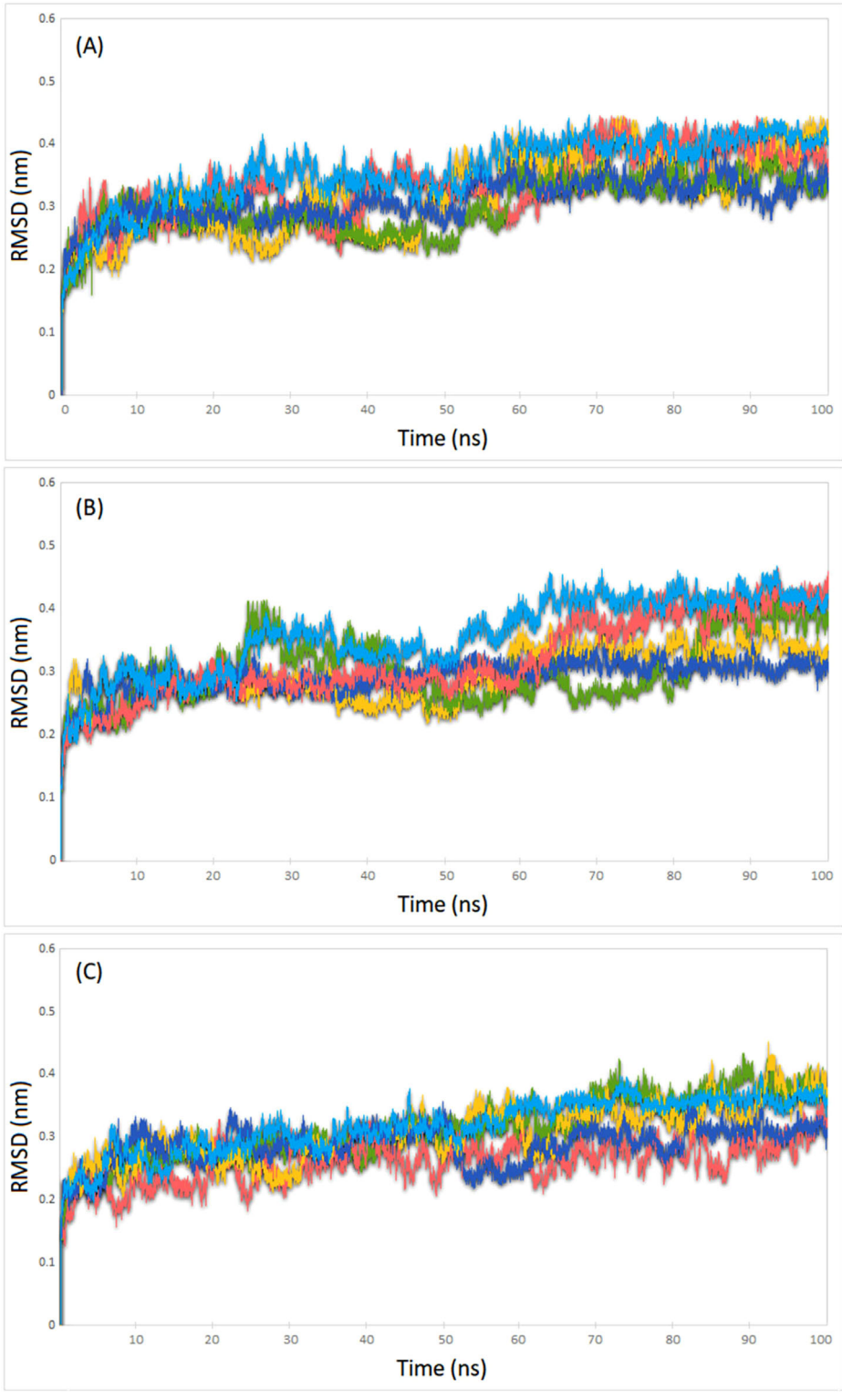


Figure 3. The RMSD plot of the ER, PR, and AR proteins complexes in interaction with selected compounds: (A) the ER complex with Ellagic acid (dark blue), Hydrocinnamic acid (yellow), Carvonhydrat (green), and β-Damascenone (pink), and Raloxifene (control) (light blue), (B) the PR complex with Ellagic acid (dark blue), Hydrocinnamic acid (yellow), Cinnamic acid (green), and β-Damascenone (pink), and Levonorgesteol (control) (light blue), and (C) the AR complex with Ellagic acid (dark blue), Hydrocinnamic acid (yellow), and Carvonhydrat (green), Phenylacetic acid (pink), and Methyltrienolone (control) (light blue). The RMSD plot of the complexes are represented based on the average of the triplicate MD simulation runs.

