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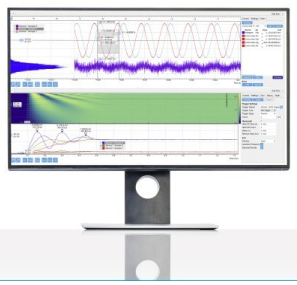



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# Study on Molecular Docking of Red Betel (*Piper Crocatum* Ruiz & Pav.) Active Compound and Tamoxifen Drug as an Inhibitor of Estrogen Receptor-α (ER-α) that Plays a Role in Breast Cancer

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**Abstract.** Breast cancer is the second leading cause of death due to cancer among women. The most common trigger of cancer is an excessive expression of Estrogen Receptor-α (ER-α) which plays a significant role in the growth, development, and pathophysiology of the breast. Tamoxifen is one of Selective Estrogen Receptor Modulators (SERMs) used to treat breast cancer patients, but this drug harmfully impacts on the uterus so that a safe alternative treatment is needed by using herb materials, such as Red Betel. This research aimed to predict the potency of Red Betel Kaempferitrin, β-amyrin, Piperbetol, Piperine, and Sesamin compounds as an inhibitor of Estrogen Receptor-α (ER-α) through molecular docking method. Potency Activity test and ADMET test employed some software and web server. The results of docking between five Red Betel compounds and ER-α were at the similar site to Tamoxifen drug through alkyl and hydrogen bond with the lower affinity value of the Red Betel compound than the control drug. Red Betel active compound has the potency as an anti-neoplastic and anti-oxidant. The Red Betel active compound has a good ADMET profile. This study concluded that the five Red Betel compounds are potential to be breast anticancer drug.

## INTRODUCTION

Breast cancer is the second leading cause of death due to cancer among women. Breast cancer usually begins with hyper-proliferation ductus and develops to a benign tumor or even carcinoma metastasis after stimulated by various carcinogenic factors continually [1]. Estrogen shows a selective action of tissue that is essential in biomedical to the growth, development, and pathophysiology of the breast [2,3]. Estrogen Receptor is classified into sub-types, namely Estrogen Receptor-α (ER-α) and Estrogen Receptor- β (ER-β) [3]. One of the most common triggers of breast cancer is an excessive expression of Estrogen Receptor-α (ER-α) [4]. ER-α is related to the development of breast cancer that makes ER-α as an interesting drug target. Almost 70% of breast cancer patients have positive Estrogen Receptor-α (ER-α) [5]. Estrogen is correlated to ER and induces the conformation change oriented to the receptor dimerization to be Estrogen-Responsive Elements (EREs) so that it causes gen expression bringing about cell proliferation [6].

Tamoxifen is one of the Selective Estrogen Receptor Modulators (SERMs) and becomes the only chosen drug used for more than 30 years to heal breast cancer patients [2]. Therapy with tamoxifen as the ER-α antagonist can reduce the quality of patient's life since therapy with tamoxifen causes harmful side effect on the uterus [4,7]. The development of therapy is optimal for inhibitor and safe treatment of breast cancer by using herbal material. Herbal material that contains bioactive compound can be utilized as the alternative of Selective Estrogen Receptor Modulators (SERMs). One of these herbal materials is Red Betel used by people as a traditional drug. This study predicted the

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potency of the Red Betel compound content as the inhibitor of Estrogen Receptor-α (ER-α) based on in silico by using molecular docking method, potential activity test, and ADMET test to determine the affinity value, type, and position of bonding between ligand-macromolecule, compound potency, and its pharmacokinetic characteristic.

## MATERIALS AND METHODS

This work was based on bioinformatics. The web server and software used were PubChem, Protein Data Bank (PDB), PyMol, PyRx, and Discovery Studio. The materials were three-dimension (3D) structure of Kaempferitrin (CID: 5486199), β-amyrin (CID: 73145), Piperbetol, Piperine (CID: 638024), Sesamin (CID: 72307), Tamoxifen (CID: 2733526) of PubChem web server, and 3D structure of Estrogen Receptor-α (ER-α) obtained from Protein Data Bank (PDB) web server.

