

## ANTICANCER ACTIVITY TEST OF ETHANOL OF BENALU MANGGA LEAVES (*DENDROPTHOE PENTANDRA*) OBTAINED FROM SOME LOCATIONS IN INDONESIA AGAINST T47D BREAST CANCER CELL LINE

Astri Erdiani Putri<sup>1</sup>; Roihatul Muti'ah; Weka Sidha Bhagawan, Erna Susanti

<sup>1,2,3,4</sup> Pharmacy Department Faculty of Medicine and Health Sciences State Islamic University (UIN) Maulana Malik Ibrahim (Maliki) Malang.

### Article Info

#### Article history:

Received Jul 12<sup>th</sup>, 2017

Revised Aug 20<sup>th</sup>, 2017

Accepted Oct 26<sup>th</sup>, 2017

#### Keyword:

Anticancer  
Benalu Mangga Leaves  
(*Dendroptoe pentandra*)  
location  
T47D breast cancer

### ABSTRACT

Indonesia has so many kind of benalu (mistletoe), one of them which has potential anticancer activity is benalu mangga (*Dendroptoe pentandra*). This research aims to determine the profile of anticancer activity of benalu mangga leaves extract from several locations in Indonesia against T47D cell line using invitro technique. The profile of anticancer activity can be used as one of the Requirement developed to be fitofarmaka. The separation of the active compound from leaves mistletoe mango do with ultrasonic maseration extraction method using a solvent of ethanol 96%. Each extract derived from 5 locations i.e. Kediri East Java, Pekalongan Central Java, Denpasar Bali, Lampung Sumatra and Bulungan Kalimantan at test level of toxicity against the *cell line* T47D breast cancer by MTT method Assay. Test statistic one way analysis of variance (Anova) with software SPSS version 16.0 used assess whether there is a difference significantly anticancer activity (IC<sub>50</sub>) leaf extract mistletoe mango from five locations. The results showed that the IC<sub>50</sub> value of ethanol extracts of leaves mistletoe mango from the Kediri, Pekalongan, Denpasar, Lampung and Bulungan are 304.79 µg/ml, 4646.34 µg/ml, 417.01 µg/ml, 540.91 µg/ml, and 287.39 µg/ml. The results of the Anova statistical analysis shows that there are significant differences between the location of Kediri-Pekalongan, Bulungan-Pekalongan, Pekalongan and Denpasar-Lampung-Sumatra. The extracts of leaf mistletoe mango (*D. pentandra*) from Bulungan, Kediri and Denpasar location are potential to be anticancer agent

Copyright © 2017 Green Technology.  
All rights reserved.

### Corresponding Author:

Astri Erdiani Putri,  
Pharmacy Department  
Faculty of Medicine and Health Sciences  
State Islamic University (UIN) Maulana Malik Ibrahim (Maliki) Malang  
Email: [erdianiputri17@gmail.com](mailto:erdianiputri17@gmail.com)

## 1. INTRODUCTION

Cancer characterized by the growth of abnormal cells. The Data of the World Health Organization (WHO) 2010 year mention that cancer is the number-two cause of death after cardiovascular disease (Health, 2012). One type of cancer was the cause of death in the world is breast cancer (Empress, 2009).

Treatment for breast cancer that is widely used today is the method of chemotherapy, radiation, and surgery. These methods aim to remove cancerous tissue or turn off the cancer cells. However, these methods have not been fullest even give side effects on normal cells that surround cancer cells or other organs. (Lockhsin *et al.*, 2007). Therefore, methods of cancer treatment very need to be developed.

One method of cancer treatment that has existed and still continue to be developed is the use of anticancer agents from natural materials. The use of natural materials are relatively more secure because of the side effects is relatively small. The use of a medicinal nature as one using natural ingredients, namely by making use of something previously not usable into something useful, namely loranthus mango.

Research by Darmawan dkk., (2006) showed the methanol extract of benalu mangga leaves (*Dendrophthoe pentandra*) activity captures free radicals with  $IC_{50}$  23.944  $\mu$  g/ml. The research by Helda (2015) The fraction sitotoksicity from benalu mangga leaves (*D. pentandra*) against the *cell line* showed the most active was fraction klorofom with the value of the  $IC_{50}$  88,533  $\mu$  g/ml. From these studies can be reference that benalu mangga leaves (*D. pentandra*) potential anticancer agents and also have the potential to developed into a biopharmacy products.