Nuclear receptors (NRs) are among the key cellular transcription factors for the regulation of essential genes involved in different cell functions such as metabolism, differentiation, detoxification, death, and survival (Parvizpour et al., 2021). ER and PR are two NRs that have a major role in carcinogenesis and breast cancer progression. It has shown that both receptors are directly involved in survival promotion in about 60%–70% of breast cancer patients. They are used in the classification of breast cancers and prediction of response to specific therapies. AR is a member of the

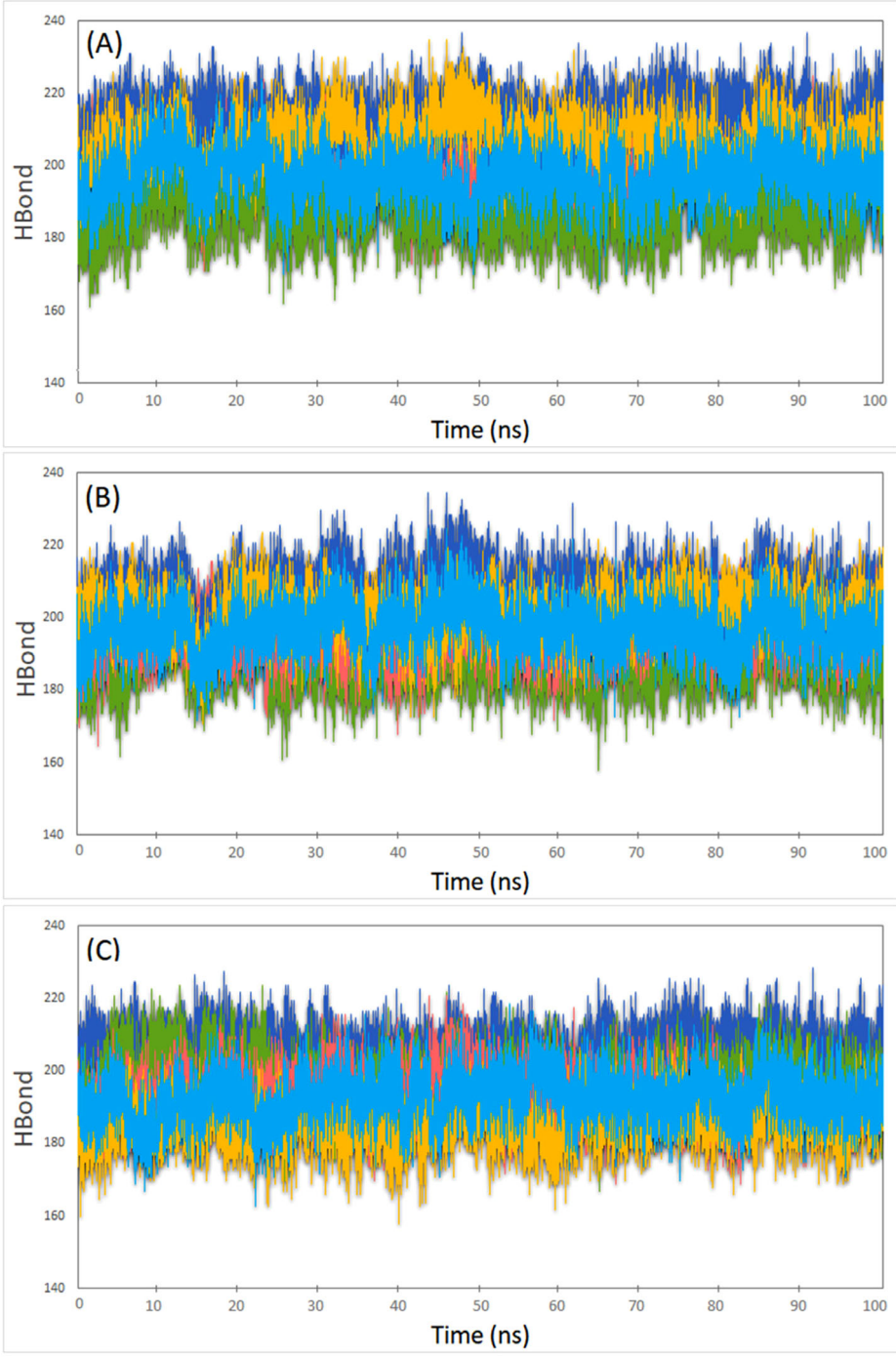


Figure 4. The H-Bond plot of the ER, PR, and AR proteins complexes in interaction with selected compounds: (A) the ER complex with Ellagic acid (dark blue), Hydrocinnamic acid (yellow), Carvonhydrat (green), and β-Damascenone (pink), and Raloxifene (control) (light blue), (B) the PR complex with Ellagic acid (dark blue), Hydrocinnamic acid (yellow), Cinnamic acid (green), and β-Damascenone (pink), and Levonorgesteol (control) (light blue), and (C) the AR complex with Ellagic acid (blue), Hydrocinnamic acid (yellow), and Carvonhydrat (green), Phenylacetic acid (pink), and Methyltrienolone (control) (light blue).

steroid receptor superfamily that is expressed in human tissues while the third-highest expression of AR has been found in the breast tumor tissues. Overexpression of AR in 70%–90% of breast cancer patients shows its predictive or prognostic role that can be a true target for drug development. The above biomarkers are considered as the prognostic factors for diagnosing breast cancer.

Computer-aided drug design (CADD) is an outstanding approach that is widely used to discover, analyze, and develop drugs through in-silico methods such as pharmacophore modeling, virtual screening, molecular docking, and dynamic simulation (Masoudi-Sobhanzadeh et al., 2021). In this way, similar active molecules against the desired protein are identified by pharmacophore modeling. The binding affinity of a compound with a target macromolecule can be assessed simply by the molecular docking study. The study reveals the biological activity of a compound whenever it binds with the target protein and triggers a specific response. Employing conventional methods for calculating the binding capacity is costly and time-consuming

