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Antiproliferation activities of Indonesian java chili, *Piper retrofractum* Vahl., against breast cancer cells (MCF-7)

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ABSTRACT

Cancer is a major killer in the world. Breast cancer is the first rank in the number of patients treated in Indonesia (28%), followed by cervical cancer (12%). Piperine is an active component in Java chili fruit (*Piper retrofractum* Vahl.) that serves as an anticancer ingredient. By extracting fruit Java chili and tested in vitro against breast cancer cells MCF-7 by means of the MTT-assay. From fifth locations Java chilies example of this research. Based on the analysis of phytochemicals, all Java chili extract contains flavonoids and alkaloids in addition to steroids. Java chili fruits extract the highest anticancer activeness as indicated by the Java chili fruits extract derived from Air Naningan 2 Lampung with IC50 value of 4.35 µg/ml.

Key words: Java Chili, anticancer, *Piper retrofractum*, MCF-7

INTRODUCTION

The common name of *Piper retrofractum* is called Java Chilli, a perennial shrub plant of the genus *Piper* (*Piperaceae*). It is an outstanding pharmaceutical species which grows naturally in Indonesia, Malaysia, Thailand, Vietnam and India. The beverage making from Java Chili fruits has been long used by Indonesia society for relaxing, recovering strength of the body, improving sleeping and aprodisiacum. Indonesia society consumes Java Chilli drink (with added ginger) (called *Bandrek*) in social, ritual and ceremonial activities. The pharmacological poisonous action and side effects of Java Chili have not been found to be significant in the optimum doses used. The growing of Java Chili as a cash crop in Java Island and others island in Indonesia is considered a national treasure as a natural resource.

The piper species have the ability as an anticancer agent or anti-proliferation agent against cancer cells. Research conducted by [1] found that the *Piper bettle* was able to inhibit the growth of cancer cells MCF-7. While [2] study found the *Piper crocatum* was an anti-proliferation agent for human cancer cells T47D. In the research conducted by [3] it was shown that the *Piper rectofractum* Vahl. was able to inhibit the growth of breast cancer cells MCF-7.

The piper species exploited by humans for traditional medicine ingredients and spices with a wide variety of trade names. The aim of the research is to study of anti-proliferation activities againts breast cancer cells MCF-7. Fifth of Java chili fruits was collected from various locations in Central Java and Lampung.

MATERIALS AND METHODS

Materials

This study using Java chili fruits from Giritontro, Paranggupito 1 and Paranggupito 2 Central Java and from Air Naningan 1 and Air Naningan 2 Lampung, and MCF-7 cell cancer from Agency for the Assessment and Application of Technology, Indonesia.

Methods

Stages to be carried out in the study is the sample preparation, extraction of Java chili fruits, phytochemicals analysis, creation of work culture, observation influences the number of samples tested for MCF-7 cell population or testing the effect of adding the sample to the cell viability of MCF-7 cell on inhibition concentration.

Sample Preparation

Java chili harvested and dried by the sun drying. Once dried, crushed chilies Java as desired. After that, the Java chili fruit is ready extraction.

Sample Extraction

Java chili fruits extraction process was a modified method [4-7]. And the extraction process using a microwave as a tool for extraction.

Qualitative testing of chemical components (Phytochemical Analysis).

Qualitative test Java chili extract includes testing the presence of alkaloids, flavonoids, terpenoids, steroids, saponins and tannins [8].

Test alkaloids. A total of 100 mg of extract of Java chili is inserted in a test tube, then add two drops of ammonia and 5 ml of chloroform and filtered. The filtrate was added to 1 ml of screening results H₂SO₄ 2 M, then the fraction of added acid reagent. The existence of alkaloids in Java chili extract is indicated by the formation of a red precipitate the reagent.

