INTRODUCTION

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 last 50 years [1]. Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects [2]. Indonesia has the potential diversity of plant species as medicinal plants. The World Health Organization reported that breast cancer is one of the leading causes of death and the most common cancer type among women worldwide in 2012 [3]. Moreover, breast cancer ranks as the 5th cause of death from cancer overall (552,000 deaths), is the most frequent cause of cancer death in women in less developed countries (324,000 deaths, 14.3% of total), and the 2nd cause of cancer death in developed countries (198,000 deaths, 15.4%) after lung cancer. A previous study reported that breast cancer is predicted to be a leading new cancer cases and the 2nd most common death cause of women suffering from cancer in the US [1]. Therefore, research and development in cancer detection and treatment is importantly required to solve those problems.

Breast cancer occurs when breast cells start to grow uncontrollably. These cells can invade nearby tissues and spread throughout the body. Each type of tissue in the breast can form a cancer, but the cancer usually arises in the milk ducts or glands. Factors that influence the risk of breast cancer are the length of exposure to hormones (e.g., menstruation at an early age or late menopause), reproductive factors (e.g., no children and first pregnancy at an advanced age), dietary factors and lack of physical activity (e.g., obesity and dietary fat), radiation during breast development, hormone replacement therapy on chronic use, as well as congenital genetic factors associated with breast cancer like the presence of gene mutations [4].

Poguntano (Picria fel-terrae Lour.) has been used as drug of colic, malaria, diuretic, fever, and skin disease [5]. Modern pharmacological investigations indicated that the extract of P. fel-terrae Lour. exerts diuretic, antispetic, hepatoprotective, cardioprotective, antioxidant, anti-inflammatory, anthelmintic, and analgesic activities [6-15]. Moreover, P. fel-terrae inhibits hepatitis B (HB) e-antigen excreted by HepG2 2215 cell lines, suggesting to have anti-HB virus activity [16]. It can be developed as cochemotherapeutic regimen for breast cancer by inducing apoptosis and cell cycle arrest and suppressing cyclin D1 and Bcl-2 expression based on the recent studies [17,18]. The aim of this study was to determine cytotoxic activity of n-hexane, ethyl acetate, and ethanol fractions of P. fel-terrae Lour. herbs toward 4T1 and MCF-7 cell line.

METHODS

Plant and chemicals material

Fresh herbs of P. fel-terrae Lour. were collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia. P. fel-terrae Lour. was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium. Chemicals used were distilled water, dimethyl sulfoxide (Sigma), and [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma).

Preparation of ethyl acetate fraction (EAF)

The air-dried and powdered herbs of P. fel-terrae Lour. (1 kg) were repeatedly fractionated by cold maceration with n-hexane (3×3 day, 7.5 L). The powder was dried in the air and fractionated with ethyl acetate (3×3 day, 7.5 L). The powder was dried in the air and fractionated with ethanol 96% (3×3 day, 7.5 L) at room temperature with occasional stirring. The filtrate was collected and then evaporated under reduced pressure to give a viscous fraction and then freeze-dried to dry [17,19,20].

Cytotoxicity assay

The cells were treated with n-hexane, ethyl acetate, and ethanol fractions. In this test, 4T1 and MCF-7 cell line were grown in Dulbecco’s modified eagle medium, medium containing 10% fetal bovine serum (Gibco), and fungizone 0.5% (Gibco) in a flask in a humidified atmosphere (5% CO₂) at 37°C. The inoculums seeded at 1×10³ cells/mL at an optimal volume of 0.1 mL per well. After 24 h incubation, the medium was discharged and treated with n-hexane, ethyl acetate, and ethanol fractions. After incubation 24 h, the cells were incubated with 0.5 mg/mL MTT for 4 h in 37°C. Viable cells reacted with MTT to produce purple formazan crystals. After 4 h, SDS 10% as stopper

ABSTRACT

Objective: This study was carried out to investigate the cytotoxic activity toward 4T1 and MCF-7 cell lines of Picria fel-terrae Lour. P. fel-terrae Lour. herb powder was extracted by maceration method with n-hexane, ethyl acetate, and ethanol solvent. In vitro study was using MTT method toward 4T1 and MCF-7 cell lines.

Results: The inhibitory concentration 50% was 234.10 ± 7.85, 50.49 ± 1.07, and 212.53 ± 7.55 µg/mL for 4T1 and 84.62 ± 1.44, 56.79 ± 0.22, and 235.51 ± 4.77 µg/mL for MCF-7 cell lines, respectively.

Conclusion: The results reveal that P. fel-terrae Lour. herb fractions provide effective as anticancer. Our further study is to assess the mechanism of ethyl acetate fraction in inhibit angiogenesis and metastatic in breast cancer.

Keywords: Cytotoxicity, Picria fel-terrae Lour., Herbs, Fractions, Cell lines.
Table 1: IC$_{50}$ value of n-hexane, ethyl acetate, and ethanol fraction of P. fel-terrae Lour. herbs toward 4T1 and MCF-7 cells

<table>
<thead>
<tr>
<th>Fraction</th>
<th>IC$_{50}$ (µg/mL)</th>
<th>4T1</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>234.10±7.85</td>
<td>84.62±1.44</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>50.49±1.07</td>
<td>56.79±0.22</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>212.53±7.55</td>
<td>235.51±4.77</td>
<td></td>
</tr>
</tbody>
</table>

IC$_{50}$: Inhibitory concentration 50%

(Sigma) in 0.01N HCl (Merck) was added to dissolve the formazan crystals. The cells were incubated for 24 h in room temperature and protected from light. After incubation, the cells were shaked, and absorbance was measured using enzyme-linked immuno-sorbent assay reader at λ 595 nm. The data which were absorbed from each well was converted to percentage of viable cells [20,21].

The equation to determine viability of cells:

Viability = \frac{Abs of treatment - Abs of medium}{Abs of control cells - Abs of medium} \times 100%

RESULTS AND DISCUSSION

Plant authentication

Plant authentication was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor, and the voucher specimen was deposited in herbarium with number of 332/IPHI.1.01/II.07/II/2016 and was showed species of P. fel-terrae (Lour.).

Inhibitory concentration 50% (IC$_{50}$)

MTT method was used to determine cell viability after incubation for 24 h. Cytotoxic activity of n-hexane, ethyl acetate, and ethanol fraction of herbs of P. fel-terrae Lour. was shown in Table 1.

In every treatment, n-hexane, ethyl acetate, and ethanol fraction were shown to inhibit cells growth. The highest IC$_{50}$ value was obtained from EAF of P. fel-terrae Lour. herbs of 50.49 ± 1.07 µg/mL toward 4T1 cell lines and 56.79 ± 0.22 µg/mL toward MCF-7 cell lines. The estimated cytotoxicity of natural product is related to content of active compound in these plants including P. fel-terrae Lour. flavonoids, saponins, and tannins estimated as active compounds [13].

ACKNOWLEDGMENTS

We gratefully thank to Research Center University of Sumatera Utara through Hibah Talenta “Hibah Penelitian Unggulan Universitas” Research Grant 2017 “No: 5358/UNS.1.R/PPM/2017” for financial support in the study.

REFERENCES