INTRODUCTION

The diversity of medicinal plants in Indonesia is one of the chances in development potential of Indonesia in the global 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% (IC_{50}) values 76.322 µg/mL, 143.291 µg/mL, and 88.997 µg/mL, respectively [4], and it showed cytotoxic effect on MCF7 cell lines, too [5]. The previous studies had shown that the 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 GF_{254} (Merck).

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), 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 −78.5576; −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.
thin layer chromatography with silica gel GF 

 as stationary phase and n-hexane-ethyl acetate 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% CO₂.

**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-sitosetrol 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**

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, 1M17 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-sitosetrol, 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 2.2.

**RESULTS AND DISCUSSION**

**Isolation and characterization of β-sitosetrol**

The purity of β-sitosetrol 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-sitosetrol 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. H-3 and the characteristics of the C-3 atom; singlet peak at δH 1,05 ppm. Carbon 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].

**Table 1: Docking score between ligand and protein target**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Ligand name</th>
<th>Docking score</th>
<th>PI3K</th>
<th>EGFR</th>
<th>HER-2</th>
<th>ER-α</th>
<th>ER-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ZSTK474</td>
<td>-94.7491</td>
<td>-91.7920</td>
<td>-50.5576</td>
<td>-89.5350</td>
<td>-52.0080</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Tamoxifen</td>
<td>-91.7470</td>
<td>-92.8195</td>
<td>-90.4908</td>
<td>-78.5570</td>
<td>-68.7717</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>β-sitosetrol</td>
<td>-91.7470</td>
<td>-92.8195</td>
<td>-90.4908</td>
<td>-78.5570</td>
<td>-68.7717</td>
<td></td>
</tr>
</tbody>
</table>

EGFR: Estimated glomerular filtration rate, HER: Hyperemesis education and research, ER-α: Estrogen receptor-α, ER-β: Estrogen receptor-β.
PISK pathway plays important roles in tumor initiation and progression, including those in proliferative activity and in apoptosis. PISK 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 PISK pathway is currently being studied in clinical trials [26]. There are strong relationship between EGFR, PISK, PI3K/akt/mTor and Er-α, Er-β dan HER-2 pathways that are potential to develop as anticancer.

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, ER-β and HER-2 using Yasara.

REFERENCES