Test triterpenoids and steroids. A total of 100 mg of Java chili extract included in a test tube, then add about 5 ml of hot ethanol and filtered. The filtrate was evaporated screening results, then add 1 ml of di-ethyl ether. Having shaken with 'vortex', then add 1 ml of concentrated H₂SO₄ and 1 ml of CH₃COOH. The formation of red or gray color indicates the presence of tri-terpenoids and green color indicates the presence of steroids in Java chili extract.

Test flavonoids. A total of 100 mg of extract of Java chili is inserted in a test tube and add 5 ml of water, and then do the filtering. The filtrate obtained was added Mg powder, 1 ml of concentrated HCl, and 1 ml amilalkohol. Then stirred perfect causing different layers. Colors are formed between the two solutions iso-amyl ethanol indicate flavonoids.

Test tannins. A total of 100 mg of extract of Java chili is inserted in a test tube, then add 5 ml of water and filtered. Filtrate screening results are added 3 drops of 1% FeCl₃. The formation of blue or blackish green indicates Java chili extract tannins.

Test saponins. A total of 100 mg of extract of Java chili is inserted in a test tube and then add 5 ml of water and filtered. The filtrate was shaken with a perfect screening results and allowed to stand for 10 minutes. The formation of a stable froth showed saponin in Java chili extract.

Cytotoxic test against Breast Cancer Cells

Antiproliferasi an activity testing is performed in cell culture with the MCF-7 cancer test using methylene blue as reported by [4-5]. Doxorubicin is used as a positive control and DMSO solution as a negative control. Condensed extract is dissolved in solvent DMSO to make stock solutions of 10%. Stock solution is diluted in RPMI 1640 medium to make solution substock at 1%. For dilution tests are carried out in order to obtain a final concentration of multilevel test solution of 250, 100, 50, 10, and 1 ug / ml. Then 20 mL test solution of various concentrations is added to the plate wells which already contain cancer cells and then incubated for 24 h at a temperature of 37°C 5% CO₂. After addition of test solution back then incubated the cells for 24 h. The number of living cells per well was calculated using an ELISA Reader at a wavelength of 570 nm. IC₅₀ values are determined from the graph of percent living cells against the concentration of the test sample. The percentage of MCF-7 cell death of each test solution and the comparison is computed using the formula: % Cell death = (Ab-Au)/Ab x 100%. Description: Ab : Absorption blank (DMSO) and Au : Absorption test solution.

RESULTS AND DISCUSSION

Java chili fruit before picked (harvested) and made crude drugs can be seen in Figure 1. Java chili fruit harvested before ripe and dried after it is milled and screened compatible with the desired size of the crude drugs. After harvested fruits Java chili can be sun drying and grinding (Figure 2). Java chili fruit extraction has been carried out and managed to obtain extracts with the highest yield obtained from the location Paranggupito 1 and lows of Sumenep each at 16.40 and 11.55% (Table 1). Differences are indicated by the yield value is an application of the levels of ingredients that can be extracted by 70% ethanol in the Java chili fruit. This is in accordance with the research [10].that the location will affect the yield results.



Figure 1. Profile Java Chili (*Piper retrofractum* Vahl.) fruits and leaf morphology