Development of raw medicinal plants become pyhtopharmaka products are experiencing obstacles, one of them caused multi component compounds in plants. The factors caused the variations can be distinguished into two internal and external factors (Verma and Shikla, 2015). One of the external factors that greatly affect the content of secondary metabolites of plants is the difference growing location.

This research was conducted in order fulfill candidate pyhtopharmaka products, the criteria are viewed from the strength of the potential anticancer activity and *safety*, so it's important to knowing the profile of anticancer activity in T47D cells against invitro of benalu mangga leaves extract (*d. pentandra*) from several locations in Indonesia . By knowing the profile of anticancer activity of extracts of leaves of benalu mangga leaves (*D. pentandra*) from several locations in Indonesia, then it can find out if there is a difference between anticancer activity of extracts and of where is the location that has the most potential anticancer activity to developed pyhtopharmaka products.

## 2. RESEARCH METHOD

### Implementation Of The Research

This research was carried out in January-April 2017 in the biology laboratory, Pharmacy and laboratory chemistry UIN Maliki, Protho Parasitology in Gadjah Mada university, Yogyakarta.

### Tools and materials Research

The tools used include glass set of tools, analytical balance, filter paper, *moisture analyzer* HC 103, ultrasonic *cleanser* sonikasi *rotary evaporator* bottle, vial, *vortex* , mikropipet 200, 1000  $\mu$  L, small test tubes, small tubes, racks of 96-well *plate*, *Conical Tube*, *Yellow tip*, *Blue tip*, *Culture Dish*, *Hemocytometer* and *ELISA reader*

The materials used are loranthus leaves 96% ethanol, Mango, trypsin-EDTA (trypsin 1 x 0.25%), RPMI culture media, DMSO, MTT. 5 mg/mL (50 mg 10 mL PBS and MTT), SDS 10% in 0.1 N HCl.

## RESEARCH PROCEDURE

### The Collection Of Sample Research

Sample Collection to get samples of research in accordance with the specified characteristics. The sample in this research is plants benalu mangga (*D. pentandra*) from 5 locations in Indonesia, namely from the Kediri, East Java, Pekalongan, Central Java, Bali, Lampung and South Sumatra Bulungan Regency of North Borneo.

### Sample preparation

Sample benalu mangga leave washed to clean, dried. The sample cut into small and mashed with a blender until smooth (the powder) and sifted with a size of 60 mesh.

### Analysis Of Moisture Content

After the *Moisture Analyzer* turned on and the display shows the display 0.000 g, tool cover is opened and the *sample pan* is inserted into the empty *sample pan handler*. The cover is automatically lowered and the tool the tool display shows 0.000 Tower or on the screen. Then a number of  $\pm$  0.500 g of powder simplisia is inserted into the *sample pan* and cover the tool is lowered. Automatically, the tool will start the measurement to read the MC% measurement results on the screen.

### Ultrasonic Extraction And Maceration

The extraction of benalu manga powder (*D. petandra*) is carried out by using 96% ethanol as a solvent. A comparison between the material and the solvent is 1:10. Method of extraction is the extraction of *ultrasonic cleanser* with long extraction of 20 minutes (Handayani *et al.*, 2016). The filtrate obtained were merged into one and then is evaporated with *vacuum rotary evaporator* until the concentrated ethanol extracts obtained benalu manga leaves.

### Anticancer Assay Activity (CCRC, 2009)

#### a. Preparation of cancer cells

T47D breast cancer cells taken from the collection of the University of Gadjah Mada (UGM). Cancer cells removed from the *freezer* (-80  $^{\circ}$ C), is heated in a water bath at 37  $^{\circ}$ C for 2 – 3 minutes. After melting, the cells were transferred into *conical tube* which already contains 10 ml medium RPMI (*Roswell Park Memorial Institute*),

then discentrifugasi to separate cancer cells (pellets) with RPMI medium. Pellets formed incorporated into the *culture dish* that already contains 10 mL RPMI medium and incubated for 3-4 hours at a temperature of 37 °C/5% CO<sub>2</sub>, then observed under the microscope to see if the cells are attached at the base of *culture dish* and when the number of cells in the *culture dish* reaches 70 – 85% (konfluen) of harvested cells.

