Toxicity Test of Ehanol Extract 96% Malayan Mistletoe Leaf (*Dendrophthoe pentandra*) from Various Regions in Indonesia Against Vero Cells

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ABSTRACT

Different types of benalu can be found in Indonesia, one of benalu that has anticancer potency is benalu mangga (*Dendrophthoe pentandra*). This study aims to determine the difference of toxicity of ethanol extract 96% *Dendrophthoe pentandra* leaf from Kediri, East Java; Pekalongan, Central Java; Gunung Batin Baru, Lampung and Selor Hilir, Kaltara towards normal cell line (vero). Selection of the five regions based on the elevation where the plant grows. Ultrasonic extraction is performed to separate the active compound of *Dendrophthoe pentandra* leaf. The solvent used in the extraction is 96% ethanol. Each extract tested its toxicity using vero cell done by MTT method (microculture tetrazolium salt). The living cells form crystals of purple formazan and absorbance readings are performed using ELISA reader to determine the percentage of living cells. The results obtained from this research are LC_{50} values from East Java, Central Java, Sumatra and Kalimantan respectively 26.61; 1583.75; 4845.32 and 798.28 µg / ml. The data analysis conducted by using one-way ANOVA showed that toxicity levels between regions have a significant difference.

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1. INTRODUCTION

The use of traditional medicine in Indonesia is part of the nation's culture and widely used by the community, but in general its effectiveness and safety have not been fully supported by the research. Though natural resources in the form of medicinal plants is a national asset that needs to be explored, researched, developed and optimized utilization (Depkes, 2007). Various research institutions began to be interested to investigate herbal medicines, both of which are often consumed by society and which have never been consumed. Research begins with the identification of active compounds from various parts of plants to test activity and toxicity test or safety dose of herbal medicine. Much of the research was conducted using experimental animals (Thomas, 2011; Flower et al., 2012).

One of the plants used by the community as a traditional medicine is benalu mangga (*Dendrophthoe pentandra*). *Dendrophthoe pentandra* (*D. pentandra*) is one of the plant species of the Loranthaceae family. Benalu is a semiparasit plant spread over the tropics that grows on an old branch of mango tree as its host. *D. pentandra* generally live in rainforests and lowland plantations up to 500m above sea level (Uji et al., 2007).

Benalu is a parasite group that was initially considered not useful, it is related to the nature of parasites that can damage the host plant. However, parasites have many benefits, such as cough, diabetes, hypertension, cancer, diuretics, smallpox, ulcers, skin infections and postpartum treatment (Artanti et al., 2012). The people of Sulawesi use *D. pentandra* as an anticancer drug (Ishizu et al., 2002).

The compounds contained in the parasitic extract are flavonoids, amino acids, carbohydrates, tannins, alkaloids and saponins (Katrin, 2005). According to Syazana et al., (2004) the methanol extract of *D. pentandra* contains alkaloids, flavonoids, tannins, terpenoids and saponins. The main flavonoids found in parasites and functioning as anticancer are quercetin compounds.

D.pentandra plant used by the community as a traditional medicine or known as herbal medicine needs to be reviewed. Because according to BPOM, (2011) there are some traditional medicine that is not used again for treatment because it gives an unwanted effect. In addition, natural ingredients may contain toxic compounds. Good herbs have selective toxicity, in other words can cause therapeutic effects without damaging normal tissue cells.

This research uses *D.pentandra* leaves taken from various regions in Indonesia: Lampung Tengah (Sumatra), Bulungan (Kalimantan), Kediri (East Java) and Pekalongan (Central Java). Selection of the four sites based on differences in elevation where *D. pentandra* grows. Central Lampung has 27 m dpl, Bulungan has 80 m dpl, Kediri has 222 m dpl, Pekalongan has 8 m dpl. The purpose of this study was to determine the safety of *D.pentandra* extract against normal cells and to know which areas had the lowest toxicity.