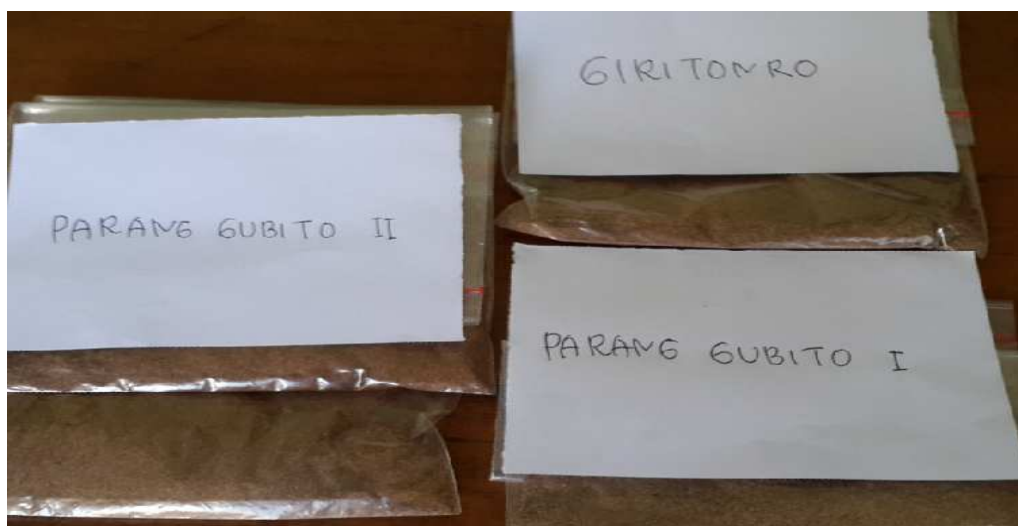


Figure 2. Ground of Java chili from thirth location in Central Java

Table 1. Results of Java chili extraction using micro wave heating and maceration with 70% ethanol

No.	Location	Yield (%)
1.	Giritontro, Cental Java	12.00
2.	Paranggupito 1, Cental Java	16.40
3.	Paranggupito 2, Cental Java	14.90
4.	Air Naningan 1, Lampung	11.55
5.	Air Naningan 2, Lampung	12.40

The content of chemical compounds every location is different, especially in the number, qualitatively evident from the appearance of chemical compounds contained in extracts of Java chili (Table 2). All of Java chili extracts containing steroid compounds, although in terms of the number of different quality. Other compounds such as saponins, alkaloids and flavonoids present in all Java chili extract, but all Java chili extract does not contain tannin compounds. Other plant components useful as anti-cancer is *Abrus precatorius* Linn contains flavonoids [9]. It is apparent there is no positive reaction from the tannins that do not show the color blue or blackish green in the form of the test solution. Figure 3 shows the existence of a positive reaction (+) qualitative analysis of flavonoids in fruit extract of Java chili. Positive reactions are shown as in Figure 3 are summarized in Table 2.

Table 2. The results of the phytochemical analysis of extracts of Java chili

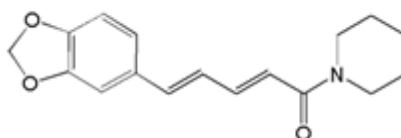
No.	Lokation	Compounds of Chemically						
		Alkaloids			Steroids	Flavonoids	Tanins	Saponins
		Dragendorf	Meyer	Wegner				
1.	Giritontro, Central Java	+	+	+	++++	+++	-	+
4.	Paranggupito 1, Central Java	+	+	+	++++	+++	-	+
5.	Paranggupito 2, Central Java	+	+	+	++++	+++	-	+
8.	Air Naningan 1, Lampung	+	+	+++	++++	+++	-	+
9.	Air Naningan 2, Lampung	+	+	+	++++	+	-	+

Informations : - = not detected, + = fair detected, ++ = good detected, +++ = very good detected, ++++=very very detected

**Figure 3. A positive result of flavonoid compounds in the test solution**

Differences liveliness Java chilies as anticancer material may be caused by the difference in ingredients especially piperine substances. It is as mentioned by [11] that piperine from Piper groups which include amide alkaloids are active components in inhibiting cancer. Figure 5 below shows the chemical structure of piperine {1-5- (1,3)-benzodioxol-5-yl) -1-oxo-2,4-penta-dienyl] piperidine}. Other content in Java chili active as anticancer ingredient is from the class of alkaloids, such as piplartin. The content of sterols also will affect the role of inhibition of cancer cell [4, 12-13].

The content of piperine or piplartin will be different occur due to the influence of the location such as climate and soil to support the growth of Java chili. Value inhibition of MCF-7 cancer cells can be seen in Table 3.

