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Research Article

CYTOTOXIC ACTIVITY OF TRITERPENOID FRACTION OF INDONESIAN PROPOLIS ON HUMAN BREAST CARCINOMA CELL LINES

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ABSTRACT

A study to identify chemical compound of Indonesian propolis and to examine its cytotoxic activity of human breast cancer cells (MCF-7 and T47D) had been conducted. Identification was conducted by using spectrometries of UV, IR, NMR (^{1}H-NMR and ^{13}C-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 IC_{50} value of 4,57µg/ml and T47D of 10,23µg/ml.

Keywords: cytotoxic, propolis, α-myrin

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 (Banksnota *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 prenilated compounds. Bankova (1998) suggested that more that 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 included flavonoid, chalcone, aliphatic acid, short-chain essential oil, aromatic acid, benzoate acid and its derivatives, aldehyde, alcohol, cinnamate acid and its derivative, phenol and heteroaromatic compound, terpene, sequesterpene, 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 (SiO_2). *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 then re-crystallized by means of appropriate eluents. The isolate was decanted with *n*-hexane and washed several times with the eluents. Pure compound resulted from isolation were identified using spectrometries of UV, IR, NMR (^{1}H-NMR and ^{13}C-NMR) and mass spectra by Gas Chromatography Mass-Selective (GC-MS).

3. Cytotoxic Assay with MTT Method As much as 100 µL of cell suspension of MCF-7 and T47D with a cell density of 5 x 10^4 cell/100 µL was distributed into a 96-well plate and incubated along with extract fractions (100, 80, 60, 40, 20, 10, 1 µg/mL) for 48 hours. In relation to control solution, 100 µL of cell suspension added with RPMI 1640 and natrium phosphate of 5 mM pH 7,2 was used as positive control, while 100 µL of cell suspension along with RPMI 1640 were used as negative control. Furthermore, the cells were incubated in an incubator with CO2 flow of 5% and under a temperature of 37°C. At the final stage of incubation, each well was added with 100 mL of MTT 2,5 µg/mL in an RPMI medium. Then, cells were re-incubated overnight under 37°C. Living cells would react with MTT turning purple. Reaction of MTT was stopped by a stopper reagent and then incubated overnight under ambient temperature. Absorption was read with ELISA Reader on a wavelength of 550 nm.

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 (SiO_2), 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.

Table 4: Results of fractionation with Column chromatography for Fraction A

No	Unification	Sub-fraction	Color	Rf
1.	Fractions 1 - 7	FE-1	Blue	0.56
2.	Fractions 8 - 9	FE-2	Orange-red	0.49
3.	Fractions 10	FE-3	Orange-red	0.34
4.	Fractions 11 - 13	FE-4	Yellow	0.27

5.	Fractions 14 - 16	FE-5	Yellow	0.18

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 I47D cells

Fractions/Isolates	MCF-7 Cells (µg/mL)	T47D Cells (µg/mL)
Ethylacetate	47.58	79.44
fraction	29.09	49.45
-FE-1	18.74	27.78
-FE-2	22.25	47.48
-FE-3	16.58	35.34
-FE-4	10.88	24.25
-FE-5	4.57	10.23
Isolate FE_{5-2}		

Table 2 shows that isolate FE_{5-2} had a cytotoxic activity of 4.57µg/mL on MCF-7 cells and 10.23µg/mL on T47D cells. Results of cytotoxic assay for Isolate FE_{5-2} compounds were much better than those of FE-5 fractions, namely 10,88µg/mL on MCF-7 cells and 24,25µg/mL on T47D cells.

Isolate FE 5-2 was obtained as a white crystal and UV spectrum showed that peak absorption occurred at wave length of 275 nm, characterized with alkena chromophore group. The IR spectrum showed absorptions at $\lambda_{max.}$ 3411 cm^{-1} due to hydroxyl group. The molecular formula of the compound was determined to be $C_{30}H_{50}O$ 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 (s) and 2 as doublet (d) at δH 0.79 (s); 0.79 (d); 0.80 (s); 0.95 (s); 0.99 (s) 0.99 (d); 1.2 (s) and 1.06 ppm (s). Investigation of chemical shift at δH 3.22 ppm (t,) exhibited a typical of hydroxyl group adjacent to the proton of H-3 and the signal at δH 5.22 ppm (d, H-12) was indicated as proton of olefenic at H-12.

The ^{13}C-NMR and DEPT spectra of isolate FE-5-2 revealed 30 carbon signals consisted 8 methyl carbons (CH_3), 9 methylene carbons (CH_2), 7 methine carbons (CH) and 6 quartenary carbons.

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

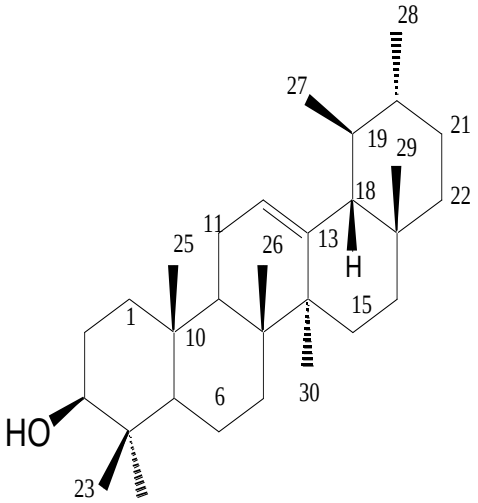

Figure 1: α-amyrin, triterpenoid from propolis

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