PROCEEDING

The 1st University of Muhammadiyah Purwokerto - Pharmacy Internasional Conference (UMP-PIC)

5-6 June 2015

"Colaboration Approach to Improve Research and Management of Chronic Diseases"

ISBN 978-602-73538-0-0

Faculty of Pharmacy

UMP

THE 1ST UNIVERSITY OF MUHAMMADIYAH PURWOKERTO – PHARMACY INTERNATIONAL CONFERENCE (UMP-PIC) PROCEEDING

5-6 JUNE 2015
Horison Hotel, Purwokerto, Central Java, INDONESIA

Editors:

Dr. Asmiyenti Djaliasrin Djalil, M.Si.
Dwi Hartanti, M. Farm., Apt.
Pri Iswati Utami, M.Si., Apt.
Susanti, M.Phil., Apt.

FACULTY OF PHARMACY
UNIVERSITY OF MUHAMMADIYAH PURWOKERTO
Purwokerto, Central Java, INDONESIA

THE 1ST UNIVERSITY OF MUHAMMADIYAH PURWOKERTO – PHARMACY INTERNATIONAL CONFERENCE (UMP-PIC) PROCEEDING

ISBN: 978-602-73538-0-0

Published by:
Faculty of Pharmacy
University of Muhammadiyah Purwokerto
Jl. Raya Dukuhwaluh, PO Box 202, Purwokerto 53182
Telp : (0281) 636751, 630463 psw 231-232
Fax : (0281) 637239
Email : farmasi@ump.ac.id
Website : www.ump.ac.id

CONTENTS

Greetings from the Dean Faculty of Pharmacy
University of Muhammadiyah Purwokerto

It is a great honour for Faculty of Pharmacy, University of Muhammadiyah Purwokerto to welcome all of invited speakers and researchers at The First University of Muhammadiyah Purwokerto – Pharmacy International Conference (UMP-PIC). Faculty of Pharmacy, UMP would like to held this confence periodicly every two year. For the first time, we have a topic, "Collaborative Approach to Improve Research and Management of Cronic Diseases". The aim of this conference is to promote the utilization and research development of cronic diseases toward patients management including prevention, therapy and health promotion.

Chronic diseases, such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes, are by far the leading cause of mortality in the world, representing 68% of all deaths in 2012. Out of the 38 million people who died from chronic disease, more than 40% of them (16 million) were premature deaths under age 70 years. Almost three quarters of all Chronical diseases deaths (28 million), and the majority of premature deaths (82%), occur in low- and middle-income countries. This invisible epidemic is an under-appreciated cause of poverty and hinders the economic development of many countries that is why then called non communicable diseases (NCD) (WHO, 2014).

I hope that all scientists and researchers participating in this event will present and discuss current international strategies for prevention and control of chronic noncommunicable diseases (NCD), to make participants familiar with the Finnish experiences from the North Karelia Project and to train in planning, implementation and evaluation of NCD prevention interventions: 'from theory to practice'. I am sure that this event will give advantages for all of us.

Lastly, I would like to express my deep gratitude to all participants for their contributions to the success of this event. I hope all of you will have a fruitful meeting and a pleasant stay in Purwokerto.

Dr. Nunuk Aries Nurulita, M.Si., Apt.
Dean of Faculty of Pharmacy
University of Muhammadiyah Purwokerto

Greetings from the Chairperson of the Organizing Committee
The 1st University of Muhammadiyah Purwokerto – Pharmacy International Conference (UMP-PIC)

Dear Colleagues,

The Organizing and Scientific Committee of the 1st UMP-PIC (University of Muhammadiyah Purwokerto-Pharmacy International Conference) is very pleased to announced its 1st Biannual Conference which will take place from June 5-6, 2015 at the Horison Hotel, Purwokerto, Central Java, Indonesia. The theme of this meeting is "Collaborative approach to improve research and management of chronic diseases". It will focus on the emerging role of pharmacists in chronic care. We have been able to invite experts in the field of science and cinical pharmacy to share about their experience in managing chronic diseases. Conference sessions will include plenary lectures, oral and poster presentations.

The 1st UMP-PIC invites pharmacists and pharmaceutical scientists from all over the world to delve into research and healthcare management in chronic diseases. All the organizing and scientific committee are greatly looking forward to welcoming the participants of the 1st UMP-PIC in Purwokerto, Central Java, Indonesia to experience the wonderful Indonesian hospitality. Come to the 1st UMP-PIC and gather at the most important meeting for our global network of pharmacists. We look forward to seeing you here!

