PROGRAM BOOK AND ABSTRACTS

International Symposium on Medicinal Plant and Traditional Medicine

Indonesian Traditional Medicine for Human Welfare

Tawangmangu, June 4th - 6th 2014

Jointly organized by

PREFACE

Dear Colleagues,

On behalf of the organizing committee, we would like to express our great honor and pleasure for your attendance and your active participation on "International Symposium of Medicinal Plant and Traditional Medicine for Human Welfare" held in Tawangmangu, June, 4-6th, 2014, organized by Medicinal Plant and Traditional Medicine Research and Development Center (MPTMRDC), National Institute of Health Research and Development, Ministry of Health Republic of Indonesia collaborating with National Working Group of Indonesia Medicinal Plant.

We were honored to have eight keynote speakers both from Indonesia and overseas who are playing a leading role in the frontline of each research field. In this symposium, we particularly focused on medicinal plant and traditional medicine with *Litsea cubeba* and *Equisetum debile* as specific topics. It was a great success to provide the excellent opportunity to discuss how medicinal plant and traditional medicine contributes for human welfare. All accepted articles will be published in the proceeding of symposium after reviewed by competent reviewers.

Ministry of Health, Republic of Indonesia through "Saintifikasi Jamu" program aimed to provide scientific evidence based of Jamu efficacy and safety for utilization in health services. In order to support that program, research and development of medicinal plant and traditional medicinal especially Jamu, from upstream to downstrean by collaboration among research institutes, universities and all stakeholders absolutely needed. In this symposium, the distinguished invited speakers will present their latest findings regarding the symposium theme, and we hope that the participants will enthusiastically discuss the newly raised research and clinical questions with the leading experts.

We hope you will have an excellent opportunity to acquire advanced knowledge and information of medicinal plant and traditional medicine and to strengthen mutual friendship during the symposium.

Sincerely yours,

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DAY 2, JUNE 5th 2014
ROOM 4
MICROBIOLOGY AND BIOTECHNOLOGY
Moderator : Dr. Gemini Alam
Assistant : Elok Widyawati

No.	KODE	TITLE	AUTHOR/s	TIME
1.	O-MBM-009	Isolation and praclinical trial of cinnamon oil for getting phytopharmaca of anticancer (Toxicity effect of cinnamon oil on male rat) and cytotoxicity effect of cinnamon oil on WidR culture cell)	Herdwiani W, Fransiska L, Rica, Yolanda CS, Sari W, Imama, Zullies, Hertianti	13.10 -13.20
2.	O-MBM-010	Antibacterial activity of methanolic extract of *Plumeria acuminata* stembark against *Escherichia coli* and *Staphylococcus aureus*	Harwoko, Nuni Anindita, Eka Prasasti Nurrachmani	13.20 -13.30
3.	O-MBM-011	The antibacterial effectivity of *Garcinia mangostana* L., *Phyllanthus niruri* L., *Allium sativum* L. and *Lumbricus rubellus* to reduce the bacterium *Salmonella* sp. in efforts to prevent and minimize the incidence of salmonellosis in poultry products	Susan Maphilindawati N., Sri Suryatmiati P., Andriani, Masniari Poeloengan	13.30 -13.40
4.	O-MBM-012	The formulation and antibacterial activity of hand sanitizer gels containing lampes (*Ocimum sanctum* L.) leaves extract as an active compound	Alasen Milala Sembiring, Mariana Wahjudi & Meriyanti S.	13.40 -13.50
5.	O-MBM-014	Combination of *Calotropis gigantea* radix extract and artemisin as an antimalarial agent against *Plasmodium berghei*	Asnal Fatati, Roihatul Muti'ah, Elok Kamilah Hayati	13.50 -14.00
6.	O-PMP-009	Antidiabetic effects of combination of andrographolide and curcumin in insulin resistance rats	Indah Solihah, Suwijiyo Pramono	14.00 -14.10
		DISCUSSION		14.10 -14.40
7.	O-MBM-015	Protective effect of bixin isolated from *Bixa orellana* L. seeds on UV B-induced inflammation and immunosuppression of the skin	Atina Hussaana, Suparmi, Hani Afnita	14.30 -14.40
8.	O-MBM-016	Cell cycle arrest and apoptosis inducing-activities of *Melia azedarach* ethanolic extract againts MCF-7 breast cancer cell line	Dina Fatmawati, Titiek Sumarawati, U. Dian Indrayani, Sumarno, Chodidjah, A. Suprijono	14.40 - 14.50
9.	O-MBM-017	Comparative cytotoxic activity of aqueous and ethanolic extract from mangosteen rinds to various cancer cell lines	Sari Haryanti, Elok Widayanti, and Yuli Widiyastuti	14.50 -15.00
10.	O-PMP-019	The effect of asthma herbs formula to the liver functions	Agus Triyono, Peristiwan Ridha	15.10 -15.20
		DISCUSSION		15.20 -15.40