#### b. Cell Harvest

Discarded media kultur in advance, plus ± 5 mL of PBS (*Phosphate Buffered Saline*) as well as dihomogenkan and then thrown back, added trispsin evenly and incubated for 3 minutes, 5 mL RPMI medium added to menginaktifkan cells and do resuspensi, observed under the microscope *inverted*, and then incubated in an incubator CO<sub>2</sub> for 24 hours.

#### c. Test sitotoksicity

Samples weighed as much as 10 mg, dissolved in 100 µl sample to divortex and dissolves perfectly. Diambil cells of the incubator, then disposed of by way of cell media reversed *plate* 180° above the place of exile and pressed slowly above the tissue for the rest of the fluid drop sweet, put 100 µ L PBS into all sumuran a filled cell and thrown back, and then put the sample solution as much as 100 µ L with a concentration of 500, 250, 125, 62.5, µ g/mL 31.25 (Hidayati, dkk, 2011) and repeated as much as 3 x (triplo), incubated dive back 24 hours.

#### d. The awarding of the reagent MTT

Discarded media cells washed with PBS, MTT solution added (reagent 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazoliumbromide) yellow 100 µ L to each sumuran. Incubation is back for 3 – 4 hours in an incubator (until formed crystals of formazan or color changes to blue). When crystals of formazan formed, has observed the conditions of the cell with the microscope *inverted* and then added *the stopper* SDS (*Sodium Dodecyl Sulfate*) 10% in 0.1 N HCl, wrapped *plate* with aluminum foil and incubated again in a dark place (room temperature) overnight

#### e. Absorbance Readings and data analysis

The presence of indicator color changes after the grant of the reagent absorbance readings done, MTT using ELISA *reader*, then calculated the percentage of living cells with the following equation:

$$\times 100 \%$$

Description:

A = absorbance treatment (cell culture + media + sample)

B = control absorbance media (media culture)

C = negative control absorbance (cell + media kultur)

Data from the percentage of living cells are then analyzed to find out the value of IC<sub>50</sub> probit analysis with SPSS, then the results of the IC<sub>50</sub> analyzed with *statistics one way analysis of variance* (Anova) with *software* SPSS version 16.0 for the purpose of assessing whether there is a difference significantly anticancer activity (IC<sub>50</sub>) leaf extract loranthus mango from five different sampling locations i.e. Kediri, Pekalongan, Denpasar, Lampung and Bulungan.

### 3. RESULTS AND DISCUSSION

#### The Collection Of Sample Research

The results of the collection of the sample is obtained benalu mangga leaves (*Dendrophthoe pentandra*) from 5 locations grow, namely, Kediri, East Java, Pekalongan, Central Java, Bali, Sumatra, Lampung and Bulungan Regency of North Borneo.

#### Sample Preparation

The results of sample preparation is obtained powder of the leaves of different colors of benalu mangga leaves. Powder from Lampung Kediri and young green, Bulungan and Denpasar, dark green and Brown Pekalongan.

Moisture content determines the *acceptability*, freshness, and durability of powders (Winarno, 2002). The average moisture content of benalu mangga powder (*D. petandra*) of Kediri, East Java (8.00), Pekalongan, Central Java (8.88), Denpasar Bali (6.53), Lampung and South Sumatra (8.03) and Bulungan Kalimantan (6.49). The average moisture content of benalu mangga powder (*d. petandra*) from 5 locations in Indonesia under 10%. The results of the analysis of moisture content of powder to 5 is already set by the BPOM.

### Analysis Of Moisture Content

The results of analysis of water content leaves loranthus Mango is shown in table 1

Location	colour	Water content I (%b/b)	Water content II (%b/b)	Water content III (%b/b)	Average Water content (%b/b)
Kediri	Green	7,72	7,92	8,37	8,00
Pekalongan	dark Green	9,14	8,53	8,97	8,88
Denpasar	dark Green	6,71	6,27	6,69	6,56
Lampung	dark Green	8,09	7,50	8,50	8,03
Bulungan	dark Green	6,27	6,69	6,51	6,49

Water content for solid dosage drugs in must have water content  $\leq 10\%$ , except for the Efervesen moisture content  $\leq 5\%$  (BPOM, 2014).

