

The in vitro assessment of anti proliferation activity of crude diethyl ether extract of *Dendrophthoe* species against to myeloma culture cell

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ABSTRACT

Herb medicine have an active substances that can dissolved on polar, semi polar and non polar liquid extract. The methanol and ethyl acetate as a polar and semi polar extract liquid were used to study of herb medicine such as *Dendrophthoe* species. Diethyl ether as a non polar liquid extract was never use to study the *Dendrophthoe* species. The aim of this study was to investigate the anti proliferation activity of *Dendrophthoe* species to myeloma culture cells after extracted by non-polar extract solution (diethyl ether). The post test only control group design was used for this research. A thirty six of microtiterplates wells were used for myeloma culture cells in RPMI medium. The wells were divided placing in two groups: treatment groups and controls groups. Each three wells of six treatment subgroups added with 100 μ l of 1.1; 5.5; 11; 22; 33 and 44 μ g/ml crude diethyl ether extract series. A RPMI solution at similar method and volume were used as control substances. The cells were assessed by inverted microscope in 200x magnified two days after. The cells were quantified analyzed for anti proliferation activity by using 1:1 methylene blue solution. The results showed that started from 11.0 μ g/ml of crude diethyl ether extract of *Dendrophthoe* species have been anti proliferation abilities of myeloma culture cells ($p < 0.05$). In conclusion, actives substances of *Dendrophthoe* species where dissolved in non polar liquid as diethyl ether has anti proliferation activities to cancer cell in vitro.

Key words: cancer, herb medicine, myeloma, *Dendrophthoe* species, anti proliferation

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INTRODUCTION

The cases of oral squamous cell carcinoma (OSCC) were known highly in the worldwide at about 80% to 90% of malignant tumor in oral cavity. The mortality rate remains high about 5% in worldwide and 2.4% to 3.57% in Indonesia.¹

Some of chemotherapeutics for reduced development of proliferates cell as carcinoma like an OSCC were seen expensive and not a lot of kinds. A practical solution to this problems as described at above is using traditional plant remedies for reduce highly cost of medication.

Dendrophthoe species (*Benalu duku*) is a traditional Indonesian herbal remedy which grows to a height of 20 cm to 30 cm (Figure 1). That trees have a some specific traditional name as follows; *Pasilan* on Depok (Jakarta) and *Dendrophthoe* on Palembang (South of Sumatera). *In vivo* and *in vitro* experimentally, the hot water extract of leaves of *Dendrophthoe* species were shown have an anti proliferation of myeloma cells.^{2,3}

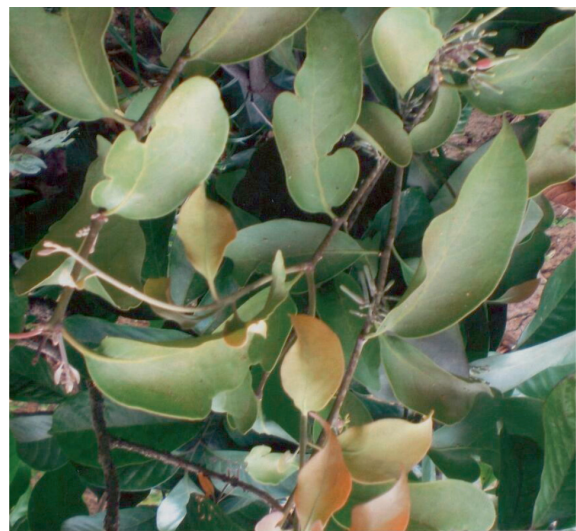


Figure 1. The leaves of *Dendrophthoe* species (*Benalu duku*) from Muara Enim (South of Sumatera island).

The ethanol, methanol and chloroform extract of *Dendrophthoe* species were known too, have an anti proliferation ability for myeloma culture cell.^{4–6} The phenomenon at above showed that leaves of *Dendrophthoe* species have a something substances dissolved in polar solution with anti proliferation ability against to cancer culture cell or analogues as an anti cancer against to carcinoma cells. Nuraini *et al.*,⁷ reported that ethyl acetate extract of leaves of *Dendrophthoe* species as a semi polar solvent had a anti proliferation activities to development of myeloma culture cells. But we did not known yet, potency of anti proliferation substances of leaves of *Dendrophthoe* species where dissolved in non-polar solvent. Diethyl ether, one of non-polar solvent was known commonly use for extract solvent of unknown herbal medicinal.⁸ By these problems as described at above, we will be exploring potency of diethyl ether extract of leaves of *Dendrophthoe* species as anti proliferation cancer culture cell to obtain concentration data where have a minimum anti proliferation ability against to *in vitro* cancer culture cell.