O-MBM-015

PROTECTIVE EFFECT OF BIXIN ISOLATED FROM *Bixa orellana* L. SEEDS ON UVB-INDUCED INFLAMMATION AND IMMUNOSUPPRESSION OF THE SKIN

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Excessive UV radiation on the skin can cause DNA damage that triggers the inflammatory response and immunosuppression. This study aimed to verify whether the bixin lotion has the effect to offer protection against inflammation and immunosuppression due to acute UVB irradiation in shaved BALB /c mice. Protection against inflammation and immunosuppression, respectively were studied in 4 groups of mice receiving the topical application of base lotion (control); bixin lotion doses of 0.5; 2.5 and 125 mg, 10 days prior to and during the UVB irradiation. Inflammation was induced by UVB irradiation 360 mJ/cm2 once a day for 3 consecutive days, whereas immunosuppression was induced for 5 consecutive days. The inflammatory response was measured as an increase in middorsal skinfold thickness at the peak response. The immune response was measured as the contact hypersensitivity (CHS) response to oxasolon sensitization.The results indicated that in all concentrations used, bixin lotion significantly decreased the middorsal skinfold thickness at 72 hours after UVB radiation ($p < 0.05$) compared to the control, but there was no significant difference between couples of the dose of bixin. Bixin lotion was also able to restore the suppression of CHS from 34.22% in the control group to 11.4; 0.5 and -67% ($p < 0.05$) at doses of 0.5; 2.5 and 125 mg respectively. Bixin lotion had potential to reduce the inflammatory edema reaction and the suppression of CHS of mice induced by UVB radiation.

Keywords: *Bixa orellana* L., photoprotection, inflammation, immunosuppression, UVB radiation

O-MBM-016

CELL CYCLE ARREST AND APOPTOSIS INDUCING-ACTIVITIES OF *Melia azedarach* ETHANOLIC EXTRACT AGAINTS MCF-7 BREAST CANCER CELL LINE

Dina Fatmawati*, Titiek Sumarawati, Ulfah Dian Indrayani, Sumarno, Chodidjah, Agus Suprijono, Israhnanto, Anita Soraya Soetoko
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Medicinal plant is main source of cancer drug development. *Melia azedarach* is one of medicinal plant has been shown to have astrong cytotoxic activities in MCF-7 breast cancer cell line with IC_{50} 20 µg/mL however, the apoptotic and cycle cell arrest effect of *Melia azedarach* was unknown. This study was aim to investigate the effect of *Melia azedarach* ethanolic extract on cycle cell arrest and apoptosisagaints MCF-7 breast cancer cell line. In this quasy experimental study, MCF-7 were divided into 4 groups including control group and study groups (treated with the ethanolic extract of *Melia azedarach* at the dose 20 µg/mL, 10µg/mL, 5 µg/mL). The cell cycle arrest effect was tested by propidium iodide and the apoptosis effect was tested against MCF-7 breast cancer cell line by annexin V/PI. Accumulation of cell in each cycle cell phase and the percentage of apoptotic cell were observed using flowcytometry. The difference among groups was analyzed using ANOVA with significant level 0.05. From qualitative image analysis data showing an increase in percentage of cell with a sub G0/G1 DNA content ($p<0,05$). In addition, the study showed a significant difference ($p<0,05$) in the percentage of apoptotic cell between control and groups. In conclusion, *Melia azedarach* induces G0/G1 cycle cell arrest and apoptosis against MCF-7 breast cancer cell line.

Keywords: *Melia azedarach*, apoptosis, cycle cell arrest, MCF-7 breast cancer cell line

CELL CYCLE ARREST INDUCING-ACTIVITIES OF *Melia azedarach* ETHANOL EXTRACT AGAINTS MCF-7 BREAST CANCER CELL LINE

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ABSTRACT

Medicinal plant is main source of cancer drug development. *Melia azedarach* is one of medicinal plant has been shown to have a strong cytotoxic activities in MCF-7 breast cancer cell line with IC_{50} 20 µg/mL however, the cycle cell arrest effect of *Melia azedarach* was unknown. This study was aim to investigate the effect of *Melia azedarach* ethanolic extract on cycle cell arrest againts MCF-7 breast cancer cell line. In this quasy experimental study, MCF-7 were divided into 4 groups including control group and study groups (treated with the ethanolic extract of *Melia azedarach* at the dose 20 µg/mL, 10 µg/mL, 5 µg/mL). The cell cycle arrest effect was tested by propidium iodide. Accumulation of cell in each cycle cell phase were observed using flowcytometry. The difference among groups was analyzed using ANOVA with significant level 0.05. From qualitative image analysis data showing an increase in percentage of cell with a sub G0/G1 DNA content ($p<0,05$). In conclusion, *Melia azedarach* induces G0/G1 cycle cell arrest and apoptosis against MCF-7 breast cancer cell line