Molecular docking technique, receptor structure was attained from Protein Data Bank (www.pdb.org) in PDB File (*.pdb) format. The 3D structure of Estrogen Receptor-α (ER-α) was used with accession number: P03372 (Code PDB: 1QKU), and then continued by sterilization using Pymol Molecular Graphics System of 1.7.4.5 Edu version. The ligand sample taken was 3D structure using PubChem application (http://www.pubchem.ncbi.nlm.nih.gov) and stored in Sybil Data Files (*.sdf.) format and then changed to *.pdb format. Docking process was conducted using PyRx-Python Prescription of 0.8 version. The docking results were clarified and visualized with Pymol software and discovery studio. The Potency Activity (PA) test technique is processing canonical smile data from PubChem with PASS SERVER web server and choosing the potency that becomes the basis of breast cancer agent. ADMET test was carried out to identify Absorption, Distribution, Metabolism, Excretion, and Toxicity in the compound used by employing pkCSM database (http://biosig. unimelb.edu.au /pkcsm/prediction). The data were the binding affinity values, the clarification of binding site, the type of bond and ligand-macromolecule amino acid residues, Probability Activity value, and pharmacokinetic values analyzed descriptively and qualitatively.

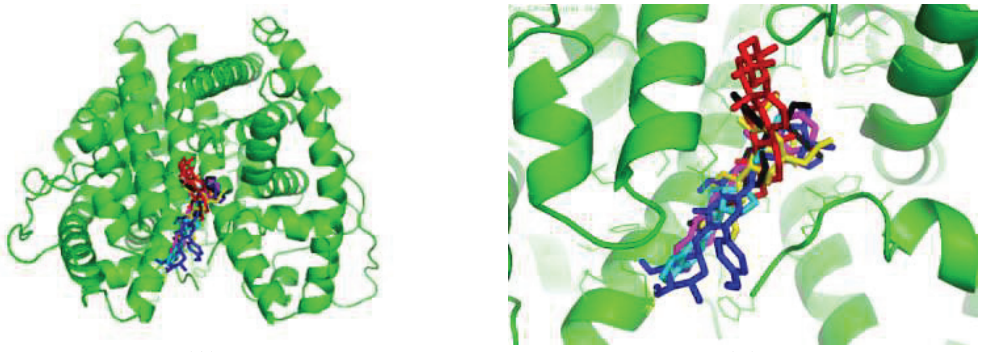
## RESULTS

The compounds used were Kaempferitrin, β-amyrin, Piperbetol, Piperine, and Sesamin, which are shown in Table 1.

**TABLE 1.** Results of LC-MS of Five Compounds from Red Betel Leaf Extract

| Compound Result | Chemical Formula | RT (min) | Composition (%) |
|---|---|---|---|
| Kaempferitin | $C_{27}H_{30}O_{14}$ | 33.483 | 4.01891 |
| Piperbetol | $C_{22}H_{26}O_6$ | 12.998 | 3.72487 |
| β-amyrin | $C_{30}H_{50}O$ | 19.319 | 3.41531 |
| Sesamin | $C_{20}H_{18}O_6$ | 12.433 | 2.61870 |
| Piperine | $C_{17}H_{19}NO_3$ | 10.111 | 1.86077 |

The clarification results of molecular docking using PyMol software showed that the five red betel compounds and Tamoxifen drug were in one site with Estrogen Receptor-α (ER-α) presented in the following Figure 1 & 2.



**(A)** **(B)**

**FIGURE 1.** A) Clarification of Binding Site of Red Betel and Control Drug Compounds in the Estrogen Receptor-α (ER-α), B) Clarification of Binding Site of Red Betel and Control Drug Compounds in the Estrogen Receptor-α (ER-α) with 12Å Magnification. Kaempferitrin (Dark Blue), β-amyrin (Red), Piperbetol (Yellow), Piperine (Purple), Sesamin (Light Blue), Tamoxifen (Black)

The docking process that employed PyRx-Python Prescription of 0.8 version software showed the binding affinity between ligand-macromolecule demonstrated in the following Table 2.

