

ANTICANCER ACTIVITY OF B-SITOSTEROL FROM *PLECTRANTHUS AMBOINICUS* (LOUR. SPRENG.) LEAVES: *IN VITRO* AND *IN SILICO* STUDIESPOPPY ANJELISA Z HASIBUAN^{1*}, PANAL SITORUS², DENNY SATRIA²¹Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. ²Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. Email: poppyanjelisa@usu.ac.id

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ABSTRACT**Objective:** β -sitosterol is the steroid compound which is an important nutrient in the diet meal, hydrophobic and soluble in organic solvents and considered as a good biomarker due to its biological activity.**Methods:** *In vitro* study was using 2,5-diphenyl tetrazolium bromide method towards T47D, MCF-7, HeLa, and WiDr cell lines. *In silico* docking using PLANTS program and visualized by Yasara program. The model of three dimension enzyme structures used in this research were epidermal growth factor receptor (EGFR), phosphatidylinositol-3-kinase (PI3K), estrogen receptor-alpha (ER- α), ER-beta (ER- β), and human EGFR 2 (HER-2). Two and three dimensions of β -sitosterol, ZSTK474, and tamoxifen as the standard were generated using Marvin Sketch program.**Results:** β -sitosterol was found to have inhibitory concentration 50% of 0.55; 0.87; 0.76, and 0.99 mM. β -sitosterol and ZSTK474 were inhibited EGFR and PI3K with docking score -92.8195; -91.7920 and -91.7470; -94.7491 β -sitosterol and tamoxifen were inhibited ER- α , ER- β and HER-2 with docking score -78.5570; -89.535, -68.7717; -52.008 and -90.4908; -50.5576, respectively.**Conclusion:** Based on the results above that shows β -sitosterol provide effective as anticancer.**Keywords:** β -sitosterol, Inhibitor, Anticancer, *In vitro*, *In silico*.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i5.16931>**INTRODUCTION**

The diversity of medicinal plants in Indonesia is one of the chances in development potential of Indonesia in the globalization era [1]. The use of medicinal plant extracts for the treatment of human disease is an ancient practice and thus has greatly increased in recent years. Cancer is one of the most frequent and distressing diseases which increased during the past 50 years [2]. Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects [3]. Indonesia has the potential diversity of plant species as medicinal plants.

One of these medicinal plants is *Plectranthus amboinicus* (Lour.) Spreng. The *in vitro* cytotoxic property of the crude extract of leaves was tested against HeLa, and showed that the n-hexane, ethylacetate and ethanol extracts had cytotoxic effect on HeLa cells with inhibitory concentration 50% (IC₅₀) values 76.322 μ g/mL, 143.291 μ g/mL, and 88.997 μ g/mL, respectively [4], and it showed cytotoxic effect on MCF7 cell lines, too [5]. The previous studies had shown that the n-hexane, ethylacetate extracts exhibited strong cytotoxic effect on T47D breast cancer cells with IC₅₀ value of 44.716 μ g/mL and 37.61 μ g/mL, respectively, and showed the synergistic effect in combination with doxorubicin to inhibit the HeLa cell line [4]. It showed the same effect in combination with doxorubicin to inhibit T47D cell line [5].

β -sitosterol is the phytosterol with chemical structure similar to the cholesterol. It is an important nutrient in the diet meal, hydrophobic and soluble in organic solvents and considered as a good biomarker due to its biological activity [6]. Broadly, β -sitosterol is used as an antioxidant and an antidiabetic agent [7]. It is also considered to be highly effective in the treatment of prostate enlargement [8], to boost the function of T cells and primes the immune system to function and operate more efficiently [9]. Human liver microsome studies show that β -sitosterol inhibits the cholesterol absorption [10]. It has shown the

antifertility [11,12], anti-inflammatory and antipyretic activity [13]. The purposes of this research were to assess the activity of β -sitosterol in inhibition the growth of T47D, MCF-7, HeLa and WiDr cell lines and the activity in inhibit of phosphatidylinositol-3-kinase (PI3K), EGFR, estrogen receptor-alpha (ER- α), estrogen receptor-beta (ER- β) and HER-2 with *in silico* method.

METHODS**Chemicals and reagents**

n-hexane, ethylacetate and methanol were purchased from Merck (Darmstadt, Germany), dimethyl sulfoxide (Sigma-Aldrich, Germany), [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma Chemical, St. Louis, MO), RPMI media and phosphate buffer saline foetal bovine serum (FBS) 10% v/v (Gibco, Grand Island, NY, USA), silica gel 60H (Merck), thin-layer chromatography (TLC) silica gel GF₂₅₄ (Merck).

