Cytotoxic Activity of Triterpenoid Fraction of Indonesian Propolis on Human Breast Carcinoma Cell Lines

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INTRODUCTION

Human breast cancer remained one of the leading causes of mortality because of cancers in the world after lung cancer, stomach cancer, hepatic cancers, and colon cancers with an incidence of 502,000 cases (Jemal et al., 2005). Human breast cancer develops when breast cells grow uncontrollably and eventually invade distant tissues. All types of tissues in the breast progressed to cancer, but cancer generally develops in ducts and glands. It took several months or even several years for cancer to progress to the extent that the tumor is large enough to palpate on the breast. The tumor can be detected using mammograms that could detect tumor early on its development (Perry et al., 1995).

Propolis is a glue-like substance formed by honey bees from plants’ resin that has anti-microbial (Bankova et al., 2000) and activity antiviral activities. It also serves as an anti-inflammation and anti-cancer (Kianmehr et al., 2001; Grunberger et al., 1988). It stimulates human immune system (Bankova, 2000; Syamsudin et al., 2009a).

Propolis has a broad spectrum of pharmacological effects because of high flavonoid contents. Greenaway et al. (1990) suggested that flavonoid is included into natural compound groups with varied phenol structures. Flavonoid is contained in fruits, nuts, barks, roots, shaft, and flowers, as well as in wine.

Chemical contents of propolis are varied depending on the vegetation families in the areas where propolis is formed (Marcucci et al., 1999). Chemical contents of propolis from four-season areas were dominated by phenolic compounds, such as flavonoid and cinnamate acid derivatives. In the tropical areas, propolis contents were dominated by diterpene and prenolated compounds. Bankova (1998) suggested that more than 180 compounds were contained in the identified propolis. Another source, Greenaway et al. (1990), suggested that more than 300 different compounds were identified in propolis. They include flavonoid, chalcone, aliphatic acid, short-chain essential oil, aromatic acid, benzoic acid and its derivatives, aldehyde, alcohol, cinnamate acid and its derivative, phenol and heteroaromatic compound, terpene, sequesterpeno, terpenoid, sterol, sugar, lactone, amylase amino acid, nucleic acid derivative, vitamin, mineral, and so on. Bankova et al. (2000) classified chemical contents of propolis into three: 1) aglycone flavonoid, (2) cinnamate acid derivative, and (3) terpenoid. The efforts of finding anti-cancerous compounds from natural sources, particularly propolis that is proven effective in inhibiting human breast cancer cells (T47D and MCF-7) in vitro through preliminary studies, provided huge opportunities to identify selective anti-cancers against human breast cancers.

Materials and methods

1. Fractionation of ethylacetate extracts Ethylacetate fractions were isolated through column chromatography analysis. Three point two grams of active fractions were introduced into the column (SiO2); n-hexane and acetone with a ratio of 7:3 were used as eluents. Every 100 ml was intercepted and then isolated based on the fractions, which were monitoring with using Thin Layer Chromatography (TLC) analysis. The sub-fractions (FE-1 to FE-5) were tested in vitro for anti-plasmodial properties. The assay showed that sub-fractions FE-5 were most active than others sub-fractions (Table 2). Therefore, only FE-5 was subject to column chromatography and eventually isolated into one isolate for chemical structure analysis.

2. Purification and Identification of Chemical Structure Purification of isolates was conducted using decantation techniques. The isolates were decanted with n-hexane and washed several times with the eluents. Pure compound resulted from isolation were identified using spectrometries of UV, IR, NMR (1H-NMR and 13C-NMR) and mass spectra; while cytotoxic test was conducted with MTT method. The study found that active isolates were triterpenoid compounds, that is α-amyrin which is active against MCF-7 cells with an IC50 value of 4.57µg/ml and T47D of 10.23µg/ml.

Results and Discussion

Active fractions with the most powerful cytotoxic activities were isolated with column chromatographic analysis. Three point two grams of active fractions were introduced into columns (SiO2), with n-hexane and acetone = 7 : 3 used as eluents. Every 100 ml was intercepted and isolated based on the fractions and monitored by using TLC analysis. Any fractions with the same Retardation of Factor (Rf) were put into one; Changes in some fractions are presented in Table 1.
The isolates were assayed for their activities in vitro using MCF-7 and T47D cells with the aim of selecting the most potential isolates. The results of cytotoxic assay for the fractions and isolates are presented in Table 2.

Table 2: Results of cytotoxic assay for the fractions and isolates on MCF-7 and T47D cells

<table>
<thead>
<tr>
<th>Fractions/Isolates</th>
<th>MCF-7 Cells (µg/mL)</th>
<th>T47D Cells (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylacetate fraction</td>
<td>47.58</td>
<td>79.44</td>
</tr>
<tr>
<td>-FE-1</td>
<td>29.09</td>
<td>49.45</td>
</tr>
<tr>
<td>-FE-2</td>
<td>18.74</td>
<td>27.78</td>
</tr>
<tr>
<td>-FE-3</td>
<td>22.25</td>
<td>47.48</td>
</tr>
<tr>
<td>-FE-4</td>
<td>16.58</td>
<td>35.34</td>
</tr>
<tr>
<td>-FE-5</td>
<td>10.88</td>
<td>24.25</td>
</tr>
<tr>
<td>Isolate FE-3</td>
<td>4.57</td>
<td>10.23</td>
</tr>
</tbody>
</table>

Table 2 shows that isolate FE-3 had a cytotoxic activity of 4.57 µg/mL on MCF-7 cells and 10.23 µg/mL on T47D cells. Results of cytotoxic assay for isolate FE-3 compounds were much better than those of FE-5 fractions, namely 10.88 µg/mL on MCF-7 cells and 24.25 µg/mL on T47D cells.

Isolate FE-5-2 was obtained as a white crystal and UV spectrum showed that peak absorption occurred at wavelength of 275 nm, characterized with alkena chromophore group. The IR spectrum showed absorptions at \( \nu_{\text{max}} \) 3411 cm\(^{-1} \) due to hydroxyl group. The molecular formula of the compound was determined to be \( \text{C}_{30}\text{H}_{42}\text{O} \) by GC-MS \( m/z 426 \) for [M]+.

The \(^1\)H-NMR spectra of compound showed there are 8 methyl groups which 6 as singlet (\( \delta \)) and 2 as doublet (\( \delta \)) at \( \delta \)H 0.79 (\( \delta \)), 0.79 (\( \delta \)); 0.80 (\( \delta \)), 0.95 (\( \delta \)), 0.99 (\( \delta \)), 0.99 (\( \delta \)), 1.2 (\( \delta \)), and 1.06 ppm (\( \delta \)). Investigation of chemical shift at \( \delta \)H 5.22 ppm (\( \delta \), H-12) was indicated as proton of olefinic at H-12.

The \(^{13}\)C-NMR and DEPT spectra of isolate FE-5-2 revealed 30 carbon signals consisted 8 methyl carbons (\( \text{CH}_3 \)), 9 methylene carbons (\( \text{CH}_2 \)), 7 methine carbons (\( \text{CH} \)) and 6 quarternary carbons.

Comparison of the NMR spectral data of the isolate with these \( \alpha \)-amyrin (Lima, 2004) showed a good agreement compound FE-5-2 therefore was assigned as \( \alpha \)-amyrin.

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References