### The extraction Maceration Ultrasonic

Extraction maceration of benalu manga leave used solvent ethanol 96%. Ethanol extract was shown on table 2.

Table 2 results of maceration to extract the ethanol

Location	powder + solvent	Colour filtrate	Colour extract	Weight of extract (g)	Rendemen (%) (b/b)
Kediri	50.7329 g + 500 ml	dark Green	Green	5.2402	10.329
Pekalongan	50.7206 g + 500 ml	dark Green	Green	1.4861	2.929
Denpasar	50.7926 g + 500 ml	dark Green	Green	4.0458	7.965
Lampung	50.7187 g + 500 ml	dark Green	Green	4.8469	9.556
Bulungan	50.7177 g + 500 ml	dark Green	Green	3.0810	6,074

The highest from 5 extracts are extracts from location Kediri, East Java with 10.33%, followed by extracts from Lampung in Sumatra, Bali, Bulungan Kalimantan, and Pekalongan Central Java with value 9.56%, 7.97%, 6.07%, and 2.93%. The difference it by several factors such as the age of the plant, harvest and process time, plant varieties, growing place environmental factors, factors of processing plants. These factors make the plants derived from a single species however grows in different places have different yield values, and active compound content is also different that would then affect activity (according to Distanta, et al 2009; Ayunda 2014).

### Anticancer Assay Activity (CCRC, 2009)

Ethanol extract tested against the *cell line* T47D breast cancer using the MTT method. Living cells elongated shaped like leaves while the dead are globose (Nala, 2013). In control cells, cancer cells much living can be seen from the number of cells that the shape is elongated like leaves. Based on data analysis with SPSS *probit analysis* obtained  $IC_{50}$  value of each extract and fractions shown in table

Table 4 : Result  $IC_{50}$  from 5 location

No	Nama	$IC_{50}(\mu g/ml) \pm SD$
1	Kediri	$304.79 \pm 26.7204402$
2	Pekalongan	$4646.34 \pm 423.280308$
3	Denpasar	$417.01 \pm 47.3979817$
4	Lampung	$540.91 \pm 0.94252431$
5	Bulungan	$287.39 \pm 20.1914535$
6	Kontrol positif	$13.461 \pm 0.434899$

Based on table 4 can be known that ekstrak which has the highest toxicity i.e. extract derived from Bulungan IC with a value of  $IC_{50}$  averaged 287.39  $\mu\text{g/ml}$  followed from Kediri, Denpasar, Lampung and Pekalongan with  $IC_{50}$  value consecutive 304.79  $\mu\text{g/ml}$ , 417.01  $\mu\text{g/ml}$ , 540.91  $\mu\text{g/ml}$ , 4646.34  $\mu\text{g/ml}$ . When compared to the value of the  $IC_{50}$  of positive control (doksorubisin) 13,461  $\mu\text{g/ml}$ , then the value of the  $IC_{50}$  of the 5 the extract is still far below. Even so, it does not mean extract ethanol benalu mangga leave (*D. pentandra*) from 5 locations is not potentially.

The value of the  $IC_{50}$  obtained reflect the sitotoksicity against the test cell. The results showed that the test solution has the cytotoxic activity by the method MTT because the  $IC_{50}$  of his entry in the range of concentrations used. Based on the criteria set forth by NCI (*National Cancer Institute*) (NCI, 2001 in Rahmawati, 2013) that an extract of the active anticancer activity stated in IC has a value of  $IC_{50} < 30 \mu\text{g/ml}$ , the moderate active in having the value of  $IC_{50} \geq 30 \mu\text{g/ml}$  and  $IC_{50} < 100 \mu\text{g/ml}$ , and is said to be inactive when the value of the  $IC_{50} > 100 \mu\text{g/ml}$ . However, that does not mean extract from plant parts that have the  $IC_{50} > 100 \mu\text{g/ml}$  not potentially developed as anticancer because according to Machana (2011), the extract is said to be inactive as anticancer if the value of the  $IC_{50} > 500 \mu\text{g/ml}$ . It can be concluded that from the 5 extracts tested anticancer activity with the most potentially MTT method is to extract from the location of Bulungan IC with a value of  $IC_{50}$  averaged 287.39  $\mu\text{g/ml}$ , Kediri with  $IC_{50}$  values 304.79  $\mu\text{g/ml}$ , and Denpasar with  $IC_{50}$  values 417.01  $\mu\text{g/ml}$ . positive control when compared to IE doksorubisin IE with  $IC_{50}$  value of average 13,461  $\mu\text{g/ml}$  then second extract is still far below the value of the  $IC_{50}$  of his. After getting the value of the  $IC_{50}$  of extracts, next is doing a Test *statistic one way analysis of variance* (Anova) with *software* SPSS version 16.0. The results can be seen in table 5.