Githa Fungie Galistiani, M.Sc., Apt.
Chairperson

Proceeding of The 1st University of Muhammadiyah Purwokerto - Pharmacy International Conference
5-6 June 2015, Horison Hotel Purwokerto
ISBN 978-602-73538-0-0

Ethanolic Extract *of Arcangelisia flava* Leaves is Cytotoxic and Selective against Breast and Colon Cancer Cell Lines

Endah Puspitasari, Dian Agung Pangaribowo, Ika Yanuar Isparnaning, Yora Utami

Faculty of Pharmacy, Jember University, Jalan Kalimantan 37, Jember 68121, Indonesia
(E-mail: *e.puspitasari@unej.ac.id*)

ABSTRACT

Previous study revealed that ethanolic extract of *Arcangelisia flava* leaves (EEAfL) was able to increase immune response on doxorubicin-treated rats with no signs of toxicity. Its use as cancer co-chemotherapeutic agent could be based on its capability to decrease chemotherapeutic agent side effects as well as increasing the effectivity of chemotherapeutic agent. This study was determined to test the cytotoxicity and selectivity of EEAfL againts several cancer cell lines as the basis for increasing the effectivity of chemotherapeutic agent indicator. The cytotoxicity assay was done by MTT method on HeLa, MCF-7, and WiDr cell lines as well as Vero cell line for the selectivity assay. EEAfL exhibited cytotoxic activity on HeLa, MCF-7, WiDr, and Vero with IC_{50} value of 467+70; 136+17; 213+79; and 1340+288 ppm, respectively. Thus it was selective on HeLa, MCF-7, and WiDr with SI value of 2.87; 9.85; and 6.29, respectively. Meaning that EEAfL is cytotoxic and selective against MCF-7 and WiDr, but not on HeLa cell line. It can be concluded that EEAfL has the chance to be developed as cancer co-chemotherapeutic agent especially for breast and colon cancer, still there are ways to go.

Key words: breast cancer, colon cancer, cytotoxic, ethanolic extract of *Arcangelisia flava* leaves, selective.

INTRODUCTION

Our previous studies revealed that ethanolic extract of *Arcangelisia flava* leaves (EEAfL) was able to increase the lymphocytes on doxorubicin-treated rats, suggesting that it increases immune response on doxorubicin-treated rats (Puspitasari and Umayah, 2013). It did not cause any signs of toxicity, biochemically nor histopathologically based on sub chronic toxicity assay (Puspitasari and Umayah, 2014). These results providing a prove that EEAfL could be used as cancer co-chemotherapeutic agent in combination with doxorubicin in order to decrease doxorubicin side effects.

Its use as cancer co-chemotherapeutic agent could be based on its capability to decrease chemotherapeutic agent side effects as well as increasing the effectivity of chemotherapeutic agent (Steward and Brown, 2013). This study was determined to test the cytotoxicity and selectivity of EEAfL againts several cancer cell lines as the basis for increasing the effectivity of chemotherapeutic agent indicator. The cytotoxicity assay was evaluated based on the IC_{50} obtained from MTT method (Doyle and Griffiths, 2000), while the selectivity was determined using selectivity index (SI) value (Prayong et al., 2008). This study was done on cervical, breast, and colon cancer cell lines, as well as on normal cell line for the selectivity assay.

ISBN 978-602-73538-0-0

EXPERIMENTAL

2.1 Plant Extract Preparation

The *A. flava* leaves were collected from Meru Betiri National Park, Jember, Indonesia. They were selected for their freshness, old age, and healthy ones. The leaves were washed thoroughly with water, then, were air dried followed by oven drying at 50 ^{0}C. The dried leaves were grounded and sieved. The ethanolic extract were prepared using 100 g of leaves powder. The ground-dried leaves was extracted with ethanol 96%. The extraction was repeated three times. The ethanol extract was evaporated under reduced pressure (Heidolph, Laborota) resulting EEAfL. EEAfL was then suspensed in DMSO never exeed than 1% for cytotoxicity assay.