Keyword: Melia azedarach, apoptosis, cycle cell arrest, MCF-7 breast cancer cell line

INTRODUCTION

Cancer is a disease of malignant neoplasms that have a wide broad spectrum and complexity. Cancer progression is characterized by increased proliferative activity and the disruption of apoptosis. It’s the second leading cause of death in worldwide including Indonesia following cardiovascular disease. On data from the world's leading cause of death in 2005, cancer is the second highest cause of death after cardiovascular diseases. Up to present, spontaneous cancer healing has been rare and almost no cancer can be cured by the spontaneous and if the cancer continues to be allowed to grow will result in the death of the sufferer (Rasjidi, 2009). Breast cancer is the most frequently diagnosed cancer and cause most of death from cancer among women worldwide yearly. About 1,3 million new cases of invasive breast cancer are expected to occur. Breast cancer incidence has been rising in many developing countries. There have been improved patient survival and quality of life (Rasjidi, 2009).

There are various approaches for breast cancer therapy such as radiotherapy, surgery, and chemotherapy however, those therapy produces side effect due to its toxicity (Tjindarbumi, 2001). Indonesia has mega biodiversity plant and some of them has been use as traditional medicinal plants. *Melia azedarach* was one of potential candidate for breast cancer therapy been shown to contain triterpenoid and limonoids (Ntalli, *et al*., 2010). Limonoids-contained *Melia azedarach* has been shown various biological properties such as antioxidant, anti-bacteria, and anti-cancer Mazumder *and* Rahman, 2008). Fasad (2008) showed that triterpenoid acts as ligand for PPARG which inhibit the activation of Retinoblastoma protein (pRb). As consequence of pRb inactivation was G1 cycle cell arrest. However, the study on the effect of ethanol extract of *Melia azedarach* as anticancer has not been conducted. In this study we purposed that ethanol extract of *Melia azedarach* has anticancer activity against MCF-7 breast cancer cell line by alterating cycle cell pattern.

MATERIAL AND METHODS

Plant material

Melia azedarach were collected from forest located in Kendal, Central Java, Indonesia during March-May 2012. The *Melia azedarach* were identified by staff of development structure of plant, Department of biology, Semarang State University, Central Java, Indonesia. The fresh seeds of *Melia azedarach* were collected, was air-dried at room temperature and chopped finely.

Preparation of Extract

Three hundred grams of dried and chopped materials were extracted with ethanol by soxchletation method for 24 hours, filtered then evaporated using rotary evaporator to produce ethanol extract of *Melia azedarach.* The ethanol extracts were dissolved in dimethyl sulfoxide (DMSO- merck) and subsequently diluted to appropriate working concentrations with RPMI culture medium (RPMI-Sigma) for proliferation inhibitor proliferative. For the Identification functional chemical group in the crude extract Thin Layer Chromatography method was performed.

Cell culture

The human breast cancer MCF-7 cell line was obtained from the parasitology laboratory of Faculty of Medicine Gajah Mada University, Yogyakarta, Indonesia. The cells were grown and maintained in RPMI supplemented with 10% (v/v) foetal bovine serum (FBS- Sigma Aldrich), 0,5% fungizone (Gibco), 2% penstrep (Gibco), and incubated at 37^0C in a humidified atmosphere and 5% CO_2 (Mooney *et al*., 2002; Tan *et al*., 2005).

Cytotoxic assay

Cytotoxicity of the extract at various concentrations (1.562-1000µg/mL) was assessed using the 3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyl tetrazolium bromide (MTT-Sigma) assay. The MTT colorimetric assay developed by Mosmann with modification was used to screen the cytotoxicity of all the ethanolic extract of gembili. Briefly, the cells were seeded in 96-well plates at a density of 104 cells/well in 100 µl culture medium. Following 24-h incubation an attachment, the cells were treated with different concentrations of ethanol extract of gembili and doxorubicin (positive control) for 24 h. The potential candidates which resulted in cell survival of less than 50% were further assessed for their IC50 (concentration that inhibits cell growth by 50%) values at the concentration ranged 1000 and 31.25 µg/ml. Each experiment was performed in triplicate. Following washing and incubation with MTT solution at 37°C for 4 h. The yellow MTT dye was reduced by succinic dehydrogenase in the mitochondria of viable cells to purple formazan crystals. The formazan crystals was diluted in 10% SDS HCl (0.1%). The result was measured using ELISA reader (595 nm) using a microplate reader (Nunc®) and presented in optical density (OD). The percentage of cytotoxicity compared to the untreated cells was determined with the equation:

Cell viability (%) =

$$\frac{\text{(control cells absorbance – media absorbance) - (treated cells absorbance – media absorbance) x 100}}{\text{(control cells absorbance – media absorbance)}}$$

Data generated were used to plot a dose-response curve of which concentration of extract required to kill 50% of cell population (IC_{50}) was determined.

Cell cycle distributrion of MCF-7 cells treated with ethanolic extract of *Melia azedarach* using flowcytometry

The harvested cells (1 x 10^6) were seeded onto 6 microwell plates (Nunc®) and incubated for 24 h at 37^0C in 5% CO_2. Cells were treated using the sample at final concentration of ethanol extract of *Melia azedarach* 20 µg/mL, 10 µg/mL, 5 µg/mL and incubated for 24 h. Cells were harvested by trypsinization and collected in 15 ml tube, followed by centrifugation at 1200 rpm for 3 minute. The supernatant was discarded and 100 µl of flow reagent was added, followed by incubation for 2 minute at room temperature. Subsequently, RNa-se solution was added at final concentration 1 mg/ml and Run FACS using Facs Calibur and Cell quest software (Becton and Dickinson).

Statistical analysis

The results was shown as mean ± SD for multiple experiments. The Statistical analysis was performed using SPSS software, version 17. The IC_{50} values for ethanol extract of *Melia azedarach* on MCF-7 cells were calculated by probit analysis. For differences between means, one-way analysis of variance (ANOVA) was used with Tukey's HSD test (for equal variances). Values of $p< 0.05$ were considered significant.

RESULTS

Cytotoxic Assay

The ethanol extract of Melia azedarach was showed strong cytotoxic activity againts MCF-7 cells with IC_{50} value was 24 µg/mL (table 1).

Table 1. the effect of ethanol extract of Melia azedarach on MCF-7 cells viability

concentration (µg/mL)	1	2	3	mean	Std-dev
500	-6.187	0	-0.941	-2.376	3.333
250	-6.187	0.941	0.188	-1.686	3.916
125	5.189	11.487	10.169	8.948	3.321
62,5	28.343	29.378	29.190	28.970	0.551
31,25	53.293	56.120	56.308	55.240	1.689
15,67	53.692	48.964	48.775	50.477	2.785

Based on the table 1 the highest mean percentage of MCF-7 cell viability (50.47%) was found for the mindi extract at the dose of 15.6 µg/mL. The lowest mean percentage of MCF-7 cell viability for Mindi Extract at the dose of 500 µg/mL. The increase in response of mean viability on the log of mindi extract dose is presented in figure 4

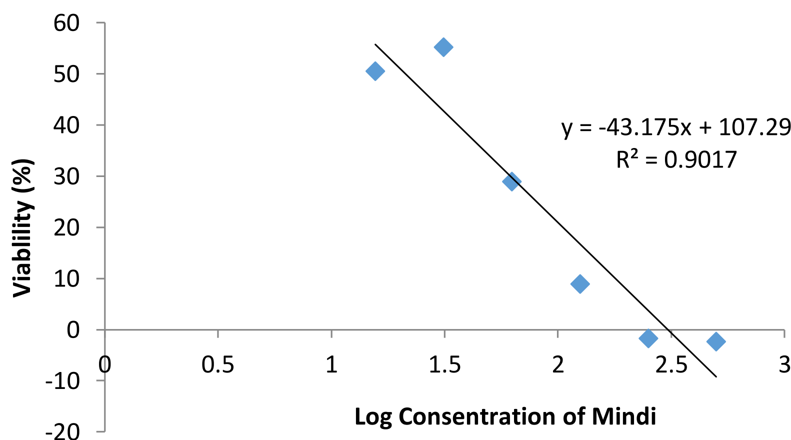

Figure 1 MCF-7 cell line Response viability on the log of mindi extract consentration

The increased ethanol extract of mindi has been shown to highly correlate with the decrease in the decreased percentage of viability in MCF-7 cell line formulated in the value equation of R2 = 0901. Probit test showd IC50 value of mindi extrct against MCF-7 cancer cell line was 24.327 µg/mL (appendix 11). Based on its IC50 value, mindi extract, is catagoried into toxic against MCF-7 brest cancer cell line.