**Figure 5. The chemical structure of piperine****Table 3. Original Java chili fruit extracts and IC50 values MCF-7 cancer cells due to treatment Java chili fruit extract**

No.	Locations	Value of IC50 MCF-7
1.	Giritontro, Central Java	181.52
2.	Paranggupito 1, Central Java	80.43
3.	Paranggupito 2, Central Java	126.09
4.	Air Naningan 1, Lampung	54.35
5.	Air Naningan 2, Lampung	4.35
6.	Doxorubicin 0.5 ppm	58.79

Based on the calculation of IC50 values obtained from the extract of Java chili origin from Air Naningan 2 is at 4.35 ug / ml. Example graph IC50 calculations can be seen in Figure 6. Conditions MCF-7 cancer cells due to administration of the ethanol extract of the fruit of chili Java is shown in Figure 7. In Figure 7 the breast cancer cells MCF-7 suffered severe damage and cause debris that is very much on extract concentration of 250 ug / ml. This indicates that the fruit extract of Java chili the origin fro Air Naningan 2 is very active in inhibiting the growth of breast cancer cells MCF-7., showed high activity in comparison with other Java chili extract. Especially when compared to the original Java chili extract Giritontro, Central Java.

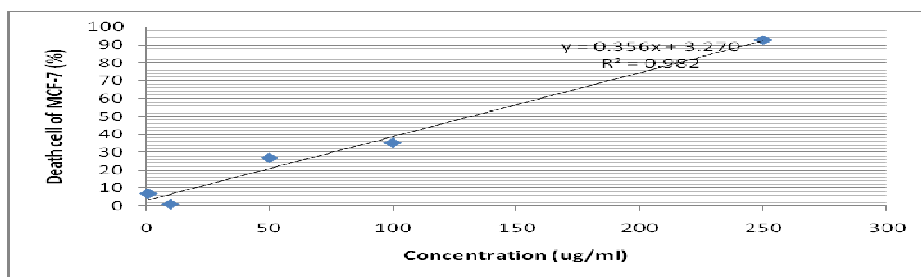


Figure 6. Graphic of IC50 calculation influence fruit extract Java chili origin Paranggupito location 2, Central Java