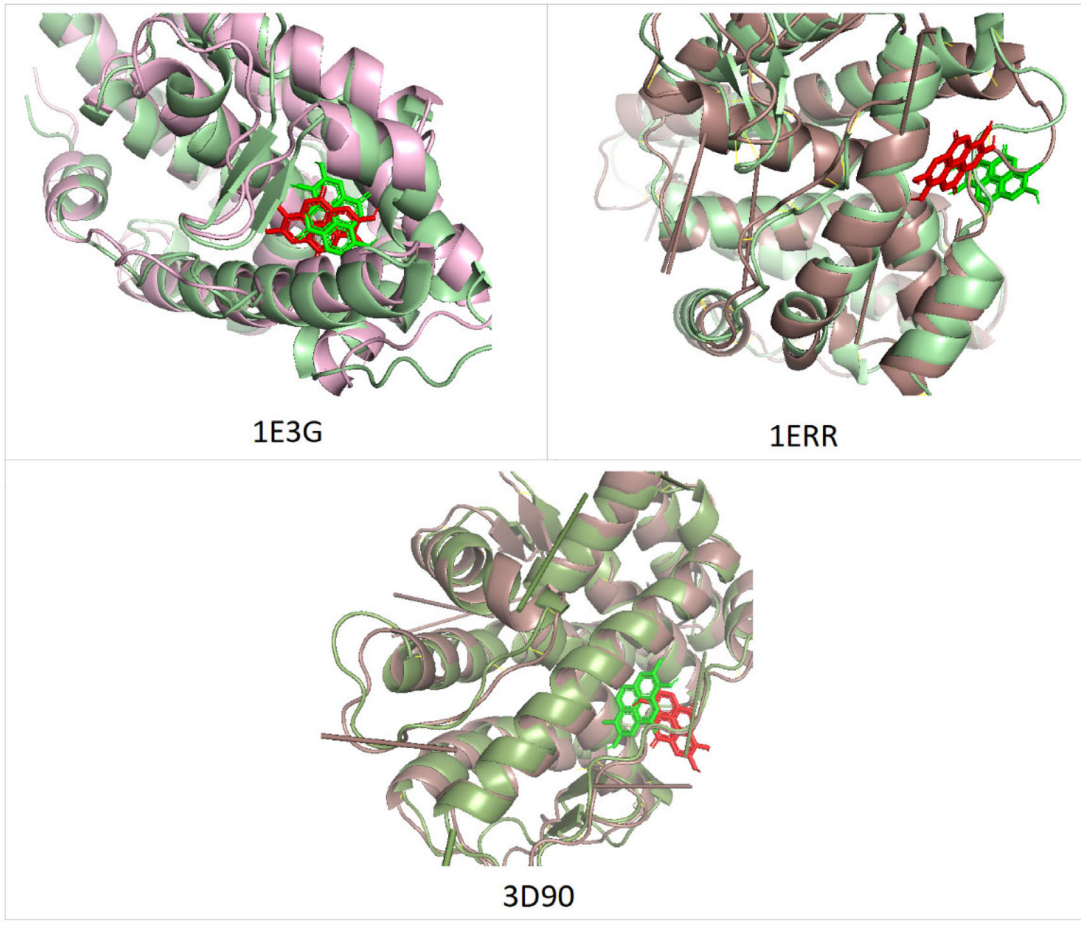


Figure 5. Conformations of the ligand-protein complex at 1E3G, 1ERR, and 3D90 binding site through selected trajectories. Proteins are represented in smudge green and dark salmon cartoon 3 D-representation corresponding to initial (0 ns) and last (100 ns) extracted trajectories, respectively. The ligand is presented in stick in initial (0 ns) and last (100 ns) extracted trajectories by red and green color, respectively.

because of the need for in-vitro and in-vivo experiments, while molecular docking simply provides the required details within a short time (Adamu et al., 2017).

MD simulation has been described for drug design and pharmacophore progress. The binding stability of the selected compounds to the target protein was studied by submitting the complex structures to the MD simulation (National Surgical Adjuvant Breast and Bowel Project, 2010). Pharmacokinetics and pharmacology properties including absorption, distribution, metabolism, and excretion (ADME) of a drug candidate as well as its toxicity are also predicted by using the available tools in CADD.

The current study was mainly conducted to employ the CADD approach for identifying the possible natural antagonist against the target protein to treat breast cancer. In this study, we employed a protocol of *in silico* approaches to the phytochemical screening of rambutan (*Nephelium lappaceum)* phytocompounds against human breast cancer. To this end, 20 phytochemical compounds were retrieved from the available databases including PubMed, Scopus, Elsevier, Frontiers, and the Malaysian Cancer National Registry Report. The most common molecular target proteins (ER, PR, and AR) which play important role in breast cancer metastasis were identified. The molecular docking study