2. RESEARCH METHOD

Material plant

The plant material used was *D. pentandra* leaf extracted using ultrasonic method using 96% ethanol solvent for 20 minutes with the solvent ratio of 1:10 (w / v) (Handayani et al., 2016). Plant determination was done at LIPI Purwodadi, Pasuruan.

Cell line

Cells used are normal vero epitel cells that are collections of the Faculty of Medicine, Gadjah Mada University (UGM) Yokyakarta.

Chemical material

Ethanol 96%, M199 culture media and Rosewell Park Memorial Institute (RPMI) media, Dimethyl Sulfoxide (DMSO), 5 mg / mL microbial tetrazolium salt (MTT), Phosphate Buffer Saline (PBS) (50 mg MTT and 10 mL PBS) Sodium Dodecyl Sulfate (SDS) 10% in 0.1 N HCl.

Toxicity Test by MTT method assay

T47D cancer cells and vero cells were placed in 96 well plates, each containing 10^4 cells. Performed the required cell calculation by multiplying the 10^4 cells needed per 100 (the fulfillment of the number of well plate) divided by the counted cells. Once distributed evenly, the cells are incubated until the normal state returns. Added variation of test solution concentration and incubated for 24 hours at incubator 5% CO_2. Each well added 100µL of MTT solution and incubated for 4 hours, formazan crystals will be formed as a result of enzymatic reactions occurring between living cells and MTT solution. The MTT reaction was stopped with a stopper reagent (10% HCl in SDS), then incubated overnight at room temperature. The next day an absorbance readings were performed with an ELISA reader at a wavelength of 595 nm (Muti'ah, 2004).

Data analysis

Calculation of LC_{50}

The absorbance data obtained from the reading by ELISA reader is converted to viability of living cells by the formula:

Percentage of live cell viability x 100%

Each viability value is analyzed with Microsoft Excel to get the LC_{50} value.

ANOVA one way multivariate analysis

The data of LC_{50} ± SD values each sample were analyzed using SPSS. The data obtained indicate whether or not the differences in toxicity of each region. It is known that between regions has a significant difference if the p value is less than 0.05.

3. RESULTS AND DISCUSSION

The more cell lines that live the greater absorbance and the more purple color is formed. Otherwise the more cell lines that die the smaller absorbance and the less purple color is formed. It can be concluded that the more toxic substances to cell line vero the lower absorbance. The result of % viability of living cells is shown in the Table 1.

Table 1. The average viability of living cells at each concentration of the test solution

Concentration	The average viability of living cells (%) ± SD			
	East Java	Central Java	Sumatra	Kalimantan
1000	100 ± 0	59.26 ± 0.62	100 ± 0	50.40 ± 5.19
500	59.98 ± 3.23	46.88 ± 6.12	91.98 ± 0.27	54.47 ± 4.48
250	72.98 ± 15.67	70.10 ± 17.01	85.34 ± 2.77	67.93 ±19.24
125	76.06 ± 7.59	77.41± 19.71	81.03 ± 15.48	42.09 ± 4.77
62,5	57.45 ± 0.46	71.36 ± 4.40	95.39 ± 7.97	92.86 ± 3.69

* Average viability ± standart deviation, 3 times replication

The percentage of living cell viability data of each test solution were analyzed by using Microsoft excel to know LC_{50} values from each test solution. The LC_{50} value is derived from the equation obtained from the four regions by three repetitions, inserted at 50% y and search the antilog of the results obtained. The result of LC_{50} of each test solution to vero cell is shown in the table 2.