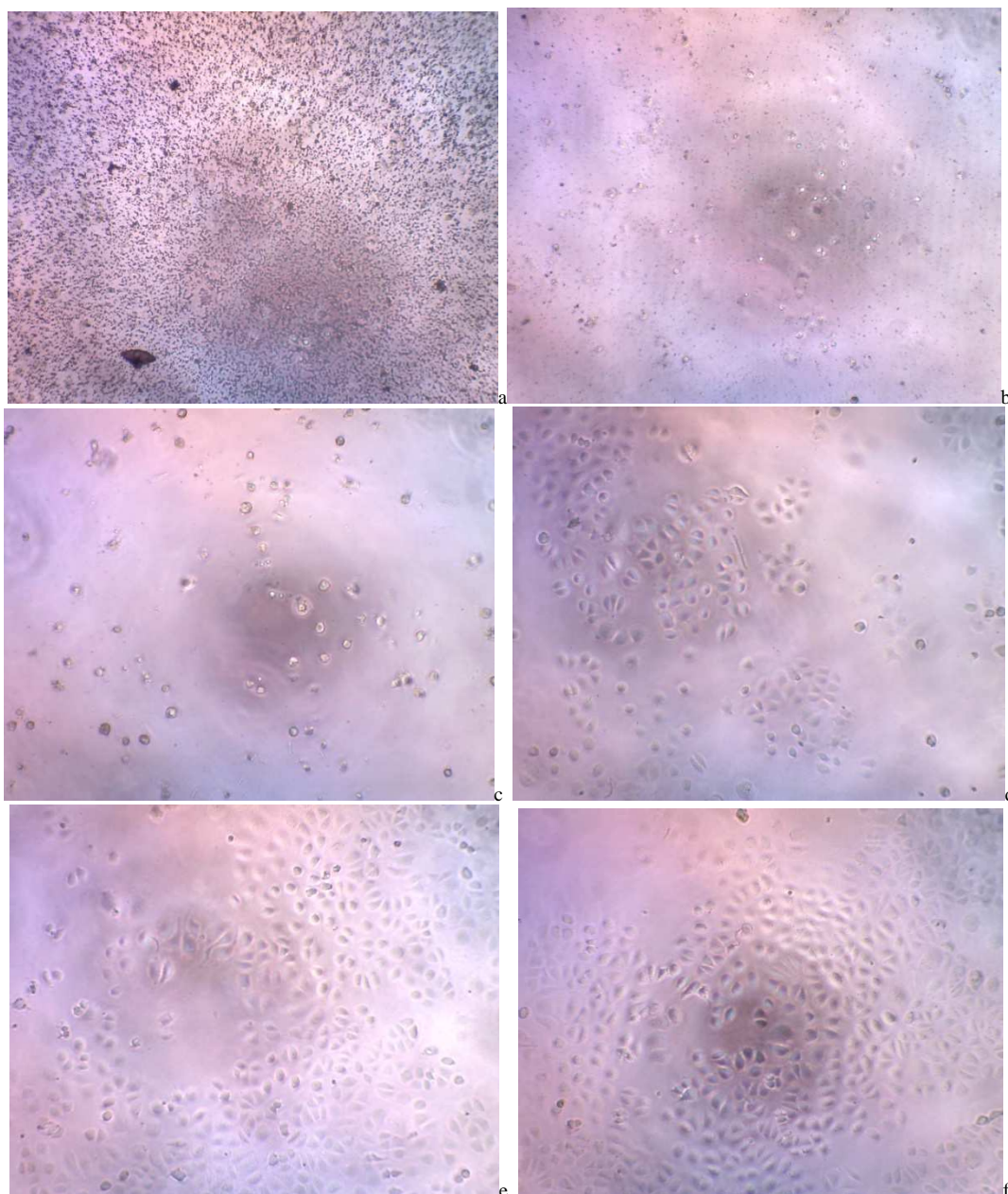


Figure 7. Conditions of breast cancer cells MCF-7 as a result of extract java chilies origin Air Nainingan 2, Lampung
 Informations : a = concentration 250 µg/ml, b = concentration 100 µg/ml, concentration 50 µg/ml, d= concentration 10 µg/ml, e= concentration 1µg/ml, dan f = control.

Most Java chili extract has the ability is nearly equal to the concentration of doxorubicin at 0.5 µg/ml in inhibiting breast cancer cells MCF-7 *in vitro*. Conditions of breast cancer cells MCF-7 as a result of extract of chili Java presented in Figure 8. In Figure 8 is apparent that the Java chili extract concentration of 250 µg / ml showed

significant inhibition of cells even cause the destruction of cancer cells very much. Inhibition of cancer cells from the extract of this Piperaceae group according to research conducted by [2-3]. When will this be implemented as a Java chili extract anticancer material it is necessary to the management of breast cancer with good handling, so that the results obtained in the healing does not cause adverse side effects.