Extraction and isolation of β -sitosterol

The *P. amboinicus* was obtained from Pematang Siantar, North Sumatera, Indonesia and was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium. The leaves of *P. amboinicus* were dried at 45°C and ground into powder. The air-dried and powdered leaves of *P. amboinicus*. (1 kg) were repeatedly fractionated by cold maceration with n-hexane (3 \times 3 d, 7.5 L). At room temperature and occasionally with stirring. The filtrate was collected and then evaporated under reduced pressure to give a viscous fraction and then freeze dried to dry [1,14-17]. n-hexane extract was fractionated with vacuum liquid chromatography using gradient eluent n-hexane: Ethylacetate (100:0; 90:10; 80:20; 70:30; 60:40; 50:50; 40:60; 30:70; 20:80; 10:90; 0:100) and methanol (100) and silica gel 60H as stationary phase. All fractions were concentrated by rotary evaporator and were dried using freeze dryer to eliminate the existence of the remaining traces of water. Then, the fractions were analyzed by

thin layer chromatography with silica gel GF₂₅₄ as stationary phase and n-hexane-ethylacetate as mobile phase. β-sitosterol was obtained using column chromatography and preparative TLC [18,19].

Cell lines and culture conditions

HeLa, T47D, MCF-7, and WiDr cell lines were kindly provided by Parasitology Laboratory, Faculty of Medicine, University of Gadjah Mada, Indonesia. The cell lines were cultured in RPMI (Hela, T47D and WiDr) and DMEM (MCF-7) mediums, supplemented with 10% (v/v) FBS, 2% penicillin-streptomycin and 0.5% fungizone in a 37°C incubator with 5% CO₂⁴⁰.

Cytotoxicity assay

Cytotoxicity was determined by the MTT colorimetric assay. Briefly, T47D, MCF-7, HeLa and WiDr cell lines were plated at 10⁴ cells/well in a 96-well plate. Each well contained 1×10⁴ cells. The culture cells were incubated in a humidified incubator at 37°C at atmosphere of 5% CO₂ and 95% air for 24 hrs. After incubation for 24 hrs at 37°C, the medium was discharged, and cells were treated by β-sitosterol with different concentration and incubated for 24 hrs. MTT 0.5 mg/mL solution was added to each well and further incubated for 4 hrs at 37°C. Viable cells react with MTT to produce purple formazan crystals. After 4 hrs, the stopper 10% SDS (Sigma Co. St. Louis) in 0.01 N HCl (Merck) was added to dissolve the formazan crystal. The cells were then incubated for 24 hrs in room temperature and protected from light. After incubation, the cells were shaken Optical density was read with an ELISA reader at λ 595 nm. The experimental data was absorbance of each well, and then converted to percentage of viable cells [1,14,20].

$$\text{Percentage of viable cell} = \frac{B - C}{A - C} \times 100\%$$

Where A, B and C are absorbance of control group, treatment group and medium (vehicle), respectively.

In silico studies

Aspire E1-470 series operated by Windows 7 Home Premium, Intel® Core™ i3 -3217U (1,8 GHz, 3MB L3 cache), 32-bit, hard disc drive 500 GB and RAM memory 2 GB DDR3 L were used to run the molecular docking process.

In silico docking using PLANTS program and visualized by Yasara program. Co Pen Drive Linux KDE program was used to connecting

Windows operation system to Linux operation system. The model of three dimensions of enzyme structure used in this research was PI3K binding pocket with the protein data bank code 3DBS, IM17 for EGFR, 3PP0 for HER-2, 3ERT for ER-α, and 1QKM for ER-β. They were obtained through from http://www.rcsb.org/pdb. Two and three dimension conformation models of β-sitosterol, tamoxifen and ZSTK474 as the standard HER-2, ER-α, ER-β and PI3K inhibitor were generated by Marvin Sketch program [21,22].

Statistical analysis

All data were expressed as IC₅₀ that analysis using probit in regression at SPSS 22.

RESULTS AND DISCUSSION

Isolation and characterization of β-sitosterol

The purity of β-sitosterol obtained was analyzed using TLC. The chromatogram gave positive results with Liebermann-Burchard reagent. Rf 0.50 indicated steroid group and confirmed with two-way TLC (mobile phase I: n-hexane:ethyl acetate 80:20 and mobile phase II: Toluene:ethyl acetate 90:10). Finally, elucidation of the structure of β-sitosterol with the data of UV spectrum (ethanol) at λ-max 268.5 nm; infra-red (KBr) with wave number 3421.72; 2920.23; 2852.72; 1566.20; 1415.75 and 1111.00 cm⁻¹. Carbon NMR spectrum (75 MHz, CDCl₃) of pure isolates indicated by the chemical shift data A1 isolate 13C - NMR spectrum showed the presence of hydroxyl group δC = 71.82 ppm (C3) and two group ena δC 121.72 (C6) and δC 140.76 (C5). 1H-NMR spectrum (300 MHz, CDCl₃) showed a multiplet δH 3.53-3.68 ppm of the H-3 and the characteristics of the C-3 atom; singlet peak at δH 1,05 ppm and δH 0.65 ppm; doublet at δH 0.95 ppm and 0.93 [7,8,19].

