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Dietary flavonoids against various breast cancer subtypes: a molecular docking study

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ABSTRACT: Breast cancer is female most frequent diagnosed cancer and the leading cause of cancer death. The consumption of dietary flavonoids is reported to cause significant breast cancer risk reduction. In vitro studies often used aglycone flavonoids rather than its conjugated form that actually present in human body. Thus its mechanism against breast cancer has not been elucidated completely. The present study aimed to investigate the possible mechanism of dietary flavonoids against breast cancer by in silico study. Conjugated flavonoids were docked to ER (estrogen receptor), HER2 (human epidermal growth factor receptor 2) and EGFR (epidermal growth factor receptor) kinase domains. The molecular docking of 22 flavonoid conjugates towards EGFR and HER2 kinase domain, and ER was successfully performed. Potential binders to proteins: epicatechin conjugates to ER (−8.7 kcal/mol), isoflavone conjugates to HER2 kinase domain (−10.7 kcal/mol), and epigallocatechin and epicatechin conjugates to EGFR kinase domain (−9.2 kcal/mol), were suggested. Supported by other studies, conjugated flavonoids may exert similar inhibitory and agonistic properties to their parent flavonoids. Taken together, the present study showed possible effects of dietary flavonoids against various breast cancer subtypes.

KEYWORDS: molecular docking simulation, diet, flavonoids, antineoplastic agents, breast neoplasms

INTRODUCTION

According to GLOBOCAN 2018 database, breast cancer is female most diagnosed cancer and the leading cause of cancer death[1]. Breast cancer is heterogenous disease, comprised of various subtypes observable by the presence of the predictive molecular markers. Breast cancer can be categorized into: Luminal A (ER^+, $PR^{+/-}$, HER^-), Luminal B (ER^+, $PR^{+/-}$, $HER2^+$), HER2 (ER^-, PR^-, $HER2^+$), basal like and claudin low (triple negatives)[2]. Each has a different prognosis and responds differently to cancer treatment. Luminal A has the best prognosis, while $HER2^+$ and triple negative breast cancer (TNBC) have the poorest[3]. Today breast cancer endorsed to be treated with endocrine theraphy, targeted theraphy, and cytotoxic chemotheraphy[4]. Breast cancer with high expression of estrogen receptor (ER) and progesterone receptor (PR) is sensitive against endocrine theraphy. ER inhibition treatment in Luminal A and B breast cancer was proven to be effective and safe[5]. While $HER2^+$ breast cancer was effectively treated using targeted theraphy with trastuzumab or lapatinib[6]. But different from previous subtypes, triple negative breast cancer is not responded very well to hormone treatment and HER2 antibody, and often treated with systemic chemotherapy. Previous study found that epidermal growth factor receptor (EGFR) kinase inhibitor, gefitinib, was able to halt the TNBC cell outgrowth in vitro[7]. Thus ER, HER2, EGFR served as important targets in breast cancer treatment. It is also possible other chemical compounds found in food may also interacts with these particular proteins. Flavonoid is the most common phytochemical compound found

ubiquitously in human diet[8,9] and has huge impact in human health. In vitro studies showed that flavonoids have wide range of biological activity antioxidant, anti-inflammatory, anti-microbial, anti-fungal, antiviral, and anti-cancer[9,10]. Consumption of flavonoids is related with less risk of cardiovascular diseases and stroke[11,12]. Other studies found that the intake of flavonoids improved the outcome of the gastric and lung cancer[13,14]. Human study on consumption of food rich in flavonoids, green tea, against breast cancer showed mixed results. Case studies has shown green tea intake was correlated with significant breast cancer risk reduction[15–17], while recent prospective cohort studies showed no correlation[18]. Soy products were rich in isoflavone and high soy intake was modestly associated with reduced breast cancer risk[19].