Table 5. One way anova test results

No	Location (i)- Location(j)	Sig.	Note
1.	Kediri-Pekalongan	0.010	significant
2.	Kediri-Denpasar	0.936	No significant
3.	Kediri-Lampung	0.867	No significant
4.	Kediri-Bulungan	0.990	No significant
5.	Pekalongan-Denpasar	0.012	significant
6.	Pekalongan-Lampung	0.014	Significant
7.	Pekalongan-Bulungan	0.010	Significant
8.	Denpasar-Lampung	0.930	No significant
9.	Lampung-Bulungan	0.857	No significant
10.	Bulungan-Denpasar	0.927	No significant

Test result analysis *one-way anova* showed that *LSD* , extracts meaningful differences are between ekstrak from the Kediri-Pekalongan Pekalongan-Pekalongan-Denpasar, Lampung, and Pekalongan-Bulungan. Based on the hypothesis that was made in the previous discussion, it can be noted that there are meaningful differences anticancer activity from 5 different locations. Anticancer activity of difference is possible because the difference of environmental conditions (ekternal Factor) as soil nutrient content, soil type, climate, time of sampling, etc.

## CONCLUSION

There is a difference in meaning the anticancer activity of extracts of leaves of loranthus mango (*d. pentandra*) between the location of Pekalongan in Central Java with Kediri, East Java, Pekalongan, Central Java with the Bulungan Regency of North Borneo, Pekalongan, Central Java with Denpasar Bali, Pekalongan, Central Java and Lampung in Sumatra. The most potentially extracts for anticancer is an extract from the location of Bulungan North Borneo (287.39  $\mu\text{g/ml}$ ), Kediri, East Java (304. 79  $\mu\text{g/ml}$ ) and Denpasar Bali (417  $\mu\text{g/ml}$ ).

## ACKNOWLEDGMENTS

This research was conducted with funding from KemenristekDikti. Thanks for the support facilities and infrastructure of the Department of Biology and Department of Chemistry, Faculty of Science and Technology, Airlangga University and for the support delivery of oil sludgefrom BPPT, Tangerang.

## **REFERENCES**

- Aderogba, M.A., Ogundaini, A.O., Eloff, J.N. 2006. *Isolation of Two Flavonoids from Bauhinia monandra (kurz) Leaves and Their Antioxidative Effects*. J. Traditional, Complementary and Alternative Medicines, 3 (4): 59-65.
- Darmawan, Akhmad dan Artanti, Nina. 2006. *Isolasi dan Identifikasi Senyawa Aktif Antioksidan dari Ekstrak Air Daun Benalu (Dendrophthoe pentandra L. Miq.) yang Tumbuh pada Cemara (Casuarina sp.)*. LIPI. Kawasan Puspiptek Serpong-Banten.
- Departemen Kesehatan RI. 2012. *Jika tidak dikendalikan 26 juta orang didunia menderita kanker*. <http://www.depkes.go/index.php/berita/press-release/>.
- Hardiyanti, Helda Dwi.2015. *Uji Antikanker Dan Identifikasi Golongan Senyawa Dari Ekstrak Etanol Dan Fraksi Daun Benalu Mangga (Dendrophthoe Pentandra) Terhadap Cell Line Kanker Payudara T47D*. Volume 10, Nomer 1:6.
- National Cancer Institute. 2001. *Measuring Cancer Death*, Cited from <http://www.cancer.gov/csr> diakses pada Maret 2007.
- Verma, N., Shukla, S., 2015. *Impact of various Factors Responsible For Fluctuations in Plant Secondary Metabolite*. J App Res med ar Plants 2 (4):105-113.