Cytotoxicity test showed that mindi leave extract has higher sytotoxicity than mindi leave extract. It might have been due to the difference in the level and type of triterpenoid in the mindi's leave and seed. Jafari (2013) showed that there is difference in triterpenoid concentration in mindis' leave and seed. The previous study showed that ethanol extract of mindi leave contains flavonoid in form quersetin dan saponin, while its fruit contains kaempherol flavonoid with time retention on HPLC as much as 57.36 dan 62.33 min (Vijayanand and Wesely 2011; Cheiffelle, 2009). Wang (2008) stated that the total concentration of triterpenoid in mindi leave and seed were 17.50±1.26mg/g and 32.40±0.95 mg/g respectively.

Cancer cell contains higher Peroxisome proliferating-activated receptor ɣ (PPARG) compared to normal cell. (Lapillone, 3003). Triterpenoid can serve as ligan Peroxisome proliferating-activated receptor ɣ thus indces poptosis in cancer cell. (Tabe et al., 2007).

Cell cycle distribution

The ethanol extract of *Melia azedarach* inhibited cell proliferation at various concentrations in dose dependent manner. In addition to study the mechanism behind the anticancer activity of ethanolic extract of *Melia azedarach* on cell cycle phases of MCF-7 were investigated. The MCF-7 cell lines were treated with extract at different concentrations of 20 µg/mL, 10 µg/mL, 5 µg/mL for 24h and percentage of cell distribution in each phase of cell cycle was shown in figure 1.

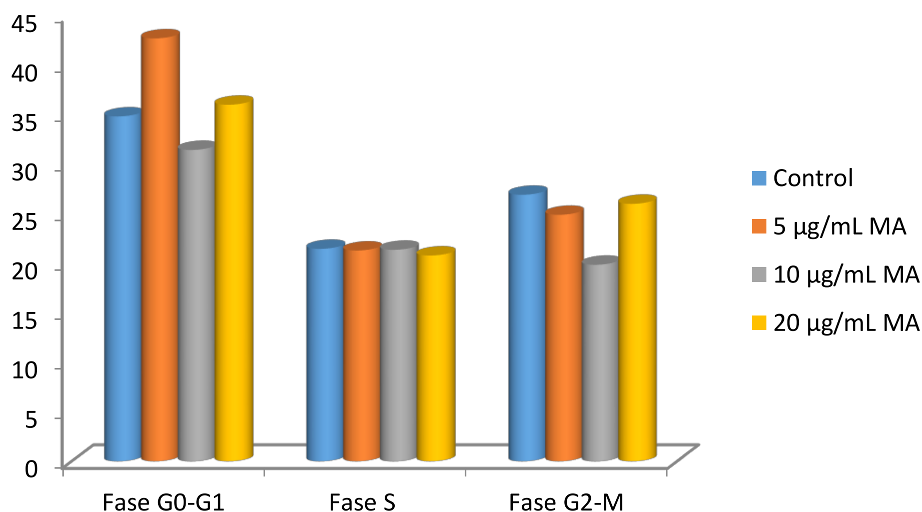

Figure 1. Cell cycle phase distribution of MCF-7 cells. MCF-7 cells (1×10^6 cells/ml) treated with ethanolic extract of *Melia azedarach* at various dose as well as RPMI were prepared for cell cycle analysis after 24 hours exposure. Cells stained with Propidium Iodide (PI) reagent were analysed with cell quest program using flow cytometer Becton and Dickinson). The data represent the mean of 3 independent experiments.

Cell cycle modulation by various natural and synthetic agents is gaining widespread attention in recent years. Given that disruption of cell cycle plays a crucial role in cancer progression, its modulation by phytochemicals seems to be a logical approach in control of carcinogenesis (Singh et al., 2002). MCF-7 cell cycle modulation was indicated by accumulation of cells in G0-G1 phase higher compared to control. Pozarowski (2004) mentions that the cells undergoing apoptosis often have fractional DNA due to DNA fragmentation. In the sub-G0 phase is only a fraction of DNA in the cell apotosis-looking.

These result suggest that ethanol extract of *Melia azedarach* has antiproliferative effect indicated by acummulation of percentage cell distribution in G0-G1 (Figure 1). The higher number of cells accumulated in G0-G1 phase was probably due to triterpenoid content of Melia azedarach acts as ligand or PPARG which inhibit activation of pRb caused G1 arrest (Fajast, 2008).

CONCLUSION

Ethanolic extract of Melia azedarach have concentration dependent anticancer activities with significant alterations on cell cycle pattern in comparison to control.

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