It is important to note that flavonoid is quickly metabolized in the body. After ingested, glycoside flavonoid found in plant materials is subjected to deglycosylation, releasing aglycone compound that readily absorbed by the intestine lining[20]. Once entered circulatory system, flavonoid is immediately transported to liver and undergoes extensive metabolism. Phase metabolism II transformed free aglycone onto flavonoid conjugates by adding glucuronides and sulphate moiety[21]. Because of this, aglycone flavonoids are rarely found in plasma. Previous in vitro studies often used aglycone flavonoids rather than its conjugated forms that present in human body. Thus its mechanism against breast cancer has not been elucidated completely, since conjugation may affect how the molecules behave. To address this, conjugated flavonoids found in plasma after ingesting food rich in flavonoid was subjected to molecular docking against ER, HER2, EGFR. The present study describes possible mechanism how the dietary flavonoids may contribute against breast cancer.

MATERIALS AND METHODS

Molecular docking towards EGFR, HER2, and ER

Conjugated flavonoids found in plasma after ingesting food rich in flavonoids as previously reported from other studies were used[22–24]. Structural data of conjugated flavonoids were retrieved from PubChem database (Fig. 1) (pubchem.ncbi.nlm.nih.gov). Co-crystallized structures of ER-4-hydroxytamoxifen (PDB ID: 3ERT)[25], HER2-SYR127063 (PDB ID: 3PP0)[26], and EGFR-gefitinib (PDB ID: 4WKQ) were obtained from RCSB database (rcsb.org). Crystal structure data were prepared by removing solvent and extracting bound ligand. AutoDock vina was used in molecular docking under default settings. The docking methodology was validated by redocking the extracted bound ligand. Chimera was used on visualization in this study. Intramolecular analysis was performed using Pose View, available at Protein Plus (proteins.plus)[27].

Table 1 Predicted binding affinity (kcal/mol) of flavonoid conjugates and known inhibitors towards ER, HER2, and EGFR.

Flavonoid conjugate	Binding affinity		
	ER	HER2	EGFR
Gefitinib	–	–	−8.8
SYR127063	–	−11.0	–
4-hydroxytamoxifen	−9.7	–	–
(-)-Epicatechin-3-gallate	−8.7	−8.5	−8.6
(-)-Epigallocatechin-3-gallate	−7.0	−8.6	−9.2
(-)-Epigallocatechin-7-gallate	−7.4	−8.9	−8.0
4'-Methylepicatechin-5-sulfate	−7.2	−8.8	−7.7
4'-Methylepicatechin-7-sulfate	−7.9	−9.6	−7.2
4'-Methyl-epigallocatechin-3'-glucuronide	−7.9	−9.9	−9.1
4'-Methyl-epigallocatechin-7-glucuronide	−6.5	−9.0	−8.2
Daidzein-4'-sulfate	−7.1	−9.4	−8.5
Daidzein-7-sulfate	−6.6	−9.2	−8.4
Epicatechin-3'-glucuronide	−8.5	−9.5	−9.1
Epicatechin-3'-sulfate	−7.7	−9.1	−8.2
Epicatechin-5-sulfate	−7.1	−8.6	−8.4
Epicatechin-7-lucuronide	−7.5	−9.3	−8.3
Epigallocatechin-3'-glucuronide	−7.7	−9.7	−9.1
Epigallocatechin-7-glucuronide	−7.2	−8.3	−8.3
Genistein-4'-O-glucuronide	−8.3	−10.7	−8.0
Genistein-4'-sulfate	−7.3	−9.7	−8.3
Genistein-7-O-glucuronide	−6.8	−9.4	−7.7
Genistein-7-sulfate	−6.4	−9.1	−8.6
Isorhamnetin-3-O-glucuronide	−7.6	−8.3	−8.8
Quercetin-3'-glucuronide	−8.7	−8.8	−8.7
Quercetin-3'-sulfate	−8.1	−9.5	−8.4

RESULTS

Molecular docking analysis

Redocking was performed to evaluate software and docking parameters used. The root mean square deviation between docked and crystal compounds was less than 2Å except for EGFR bound ligand (gefitinib). This is due to the 6-propylmorpholino moiety of gefitinib sticking out to solvent and able to move freely[28]. Thus, AutoDock Vina has favourable accuracy and proceeds the docking of flavonoid-