MATERIAL AND METHODS

Research design of this work were using experimentally, by post test only control group design with inhibition ability activities as dependent variable and crude extract of *Dendrophthoe* species and myeloma cells as dependent variable.

The air-dried stem barks of *Dendrophthoe* species leaves were collected from Muara Enim, South of Sumatera, Indonesia. A voucher specimen of *Dendrophthoe* species has been identified and deposit in Peer Groups Researcher of Herb Medicines of Department Medical-Pharmacy, Medical Faculty Airlangga University.

The leaves of *Dendrophthoe* species were pulverization and weighing carefully at about 850 g for dissolved in 2.5 L of diethyl ether p.a during the days. The mixture were processed by macerate method and filtrating with Buchner filter for obtained filtrate of diethyl ether. The residues were processing on similar method at 3 times iterates. 7.5 L filtrates were pooled on one bottle and evaporating by for obtained at about 4–5 g dried filtrate as stock sample. The sample stocks were diluted with Rose Well Park Memorial Institute (RPMI) medium and performed on

series samples as follows; a 100 mg of dried stock samples dissolved up to 100 ml of RPMI medium (100 ppm) and performed to 1.1; 5.5; 11; 22; 33.0, and 44.0 ppm of RPMI. Small volume of sample series and medium of RPMI were tested by Thioglicolate and incubate during the two days (37° C).

The myeloma cell lines (P3UI type) were obtained from the center of Veterinary Pharmaceutics, Directorate of Livestock Services, Agriculture Department, Surabaya (Indonesia). From storage of – 80° C, the cells were thawing and revival to propagates in 100 ml Roux's bottle at least 9×10^7 cells/ml as stock cells. The stock cells were distributing to 2 ml of 36 microtiterplates wells at least 2×10^4 cells/ml each well.

A 100 µl crude extract of *Dendrophthoe* species at ranging 1 to 40 µg/ml were inserting onto 18 wells of microtiterplates and it was consists of 2 ml of RPMI medium with 10% of Fetal Bovine Serum (FBS) and myeloma culture cells. A similar procedure of 100 µl RPMI solution as control substance were using for treat of myeloma in micro titer plates at control group wells. All samples were following propagates and inserting into CO₂ incubator during the 48 hours. Two days later, the cells of all samples in microtiterplates were re-suspended. A 5 µl of the samples were mixture with 1:1 of 10% methylene blue solution (w/v) and dropped on the top of haemocytometer Thoma. By inverted microscope at 200× magnified, the viable cell were counting referred to equation 1 for obtained total viable cell data as illustrated of anti proliferate ability of crude diethyl ether extract.

Total viable cells each well =

$$\frac{\text{Amount of viable cell in 5 } \mu\text{l}}{5 \mu\text{l}} \times 2095 \mu\text{l} \quad \text{Equation I}$$

The criterion of anti proliferation ability of crude diethyl ether extract of *Dendrophthoe* species were described in table 1.⁶

The data were comparing each other by MINITAB 13.3 (treatment group vs., control group) at series concentration of 1.1 ppm to 44.0 ppm. The conclusions of these researches were performed from research analyzed of independent T test at 5% significance of treatment groups vs., control groups.

Table 1. The anti proliferation ability of crude diethyl ether extract of *Dendrophthoe* species

The 24 wells of microtiterplate on 2 ml RPMI medium		
Amount of viable cell each ml	Inhibition criteria	Code
$> 1.00 \times 10^5$	The activity not appearance	–
$0.95-1.00 \times 10^5$	The lowest activity	±
$< 0.95 \times 10^5$	The strongest activity	+

RESULT

Two days later, the samples and medium were appeared still sterile and suitable for research material. Result research showed as follows; the diethyl ether extract of pulverized leaf of Benalu duku were produced anti proliferation activity to myeloma culture cell as illustrate at table 2 at below. At 1.1 $\mu\text{g/ml}$ of the crude extract of diethyl ether of *Dendrophthoe* species leaves, was not appeared an anti proliferation activities yet on myeloma