was carried out to screen the phytochemicals and select the top four compounds based on their binding affinity for each target protein. Analyzing the binding interactions between the compounds and target proteins reveals the existence of strong hydrophobic and hydrogen bonding.

The binding affinity of the compounds in interaction with the PR, ER, and AR proteins are represented in Table 2. Based on the lowest binding affinity, the ER complex with Ellagic acid, Hydrocinnamic acid, Carvonhydrat, and β-Damascenone, the PR complex with Ellagic acid, Cinnamic acid, Hydrocinnamic acid, and β-Damascenone, and AR complex with Ellagic acid, phenylacetic acid, Hydrocinnamic acid, and Carvonhydrat were chosen for further investigation.

A control ligand was selected and used as a control for each docking. The selection of control ligands was carried out based on the information provided by the Clinical Trial web server (www.clinicaltrial.gov). The control ligands are Raloxifene (pubchem CID: 5035) for 1ERR (ER), Levonorgesteol (pubchem CID: 13109) for 3D90 (PR), and Methyltrienolone (pubchem CID: 261000) for 1E3G (AR). As proved by FDA, Raloxifene is efficient for reducing breast cancer risk following its effectiveness in preventing invasive breast cancer. This efficiency in reducing breast cancer incidence was shown in a set of clinical trials designed principally to investigate Raloxifene for the prevention and treatment of invasive breast cancer (National Surgical Adjuvant Breast and Bowel Project, 2010). Levonorgestrel is a norgestrel in the form of levorotatory having synthetic progestogen with

Table 4. The drug-induced hERG inhibition, AMES toxicity, carcinogens, *Tetrahymena pyriformis* (TP) toxicity, honeybee (HB) toxicity, and rat acute toxicity (LD50 in mol/kg) of compounds.