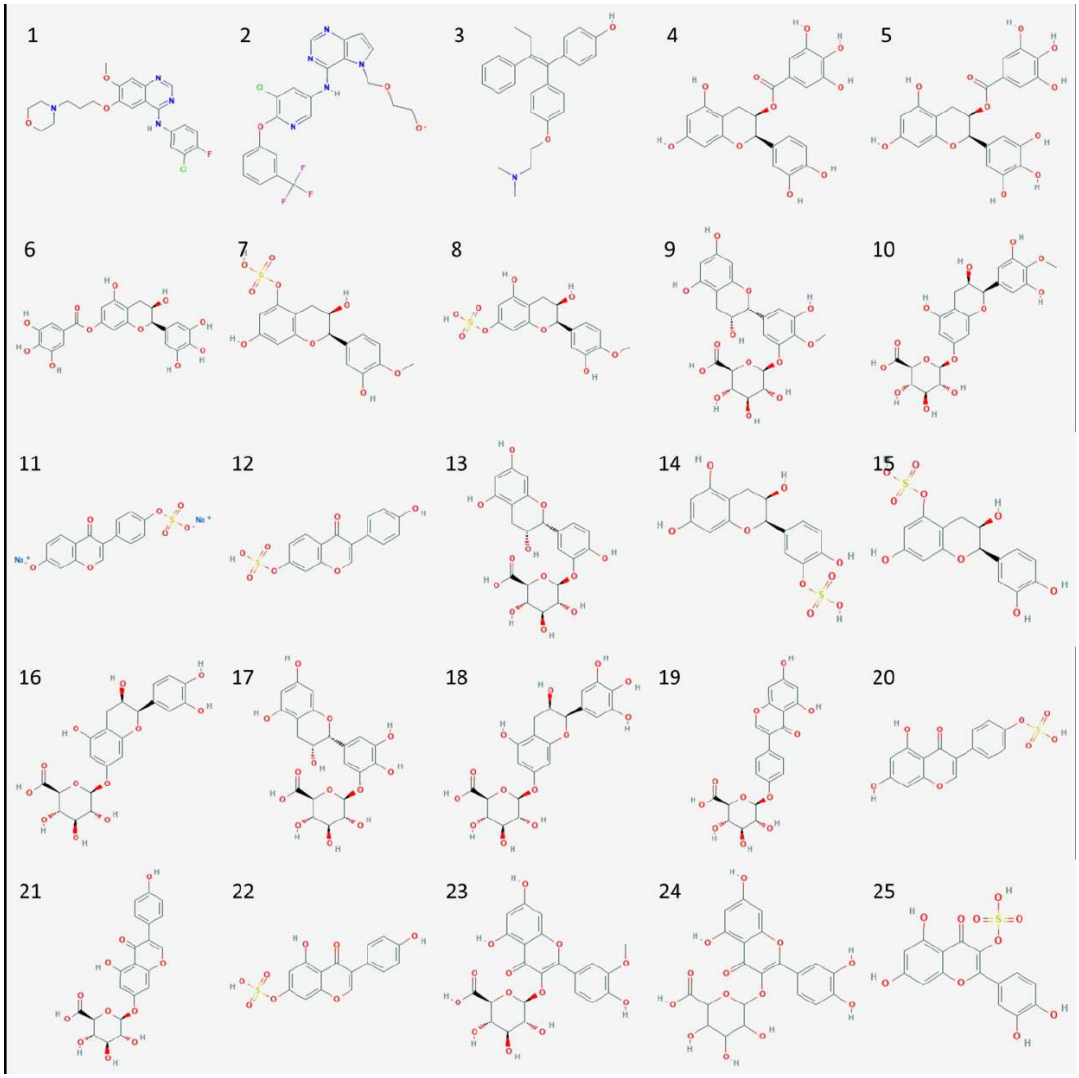

Fig. 1 The 2 dimensional structure of all studied ligands: (1) gefitinib, (2) SYR127063, (3) 4-hydroxytamoxifen, (4) (-)-epicatechin-3-gallate, (5) (-)-epigallocatechin-3-gallate, (6) (-)-epigallocatechin-7-gallate, (7) 4'-methylepicatechin-5-sulfate, (8) 4'-methylepicatechin-7-sulfate, (9) 4'-methyl-epigallocatechin-3'-glucuronide, (10) 4'-methyl-epigallocatechin-7-glucuronide, (11) daidzein-4'-sulfate, (12) daidzein-7-sulfate, (13) epicatechin-3'-glucuronide, (14) epicatechin-3'-sulfate, (15) epicatechin-5-sulfate, (16) epicatechin-7-glucuronide, (17) epigallocatechin-3'-glucuronide, (18) epigallocatechin-7-glucuronide, (19) genistein-4'-O-glucuronide, (20) genistein-4'-sulfate, (21) genistein-7-O-glucuronide, (22) genistein-7-sulfate, (23) isorhamnetin-3-O-glucuronide, (24) quercetin-3'-glucuronide, (25) quercetin-3'-sulfate.