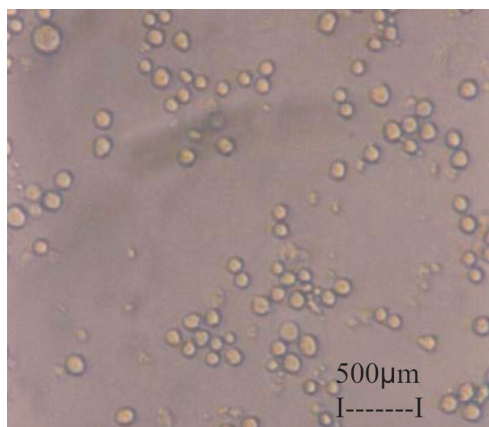


Figure 2. The myeloma culture cell after treats by crude extract diethyl ether of *Dendrophthoe* species at 11.0 $\mu\text{g/ml}$ (200 $\times$).

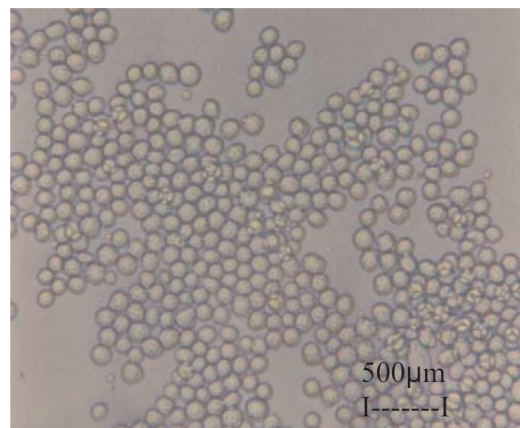


Figure 3. The myeloma culture cell after treated by RPMI medium as control (200 $\times$).

culture cell. Similar phenomena was seen on concentration of 5.5 $\mu\text{g/ml}$. But start on 11 $\mu\text{g/ml}$, the crude extract of diethyl ether of *Dendrophthoe* species leaf were seen have an anti proliferation activities against to myeloma culture cell. At figure 2 appeared clearly that since of 11.0 $\mu\text{g/ml}$ extract *Dendrophthoe* species. leaves have anti proliferation activities to myeloma cells ($p < 0.05$). Figure 3 showed that the RPMI solution was not produced anti proliferation activities on myeloma cells as control groups.

Table 2. The analysis of crude extract diethyl ether of *Dendrophthoe* species at ranging 1.1 $\mu\text{g/ml}$ to 44 $\mu\text{g/ml}$, against myeloma culture cell

Concentr.	Diethyl ether	RPMI solution	Available	p*
1.1 ($\mu\text{g/ml}$)	1.79×10^5	1.80×10^5	–	$p > 0.05$
	1.81×10^5	1.81×10^5	–	
	1.78×10^5	1.82×10^5	–	
5.5 ($\mu\text{g/ml}$)	1.77×10^5	1.60×10^5	–	$p > 0.05$
	1.77×10^5	1.78×10^5	–	
	1.76×10^5	1.84×10^5	–	
11.0 ($\mu\text{g/ml}$)	5.17×10^4	1.87×10^5	+	$p < 0.05$
	4.80×10^4	1.81×10^5	+	
	4.78×10^4	1.80×10^5	+	
22.0 ($\mu\text{g/ml}$)	4.76×10^4	1.81×10^5	+	$p < 0.05$
	3.12×10^4	1.82×10^5	+	
	3.09×10^4	1.83×10^5	+	
33.0 ($\mu\text{g/ml}$)	3.10×10^4	1.80×10^5	+	$p < 0.05$
	3.07×10^4	1.79×10^5	+	
	3.11×10^4	1.78×10^5	+	
44.0 ($\mu\text{g/ml}$)	3.09×10^4	1.86×10^5	+	$p < 0.05$
	3.08×10^4	1.84×10^5	+	
	3.07×10^4	1.81×10^5	+	

* Compared by independent t test of MINITAB 13.3

Table 3. The pharmacodynamic activities of herb medicine against to cancer cell at *in vitro* test

Herb medicine	Cancer cell	The duration of action by incubation time dependent	References
<i>Physallis angulata</i> L	Myeloma cell	48 hours	Maryati, Sutrisna ²⁰
<i>Eurycoma longifolia</i> J	HeLa cell, WiDr, Raji	48 hours	Dinar <i>et al.</i> ²¹
<i>Cola nitida</i>	Breast cancer cell (MCF-7)	24 to 72 hours	Susi <i>et al.</i> ²²

The crude extract of 22.0; 33.0 and 44.0 µg/ml were showed significance inhibit ability activities as illustrated table 2 at below. In contras, the crude extracts diethyl ether of *Dendrophthoe* species at 1.1 and 5.5 µg/ml were not showed inhibit ability activities yet.