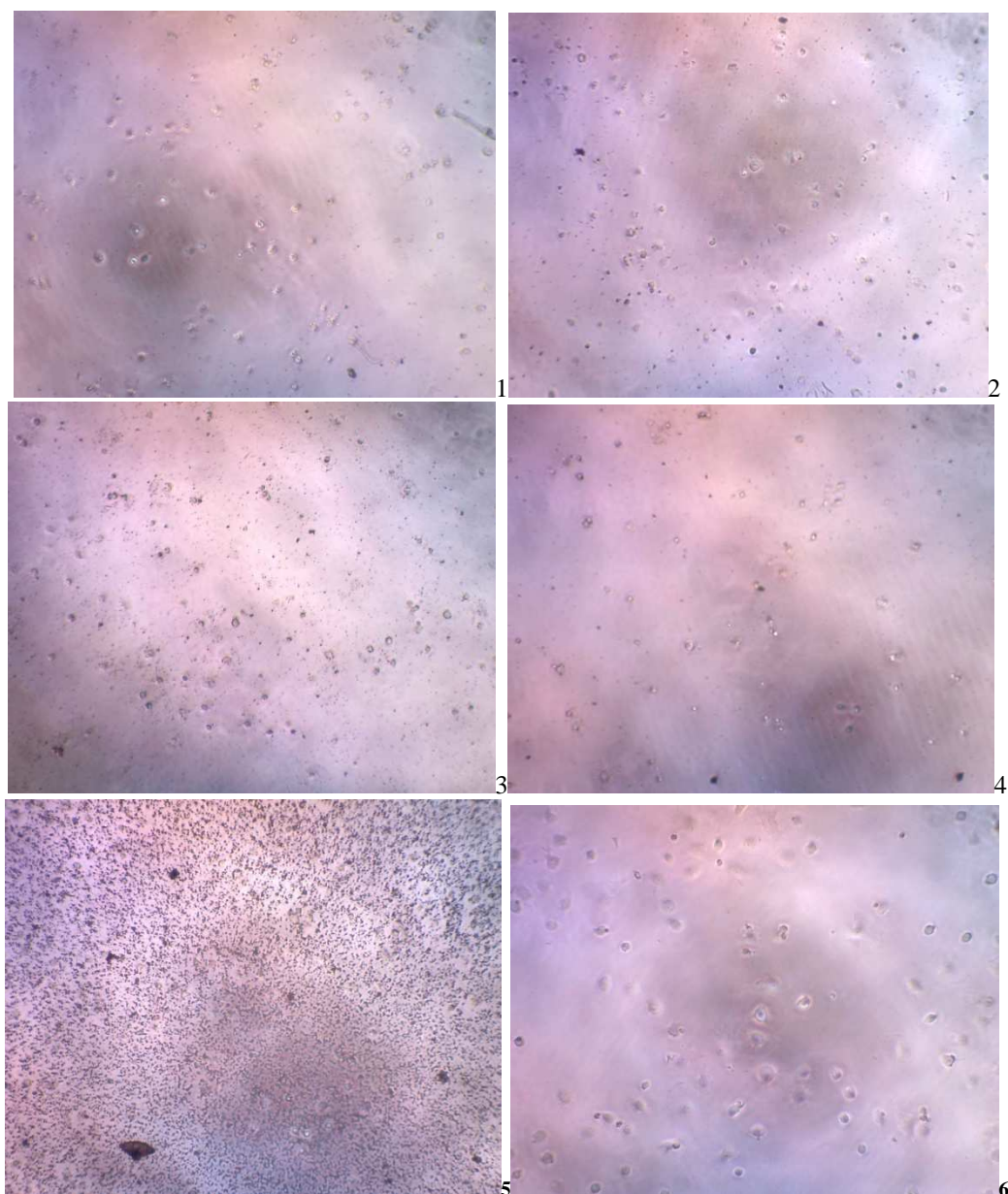


Figure 8. Conditions of breast cancer cells MCF-7 due Java chili fruit extract at a concentration of 250 µg / ml.

Description: 1-5 Java chili extract treatment results in accordance with the location of the origin of Java chili, 6 the effect of doxorubicin 0.5 µg / ml

CONCLUSION

Has successfully performed the extraction of Java chilies. Based on the analysis of phytochemicals, all Java chili extract contains flavonoids and alkaloids in addition to steroids. Java chili fruits extract the highest anticancer activeness as indicated by the Java chili fruit extracts derived from Air Naningan 2, Lampung with IC₅₀ value of 4.35 µg/ml. All Java chili fruits extract can inhibit cancer cells MCF-7 at a concentration of 250 µg/ml.

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