conjugates. The molecular docking was performed to assess possible binding conformation of flavonoid conjugates towards receptors and possible biological actions of these compounds. The molecular docking of 22 flavonoid conjugates towards EGFR and HER2 kinase domain, and ER was successfully performed. The predicted binding affinity value of flavonoid conjugates was compared to each other and receptor bound ligand (Table 1).

In the present study, based on molecular docking, epichatehin and quercetin conjugates ((-)-epicatechin-3-gallate, quercetin-3'-glucuronide, and epicatechin-3'-glucuronide) were predicted as a potential binder towards estrogen receptor. HER2 kinase domain was predicted to interact strongly towards genistein and epigallocatechin conjugates (genistein-4'-O-glucuronide, genistein-4'-sulfate, epigallocatechin-3'-glucuronide,

Table 2 Hydrogen bond formed by flavonoids conjugates and known inhibitors towards EGFR, HER2, and ER.

Flavonoid conjugate	Receptor	Hydrogen bond
4-hydroxytamoxifen	ER	Asp394 Glu353 Leu346 Thr347 Asp351 Leu387 Glue419 Gly420
(-)-Epicatechin-3-gallate		
Quercetin-3'-glucuronide		Asp351 Glu353
SYR127063	HER2	Met801 Asp863
Genistein-4'-O-glucuronide		Ser783 Thr798
Gefitinib	EGFR	Met793
(-)-Epigallocatechin-3-gallate		Glu762 Leu788 Met793 Arg841

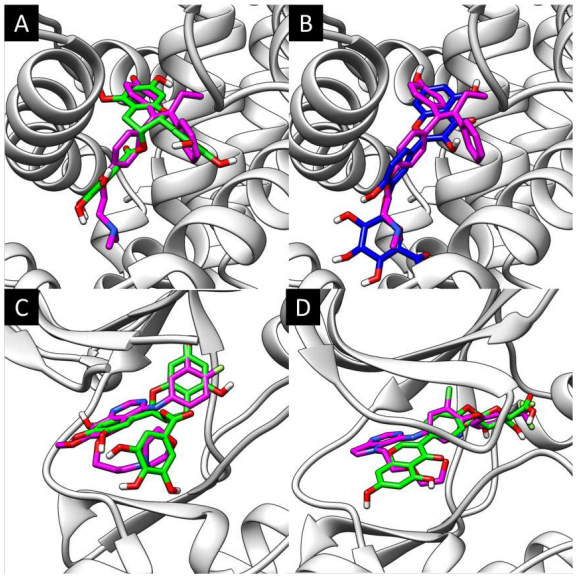

Fig. 2 Superimposed binding mode between flavonoid conjugate and known inhibitor towards ER, HER2, EGFR. (A) and (B) the binding mode of (-)-epicatechin-3-gallate (green), quercetin-3'-glucuronide (blue), and 4-hydroxytamoxifen towards (magenta) ER (light gray). (C) the binding mode of genistein-4'-O-glucuronide (green) and SYR127063 (magenta) towards EGFR kinase domain (light gray). (D) the binding mode of (-)-epigallocatechin-3-gallate (green) and gefitinib (magenta) towards HER2 kinase domain (light gray). The protein represented as ribbon, the compound as stick, and heteroatom represented in a different color from the carbon atom.

and 4'-methyl-epigallocatechin-3'-glucuronide) compared to other compounds. While several catechin derivatives ((-)-epigallocatechin-3-gallate, 4'-methyl-epigallocatechin-3'-glucuronide, epicatechin-3'-glucuronide, and epigallocatechin-3'-glucuronide) were predicted with a high affinity toward EGFR kinase domain as these compounds surpassed known inhibitor binding score.