DISCUSSION

The sample beginning at 11.0 µg/ml has a specials unknown substance with inhibition abilities to myeloma culture cell. The unknown substances were dissolved in non-polar solution (diethyl ether). Some Indonesia researcher predicted that substances with inhibition ability activities of *Dendrophthoe* species were equal to Mistellectin and Viscotoxine substances. That unknown substances better identified as Mistellectin and Viscotoxine like effects referred to mentioned by Ratna *et al.*, and Roostantia *et al.*^{11,12}

We predicted that substances where dissolved in non polar liquid extraction were appeared a complex molecules at waterproof substances of the leaves. The waterproof substances were obtained on chlorophyll region of the *Dendrophthoe* species at about 10%. On Chlorophyll region, there are some other actives substances like a phloem, chloroplast and stoma of the leaf at about 65%. Other substances perhaps an un identifying substances with anti proliferation activities as described by Hahn.^{13,14}

Arifa *et al.*,¹⁵ by Fourier Transform Infra-Red Spectrophotometer (FT-IR) have been investigated that unknown substances have a specific profile as follows; the wavelength number as stretching vibration of C = alkana was appeared at ranging 2926.28 to 2854.9 cm⁻¹. The wavelength number as stretching vibration of C = aldehyde was illustrated at 1736.09 cm⁻¹. The wavelength number of C = alkena and N–H with sharp intensity were identified at ranging 1602.99 to 1658.93 cm⁻¹. The aromatic substances were appeared at 1496.9 cm⁻¹ wavelength number as stretching vibration. The absorbance peak of C–H amine, C–O, acid compounds, carboxylate and Esther were founded at 1060.94 to 1359.94 cm⁻¹ wavelength number as stretching vibration.¹⁶ Ratna *et al.*,¹¹ predicted that unknown substances have been performing as a new compound with amino acid substances for resulted antiproliferation ability activity. But from the result research predicted that unknown substances is lead substances as futuristic compound for

anticancer purpose that where consist of amino acid, organic substances and aromatic substances.

The pharmacokinetic and pharmacodynamic of unknown substances that dissolved in diethyl ether of *Dendrophthoe* species on human and animal experiments was not seen before. But it was predicted that duration of action from unknown substances of *Dendrophthoe* species dissolved in hot water on rat with infected of myeloma still available at ranging 8 to 12 hours. The elimination half-life (T_{1/2β}) might be lowest than 1 hours as referrer to Nuraini *et al.*³ In conclusion, that result showed the available of absorption, distribution, metabolism and excretion (ADME) of *Dendrophthoe* species as herb medicine on infected subject was shortest than synthetics medicine.^{17,18}

The initial of action of unknown substances of *Dendrophthoe* species after extracted by diethyl ether against to myeloma cell at this test was obtained on a view minutes after dropped. The duration of action at *in vitro* test of *Dendrophthoe* species was appeared at 24 to 48 hours after dropped. These result showed that the pharmacodynamic action of unknown substances of *Dendrophthoe* species after extracted by diethyl ether on *in vitro* test was longer than *in vivo* test as explained by Nuraini *et al.*,³ (the duration of action at *in vivo* test at ranging 6 to 12 hours after treatment). But unknown substances of *Dendrophthoe* species that dissolved on hot water or other polar solvent at *in vitro* test as described by Roostantia *et al.*,² and Nuraini¹⁹ were showed similar of other unknown substances of herb medicine as an anti cancer as referred to table 3.

The phytochemical analysis of crude extract has revealed macromolecules of *Dendrophthoe* species to be among the chemical constituent contained within it. The macromolecules were shown to produced anti cancer activities.² However, the anti cancer effect of plants containing macromolecules actually depends on type and content of their substances in plants as described by Nuraini.¹⁹ The active substances of *Dendrophthoe* species from South of Sumatera (Muara Enim) was shown strongest than *Dendrophthoe* species from Magelang. The other substances of macromolecules as Tannins perhaps have an anti cancer activity. Tannins are polyphenolic compounds and already available on herb medicine.²³ Tannins have an antiproliferation as an interfere substance of biosynthesis of eukaryotic cell by uncoupling oxidative phosphorylation.^{24,25}