Table 2. The average LC_{50} value of *D. pentandra* ethanol extract on vero cells

Location	The Average LC_{50} ± SD
East Java	26.61 ± 16.20
Central Java	1583.75 ± 561.09
Sumatra	4845.32 ± 92.13
Kalimantan	798.28 ± 171.59

* Average LC_{50} ± Standart Deviation, 3 times Replication

The obtained LC_{50} values describe the extract toxicity of the cell line. The sample is said to be toxic if it has a LC_{50} value of less than 1000 (Meyer et al., 1982). The results showed that samples from Kediri and Kalimantan had toxic effects on normal cells while samples from Pekalongan and Sumatra had no toxic effects on normal cells. The sample from Bali was different from the four test samples, the% of viability of each concentration was 100. Therefore, no data of line equation was obtained through data analysis using excel. This is possible because the *D. pentandra* samples from Bali do not have any effect on vero cells, so that the vero cells present in the 96 well plates do not die. From the above table it is known that the samples from Kediri have the highest toxic effect while the sample from Sumatra has the lowest toxic effect.

Table 3. Sampling Location Characteristics

No	Location	Elevation (mdpl)	The Average of Temperature (°C)	Rainfall (mm)	Climate Type
1	Desa Sumbergayam kec.Kepung Kab.Kediri Jawa Timur	222	25,8	1886	Aw
2	Kec.Pekalongan Timur Kab.Pekalongan Jawa Tengah	8	26,6	2620	Am
3	Desa Nangka Utara Kec.Tonja Kab.Badung Denpasar Bali	51	26,4	1833	Am
4	Kel.Gunung Batin Baru Kec.Terusan Nunyai Kab.Lampung Tengah Sumatra Selatan	27	26,8	2122	Af
5	Kel.Tanjung Selor Ilir Kec.Tanjung Seloe Kab.Bulungan Kalimantan Utara	80	26,8	2738	Af

From the table 3, it is known that Kediri is an area that has the highest altitude of 222 mdpl. In the previous table it was found that the samples from Kediri had the highest toxicity effect. The order of toxicity levels from the highest is Kediri, Kalimantan, Pekalongan and Sumatra. From the data obtained can be deduced that the height of the place affect the level of toxicity of extract *D. pentandra*. The higher the elevation of a place, the more toxic the effects are.

In the calculation of the results of this study used 95% confidence limits. Data were analyzed using SPSS 16 for Windows. Statistical test data using One-way ANOVA because in this study data used have variables with different groups. Normality test based on Shapiro-Wilk obtained p value greater than 0,05 indicates that normal data. Based on the HSD tukey test p value obtained over 0.05 means H_0 is accepted thus stated that the data is homogeneous. Furthermore, one way ANOVA analysis can be seen from the table 4.

Table 4. One Way ANOVA Test Results

No.	Location (I) – Location (J)	Significant	Note
1.	Jawa Timur – Jawa Tengah	0.000	significant
2.	Jawa Timur – Sumatra	0.000	significant
3.	Jawa Timur – Kalimantan	0.013	significant
4.	Jawa Tengah – Sumatra	0.000	significant
5	Jawa Tengah – Kalimantan	0.012	significant
6.	Sumatra – Kalimantan	0.000	significant

From the table it is known that there are significant differences between regions of East Java - Central Java, East Java - Sumatra, East Java - Kalimantan, Central Java - Sumatra, Central Java - Kalimantan and Sumatra - Kalimantan. It is known that between regions has a significant difference if the p value is less than 0.05.

CONCLUSION

There are significant differences from toxicity test results of ethanol extract 96% *D. pentandra* Kediri - Pekalongan, Kediri - Sumatra, Kediri - Kalimantan, Pekalongan - Sumatra, Pekalongan - Kalimantan and Sumatra - Kalimantan based on LC_{50} values. *D. pentandra* from the region of Sumatra has the lowest toxicity with LC_{50} 4845.32 µg / ml. The LC_{50} values obtained from Kediri, Pekalongan and Kalimantan are 26.61, 1583.75 and 798.28 µg / ml. *D. pentandra* extract from Pekalongan and Sumatera area can be recommended as raw material for Phytopharmaca product through preclinical test and clinical trial first. *D. pentandra* toxicity to NIH-3T3 fibroblast cells and continued toxicity test in vivo should be conducted.

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