Binding mode of the compound with the highest predicted binding affinity was visualized and superimposed with the receptor known inhibitor (Fig. 2). All compounds were found to occupy the active site of the protein. (-)-Epigallocatechin-3-gallate resembled similar binding mode with gefitinib, whereas its core structure aligned with the quinazoline and aniline moiety. While genistein-4'-O-glucuronide, (-)-epicatechin-3-gallate, quercetin-3'-glucuronide only shared small geometrical similarity when compared with the bound ligand. Furthermore, hydrogen bond inferred by Pose View was compared and presented in (Table 2). Similar to gefitinib, (-)-epigallocatechin-3-gallate formed hydrogen bond with Met793. SYR127063 and genistein-4'-O-glucuronide shared no similar intermolecular interaction. Quercetin-3'-glucuronide had similar interaction with 4-hydroxytamoxifen at Glu353. (-)-Epicatechin-3-gallate had the most hydrogen bond towards ESR1.

DISCUSSION

In present study, predicted binding affinity and binding mode of conjugated flavonoids present in plasma after ingestion of dietary flavonoids against ER, EGFR kinase domain, and HER2 kinase domain were characterized *in silico*. The result shows that most compounds with predicted high binding affinity were glucuronide flavonoid conjugates. It is interesting to point out that the predominant flavonoid metabolite found in plasma after an hour ingestion of radiolabelled epicatechin was its glucuronide conjugates[29]. Thus, the potential compounds found in this study were likely exist in large concentration in plasma after consumption of dietary flavonoids.

More than 70% diagnosed breast cancer was the overexpressed ER[3]. ER plays important role in development and progression of breast cancer, since ER drives proliferation of mammary cells upon binding with estrogenic hormone[30]. ER^+ breast cancer is sensitive against endocrine therapy and is effectively treated using selective estrogen receptor modulators such as tamoxifen[31]. This study found that (-)-epicatechin-3-gallate was a potential inhibitor of ER, because of its high predicted binding affinity and similar binding mode when compared to active metabolite of tamoxifen (4-hydroxytamoxifen). Tamoxifen interacts with several amino acid residues inside the binding pocket, including Leu346, Thr347 and Leu387, forming a van der walls interaction that stabilize the com-

plex[25]. Epicatechin conjugates was predicted to be interacted with similar manner. This finding confirmed by other studies whereas epicatechin gallate was able to hamper ER activity through direct inhibition[32,33]. Quercetin conjugates also have high predicted binding affinity towards ER. But quercetin conjugates may act as an agonist rather than antagonist, since previous study found that aglycone quercetin induced cell proliferation of ER-positive breast cancer cell line through ER stimulation[34].

HER2 is a receptor tyrosine kinase which is overexpressed in 30% human breast cancer[3]. $HER2^+$ breast cancer characterized by its aggressive phenotype: high tumorigenicity and invaseness[35]. The treatment involved is either by targeting the extracellular domain using trastuzumab or its kinase domain using lapatinib[4,36]. Previous study showed that flavonoid compounds were able to inhibit human kinases[37]. Molecular docking study reported that genistein-4'-O-glucuronide had predicted binding affinity close to SYR127063. SYR127063 itself is a potent HER2 kinase domain inhibitor at IC50 of 11 nM[26]. From the experimental study, genistein was able to attenuate HER2 phosphorylation in BT474 cell line through tyrosine kinase inhibition, thus supported present finding[38].

TNBC occurs approximately 10% in breast cancer cases3. TNBC is biologically aggressive and has the poorest prognosis when compared to other subtypes[3]. Previous study reported that EGFR is a potential target for TNBC[7]. In this work, epigallocatechin and epicatechin metabolites had notable predicted binding affinity towards EGFR kinase domain. This finding supported by another study where epigallocatechin-3-gallate was able to inhibit EGFR activity[39]. Inhibition of EGFR by epigallocatechin conjugate may also affect HER2 activation, since both proteins are able to form heterodimeric complex and activate each other.

CONCLUSION

Binding affinity and binding mode between conjugated flavonoids found in plasma against ER, HER2, EGFR had been characterized in silico. Supported by other studies, conjugated flavonoids may exert similar inhibitory and agonistic properties to their parent flavonoids. Our study thus confirm and offer possible explanations how dietary flavonoids act against various breast cancer subtypes.

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