2.2 Cytotoxicity Assay

The cytotoxicity assay was done by MTT method on several cancer cell lines. HeLa was representing cervical cancer cell line. MCF-7 was representing breast cancer cell line, while WiDr was representing colon cancer cell line. The normal cell was represented by Vero cell line. Those cell lines are the collection of Parasitology Laboratorium, Faculty of Medicine, Gadjah Mada University. The cytotoxicity assay was done for 24 hours, except HeLa cell line was treated for 48 hours. Briefly, 1x10^6 cells were seeded in 96 well plate then incubated for 24 hours in 37 ^{0}C 5% CO_2 and suitable medium (HeLa was grown in DMEM low glucose, MCF-7 was grown in DMEM high glucose, WiDr and Vero were grown in RPMI) supplemented with 10% of fetal bovine serum and 1% of penicillin-streptomycin. A series of EEAfL then given to the cell line. At the determined time, the cells were washed with PBS and MTT (0.5 mg/ml) was added. The incubation was continued for 4 hours. Then the stopper reagent (10% SDS in 0.1 N HCl) was added and the absorbance was read at 595 nm. The cell viability was calculated as follows:

$$\text{Cell viability} = \frac{\text{Absorbance of treated cell} - \text{Absorbance of medium control}}{\text{Absorbance of cell control} - \text{Absorbance of medium control}} \times 100\ \%$$

The IC_{50} was determined by probit analysis based on the plot of concentration *vs* cell viability (Doyle and Griffiths, 2000).

2.3 Selectivity Assay

The selectivity index (SI) was determined based on the IC_{50} values and calculated as follows:

$$\text{Selectivity index (SI)} = \frac{\text{IC50 of normal cell line}}{\text{IC50 of cancer cell line}}$$

(Prayong et al., 2008).

2.4 Statistical Analysis

The IC_{50} were presented as mean + standard deviation (SD) from triplicate. Then they were analyzed using Anova followed by LSD (p 0.05).

ISBN 978-602-73538-0-0

RESULTS AND DISCUSSION

The EEAfL obtained was 16.1 gram from 100 g ground-dried leaves. The yield was 16.1%. The cytotoxicity assay showed that the cell viability of all four cell lines were decreased in dose dependent manner when treated by EEAfL (Fig. 1). The IC_{50} value of EEAfL on HeLa, MCF-7, WiDr, and Vero was 467+70; 136+17; 213+79; and 1340+288 ppm, respectively (Table 1). EEAfL was classified to have moderate cytotoxicity on HeLa, MCF-7, and WiDr cancer cell lines, but considered non toxic on normal cell line (Prayong et al., 2008). Based on the IC_{50}, the EEAfL exhibited cytotoxic on HeLa, MCF-7, and WiDr cancer cell line, but not on normal cell (Vero).

The methanol extract of *A. flava* stem exhibited cytotoxic activity on MCF-7 cell line with IC_{50} value of 7.7+0.6 ppm (Keawpradub et al., 2005). The distinct between these results may contributed by the active substance content in both extract. One of the active substance in *A. flava* known to have anticancer properties is berberine (Yu et al., 2007; Eom et al., 2008; Katiyar et al., 2009; Pandey et al., 2008; Kim et al., 2008). The EEAfL only contains 0.14% berberine (Puspitasari and Umayah, 2013), while berberine in methanol extract of *A. flava* stem contains 6.27% (Keawpradub et al., 2005). That is why the cytotoxic activity of methanol extract of *A. flava* stem is higher than EEAfL.

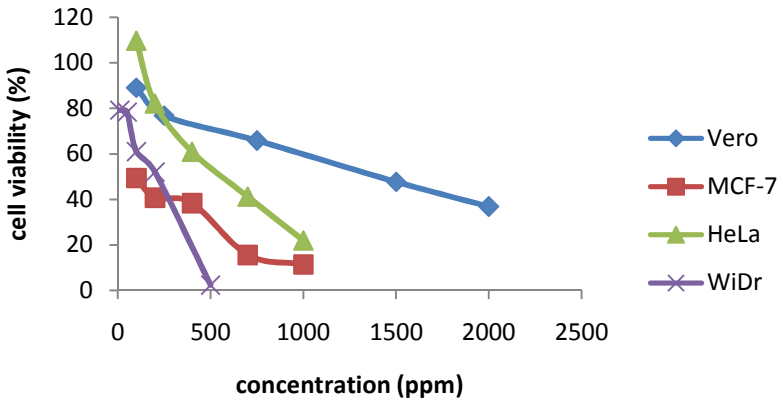

Fig 1. Cancer cell lines' viability due to EEAfL treatment for 24 hours (except HeLa cell line were treated for 48 hours).