IC₅₀

MTT method was used to determine cell viability after incubation for 24 hrs. In every treatment, β-sitosterol was shown to inhibit cells growth toward T47D, MCF-7, HeLa and WiDr cell lines. The IC₅₀ value of β-sitosterol was 0.55; 0.87; 0.76, and 0.99 mM, respectively.

Molecular docking

The root mean square deviation (RMSD) values resulted from these ligand docking were 1,5761 Å for 3DBS; 1,6970 Å for 1M17; 1,4270 Å for 3ERT; 0,3900 Å for 1QKM and 1.1930 Å for 3PP0. The RMSD was obtained <2.0000 Å indicating that the docking methods were valid [23]. In silico docking between β-sitosterol into the 3DBS, 1M17,

Table 1: Docking score between ligand and protein target

| S. No | Ligand name | Docking score | | | | |
|-------|--------------|---------------|----------|----------|----------|----------|
| | | PI3K | EGFR | HER-2 | ER-α | ER-β |
| 1 | ZSTK474 | -94.7491 | -91.7920 | | | - |
| 2 | Tamoxifen | | | -50.5576 | -89.5350 | -52.0080 |
| 3 | β-sitosterol | -91.7470 | -92.8195 | -90.4908 | -78.5570 | -68.7717 |

EGFR: Estimated glomerular filtration rate, HER: Hyperemesis education and research, ER-α: Estrogen receptor-α, ER-β: Estrogen receptor-β

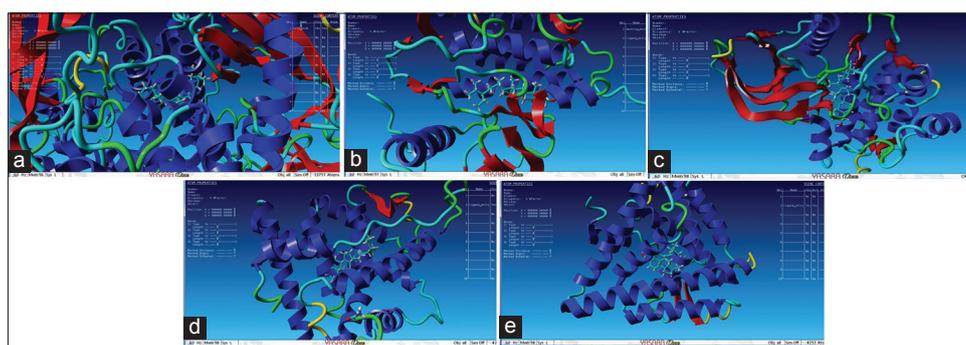


Fig. 1: Visualization of interaction between, (a) β-sitosterol with phosphatidylinositol-3-kinase, (b) β-sitosterol with epidermal growth factor receptor, (c) β-sitosterol with HER-2, (d) β-sitosterol with estrogen receptor-alpha, (e) β-sitosterol with estrogen receptor-beta

3ERT, 1QKM and 3PP0 binding pocket result in the docking score in Table 1 is showed the results of docking score. Fig. 1 is showed the results of visualization of β -sitosterol to PI3K, EGFR, ER- α , ER- β and HER-2 using Yasara.

The docking score represents the binding affinity of the ligand to the target protein. The docking of PI3K, EGFR, ER- α , ER- β dan HER-2 target with compounds using docking procedure revealed that all the computationally predicted lowest energy complexes of PI3K, EGFR, ER- α , ER- β dan HER-2 are stabilized by intermolecular hydrogen bonds and stacking interactions [21]. Docking score of β -sitosterol was lower than ZSTK474 as kinase inhibitor especially PI3K but higher in inhibition of EGFR. Docking score of β -sitosterol was lower than tamoxifen as ER- α inhibitor but higher in inhibition of ER- β and HER-2. *In silico* drug design can play a significant role in all of the stages of drug development from preclinical assessment to the end of clinical development [24]. The results were obtained at *in silico* screening have shown that it represents the best step (way) to get an accurate result in a short time and saving manner [25].

PI3K pathway plays important roles in tumor initiation and progression, including those in proliferative activity and in apoptosis. PI3K signaling is also commonly associated with the metastatic cascade in carcinoma. Although several aspects of tumor inhibition are not fully understood, numerous small molecule inhibitors targeting the PI3K pathway is currently being studied in clinical trials [26]. There are strong relationship between EGFR, PI3K, PI3K/AKT/mTOR and ER- α , ER- β and HER-2 signaling is frequently deregulated due to mutations affecting one of its upstream regulators the EGFR receptor and other components within the pathway [27-29].

CONCLUSION

β -sitosterol is steroid from *P. amboinicus* (Lour.) Spreng. It was showed to have the activity in inhibition of cancer growth toward T47D, MCF-7, HeLa and WiDr cell lines and through PI3K, EGFR, ER- α , ER- β dan HER-2 pathways and they are potential to develop as anticancer.

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