**TABLE 2.** Results of Molecular Docking in the Form of Binding Affinity

| Compound | Bonding Affinity (kcal/mol) |
|---|---|
| Sesamin | -9.3 |
| Kaempferitrin | -9.2 |
| β-amyrin | -8.3 |
| Piperine | -8 |
| Piperbetol | -6.9 |
| Tamoxifene (Drug) | -7.4 |

The molecular docking results visualized by Discovery Studio software, the amino acid residues formed from the Red Betel, and control drug compounds correlated to the amount of Hydrogen bond and Alkyl bond are shown in the following Table 3.

**TABLE 3.** Amino Acid Residues and the Type of Ligand-Macromolecule Bond

| Ligand | Amino Acid Residues | |
|---|---|---|
| | Hydrogen Bond | Alkyl Bond |
| Kaempferitin | Glu: 419, Glu: 523 | Cys: 381, Met: 427, His: 516, Lys: 520, |
| β-amyrin | - | Met: 427, Lys: 520 |
| Piperbetol | - | Lys: 520, Arg: 548 |
| Piperine | - | Lys: 381, Lys: 520 |
| Sesamin | | Met: 427, Lys: 520 |
| Tamoxifen | | His: 377, Cys: 381, Lys: 520 |

Prediction of red betel compound potency is presented in the following table 4.

**TABLE 4.** Prediction of Flavonoid Group-Compound Potency of Red Betel (*Piper crocatum* Riuz & pav.)

| No. | Potency | Pa Value | | | | | |
|---|---|---|---|---|---|---|---|
| | | Kaempferitrin | β-amyrin | Piperbetol | Piperine | Sesamine | Tamoxifene (control) |
| 1. | Anti-neoplastic | 0.847 | 0.916 | 0.792 | 0.507 | 0.797 | 0.821 |
| 2. | Anti-oxidant | 0.905 | 0.405 | 0.268 | 0.196 | 0.516 | 0.926 |

*Source: PASS SERVER* [8]

The pharmacokinetic characteristics of Red Betel and control active compounds are presented in the following Table 5.

**TABLE 5.** Prediction of Potencies of Red Betel and Control Compounds

| Parameter | | Compound | | | | | |
|---|---|---|---|---|---|---|---|
| | | **Kaempferitrin** | **β-amyrin** | **Piperbetol** | **Piperine** | **Sesamine** | **Tamoxifene (control)** |
| Absorbtion | Absorbtion in human's digestion (%) | 35.385 | 93.733 | 96.282 | 94.444 | 97.81 | 96.885 |
| | Permeability Caco-2 (log Papp in $10^{-6}$ cm/s) | 0.225 | 1.226 | 1.301 | 1.596 | 1.399 | 1.065 |
| Distribution | Volume Distribution (human) (log L/kg) | 1.487 | 0.268 | 0.107 | 0.158 | -0.17 | 0.83 |
| | Blood Brain Barrier Permeability (BBB) (logBB) | -1.823 | 0.667 | -0.522 | -0.102 | -0.862 | 1.329 |
| Metabolism | CYP2D6 Substrate | No | No | No | No | No | No |
| | CYP2D6 Inhibitor | No | No | No | No | No | Yes |
| Excretion | Total of Clearance (log mL/min/kg) | -0.102 | -0.044 | 0.295 | 0.232 | -0.126 | 0.556 |
| | Renal OCT2 Substrate | No | No | No | Yes | No | No |
| Toxicity | Ames Toxicity | No | No | No | No | Yes | Yes |

*Source*: pkCSM [9]

## DISCUSSION

Based on Table 2, the binding affinity of Red Betel compound had a lower value than Tamoxifen drug. According to the reasearch [10], the lower binding affinity indicated that binding the target protein needs less energy. Although Piperbetol has higher affinity value than Tamoxifen drug, the five Red Betel compounds could work together to inhibit the activity of Estrogen Receptor-α (ER-α). The interaction between compounds could enhance their activity and reduce the toxicity of those compounds [11]. The best ligand orders bound with Estrogen Receptor-α (ER-α) are Sesamin, Kaempferitrin, β-amyrin, Piperine, Piperbetol, and Tamoxifen, respectively.