Bioactive compound	hERG inhibition	AMES	Carcinogens	TP Toxicity	HB Toxicity	RAT (LD50)
Ellagic acid	No	No	No	Yes H	Yes H	2.6213
Furfural	No	No	No	Yes H	Yes H	2.097
2-phenylethanol	No	No	No	Yes H	Yes H	1.86
β-damascenone	No	No	No	Yes H	Yes H	1.7819
Cinnamic acid	No	No	No	Yes H	Yes H	1.7416
Vanillin	No	No	No	Yes H	Yes H	1.9642
3-phenylpropionic acid	No	No	No	Yes H	Yes H	1.9377
Phenylacetic acid	No	No	No	Yes H	Yes H	1.8134
5-Methylfuran-2 carbaldehyde	No	No	No	Yes H	Yes H	1.7307
Catechol	No	No	No	Yes H	Yes H	2.5957
Heptanoic acid	No	No	No	Yes H	Yes H	1.3275
3-Hydroxybenzoic acid	No	No	No	Yes L	Yes H	1.3983
Ethyl cinnamate	No	No	No	Yes L	Yes H	1.6755
Carvone	No	No	No	Yes H	Yes H	1.8809
Furaneol	No	No	No	Yes H	Yes H	1.8941
(E)-2-nonenal	No	No	Yes	Yes H	Yes H	1.5307
Ethyl 2-methylbutyrate	No	No	Yes	Yes L	Yes H	1.2415

progestational and androgenic activity. Levonorgestrel stimulates the hormone-receptor complex, initiates transcription, and increases the synthesis of certain proteins by binding to the progesterone receptor in the nucleus of target cells (Petit-Topin et al., 2009). Furthermore, Metribolone is a widely used ligand in androgen receptor ligand binding assays as a photoaffinity label. It was clinically investigated against advanced types of breast cancer in the late 1960s; however, its severe hepatotoxicity characteristics caused it to discontinue its production (Matias et al., 2000).

The behavior of a drug candidate in the human body is commonly determined based on its ADME properties known as pharmacokinetic parameters. Success in clinical tests of the candidate is critically dependent on its preclinical test using these parameters. As an important factor of a drug molecule, permeability across the biological barrier is dependent on the molecular weight as well as the polar surface topological area. Permeability is decreased by higher molecular weight while it is improved by a lower polar surface topological area. **A**bsorption is another key factor of the drug molecule that depends on its lipophilicity. This factor is calculated by the logarithm of the inorganic and aqueous phase partition coefficient of the target molecule (LogP). There is a correlation between the lower absorption of the drug molecule and the higher value of the LogP parameter. A drug candidate is assessed for its water solubility using the LogS parameter. The higher solubility is achieved when the molecule has a lower value of LogS. The number of donors and acceptors of hydrogen bonds beyond the proper range denotes the capacity of a drug molecule to cross the membrane bilayer. Besides, the overwhelming amount of rotatable bonds is investigated based on the oral bioavailability of the compound. The proper range of this parameter is near 10. The results in Table 3 indicate that the selected phytocompounds obtain fruitful results in terms of pharmacokinetic properties. Another important assessment for validating a drug candidate is the evaluation of its toxicity to prevent any possibility of damage to target organisms. Preclinical toxicity tests by computational methods are time- and cost-effective without the need to test on animals. The toxicity test results in Table 4 reveal that the selected phytocompounds pass the toxicity test except for (E)-2-nonenal and Ethyl 2-methylbutyrate.

The MD simulation of the selected protein-ligand complexes was carried out to investigate the stability of the drug candidates in interaction with the target proteins. In this regard, the selected compounds were exposed to the MD simulation in interaction with the target proteins. The results obtained from the MD simulation confirm the stability of the drug candidates in interaction with the target proteins.

5. Conclusion

Breast cancer is the second cause of cancer death in women after lung cancer. This high rate of mortality is an alarming situation to motivate research on finding out effective drugs for its treatment. Synthetic chemotherapeutics have not satisfied the expected outcome in cancer therapy through their production is highly expensive. The plant-derived chemotherapeutic agents have shown successful outcomes in the treatment of diseases, especially cancer. Rambutan is a potent herb that yields compounds with a variety of medicinal characteristics. All the selected phytocompounds passed the Ro5 & VP protocol. Profiling the ADMET properties of the compounds indicates that the compounds have the capability for further proceeding into the drug pipeline. Consequently, the findings of this study provide a robust base for future *in vitro* and *in vivo* works to analyze these compounds from rambutan plants for developing into drugs.

Notes

1. www.swissadme.ch
2. http://lmmd.ecust.edu.cn/admetsar2/

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Disclosure statement

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