The SI value of EEAfL on HeLa, MCF-7, and WiDr was 2.87; 9.85; and 6.29, respectively (Table 1). SI value more than 3 was classified to be high selective, while SI value less than 3 was classified to be less selective (Prayong et al., 2008). EEAfL was considered to have high selectivity on MCF-7 and WiDr, but less selectivity on HeLa cell line. The EEAfL could be taken for further bioassay guided experiment as anticancer against breast and colon cancer based on these findings, including the molecular mechanisms investigations and the possibility of the use in combination with cancer chemotherapeutic agents in order to obtain maximum effect and minimum side effects.

ISBN 978-602-73538-0-0

Table 1. IC_{50} and SI of EEAfL on several cancer cell lines

No	Cell line	IC_{50} (ppm)	SI
1	HeLa	467 + 70[a]	2.87
2	MCF-7	136 + 17[b]	9.85
3	WiDr	213 + 79[a.b]	6.29
4	Vero	1340 + 288[c]	

**The IC_{50} data was shown in mean + SD (n=3).*
***Different notation on IC_{50} showed significant difference by LSD ($p<0.05$)*

CONCLUSIONS

Based on the results, we can conclude that EEAfL is cytotoxic and selective against breast and colon cancer, but not on cervical cancer. It has the chance to be developed as cancer co-chemotherapeutic agent especially for breast and colon cancer, still there are ways to go.

ACKNOWLEDGMENT

The authors gratefully thank to Dirlitabmas, Directorate of Higher Education, Ministry of Education and Culture, Republic of Indonesia for the funding via Hibah Bersaing 2015 No. 259/UN25.3.1/LT/2015.

REFERENCES

Doyle A., Griffiths J.B. (2000) *Cell and Tissue Culture for Medical Research*, John Willey and Sons Ltd.. New York.

Eom K.S., Hong J.M., Youn M.J., So H.S., Park R., Kim J.M., and Kim T.J. (2008) Berberine induces G1 arrest and apoptosis in human glioblastoma T98G cells through mitochondrial/caspasas pathway. *Biol Pharm Bull.* 31(4):558-562.

Katiyar S.K., Meeran S.M. Katiyar N., and Akhtar S. (2009) p53 cooperates berberine-induced growth inhibition and apoptosis of non-small cell human lung cancer cells in vitro and tumor xenograft growth in vivo. *Mol Carcinog.* 48(1):24-37.

Keawpradub N., Dej-adisai S., and Yuenyongsawad S. (2005) Antioxidant and cytotoxic activities of thai medicinal plants named khaminkhruea: *Arcangelisia flava, Cosciniu blumeanum,* and *Fibraurea tinctoria Songklanakarin J. Sci. Technol.* 27 (Suppl. 2):455-467.

Kim J.B., Lee K.M., Ko E., Han W., Lee J.E., Baw J.Y., Kim S., and Noh D.Y. (2008) Berberine inhibits growth of the breast cancer cell lines MCF-7 and MDA-MB-231. *Planta Medica* 74(1):39-42.

Pandey M.K., Sung B., Kunnumakkara A.B., Sethi G., Chaturvedi M.M., and Aggarwal B.B., (2008) Berberine modifies cystein 179 of ikappa balpha kinase, suppresses nuclear factor-kapab-regulated antiapoptotic gene products, and potentiates apoptosis. *Cancer Res*. 68(13): 5370-5379.

ISBN 978-602-73538-0-0

Prayong P., Barusrux S., Weerapreeyakul N. (2008) Cytotoxic activity screening of some indigenous thai plants. *Fitoterapia* 79:598-601.

Puspitasari E., Umayah E.E. (2013) The development of standardized ethanolic extract of *Arcangelisia flava* as doxorubicin co-chemotherapeutic agent for cancer treatment. *Research Report*, Jember University.

Puspitasari E., Umayah E.E. (2014) The Development of standardized ethanolic extract of *Arcangelisia flava* as doxorubicin co-chemotherapeutic agent for cancer treatment. *Research Report*, Jember University.

Steward W.P., Brown K. (2013) Cancer chemoprevention: a rapidly evolving field. *BJC* 109:1-7.

Yu F.S., Yang J.S., Lin H.J., Yu C.S., Tan T.W., Lin Y.T., Lin C.C., Lu H.E., and Chung J.G. (2007) Berberine inhibits WEHI-3 leukemia cells in vivo*. in Vivo* 21(2): 407-412.