Table 3 presents that the five Red Betel compounds interacted with the enzyme through alkyl bond, while Kaempferitrin besides passing alkyl bond, it also passed hydrogen bond (Table 3). Hydrogen bond plays a significant role in macromolecular recognition, folding, and stability, while Alkyl bond can increase the affinity between ligand-receptor and biological activity of the ligand [10, 12]. The clarification results displayed in Table 3 also show that the amino acid residues of Estrogen Receptor-α (ER-α) connected to five Red Betels through amino acid residues of Glu-419, Met-427, His-516, Lys-520, Glu-523, Arg-548, and the amino acid residues were the same as the amino acid residues of Tamoxifen, drug namely His-377, Cys-381, and Lys 520.

Based on Table 4, the potencies of Red Betel compound as anti-neoplastic, namely Kaempferitrin, β-amyrin, Piperbetol, Sesamine had a high anti-neoplastic activity, experimentally and in silico, since the Pa value was more than 0.7. The anti-neoplastic agent is used during the treatment for most cancer patients by inhibiting the growth of tumor [13]. The potency of Red Betel compound as anti-oxidant is Kaempferitrin that has a high activity

experimentally and in silico since the Pa value was more than 0.7. The ROS level in the cell abnormalities (cancer) is even very high. This case is caused by high metabolism activity, mitochondria dysfunction, peroxisome activity, the increase in cell receptor signaling, oncogene activity, oxidases activity, cyclooxygenases, lipooxygenases, and thymidine phosphorylase, or through cross-reaction with immune cell infiltration. The anti-oxidant that will catch free radical then forms a relatively stable radical [14].

Parameter of absorption consists of the absorption in human's digestion and Caco-2 cell. The absorption in human's digestion of the Red Betel compound, which was sesamin (97.81%), had a higher value than that of Tamoxifen drug (96.885 %). According to [15], the absorption is good when the absorption value is > 80%, while it will be bad if it is < 30%. The absorption in Caco-2 cell permeability of Red Betel, that was piperine (1.596 x10-6 cm/s), had a higher value than that of Tamoxifen drug (1.065x10-6 cm/s). According to research [15], the Caco-2 permeability will be high if the predicted Papp log value is > 0.90 cm/s.

The distribution parameter consists of the volume of distribution (vd) and Blood-Brain Barrier Permeability (BBB). The Vd of Red Betel compound, that was kaempferitrin (1.487 LogL/kg), had a higher value than that of Tamoxifen drug (0.83 LogL/kg). According to [15], the Vd value is categorized low if the volume of distribution is less than -0.15 log L/kg and the volume value is considered high if it is more than 0.45 log L/kg. This compound can be equally distributed to provide a similar concentration in the blood plasma. BBB permeability in the Red Betel, which was β-amyrin (0.667 logBBB), had a lower value than the BBB permeability of Tamoxifen drug (1.329 logBBB). According to research [15], a compound is categorized being able to penetrate blood-brain barrier well if it has Log BB value > 0.3, and it cannot be distributed well if the log BB value is < -1.

In the parameter of metabolism, the compound of Red Betel is not CYP2D6 substrate and does not become a CYP2D6 inhibitor while Tamoxifen is not CYP2D6 enzyme inhibitor. The Red Betel compound could be metabolized well by CYP2D6 enzyme, while Tamoxifen drug could not be metabolized well since it influenced the function of CYP2D6 enzyme. According to [15], CYP2D6 cytochrome is responsible for the essential drug metabolism in drug metabolism reaction. Flavonoid-group compound tends to be metabolized by the P450 enzyme in the body.

The total of Clearance in Red Betel compound was lower than Tamoxifen drug (0.556 logs/ml/min/kg) so that the drug compound can be excreted faster than the Red Betel compound. The Red Betel compounds, except piperine compound, is Renal OCT2 substrate and does not cause mutagenic, and it is similar to drug compound. Red betel compound, except sesamin, does not produce toxics while the tamoxifen drug has toxics indicated in Ames toxicity parameter.

## SUMMARY

The five Red Betel and control drug compounds were bound with Estrogen Receptor-α (ER-α) on the same binding site through alkyl and hydrogen bonds with lower affinity value than that of control drug. The red betel active compounds (kaempferitrin, β-amyrin, piperine, and sesamin) can potentially replace Tamoxifen drug based on the compound potency as an anti-neoplastic and anti-oxidant. Based on the pharmacokinetic characteristic that is ADMET, red betel has balanced value with Tamoxifen drug compound.

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