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Neurogenic Differentiation of Bone Marrow Mesenchymal-Like Stem Cell Induced by *Delonix regia* Flowers Extract

✉**Kartini Eriani[1], Irma Suryani[1], Al Azhar[2], Risa Nursanty[1], Ichsan Ichsan[3], Arief Boediono[4]**

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[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Indonesia
[2]Faculty of Veterinary Medicine, Universitas Syiah Kuala, Indonesia
[3]Faculty of Medicine, Universitas Syiah Kuala, Indonesia
[4]Faculty of Veterinary Medicine, Institut Pertanian Bogor, Indonesia

History Article

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Abstract

Stem cell technology has great potential in the effort to cure degenerative diseases. This study was done to determine optimum dose of flamboyant (*Delonix regia*) flower extract to induce proliferation and differentiation of mice (*Mus musculus*) bone marrow mesenchymal-like stem cell. Bone marrow cells were collected from mice by aspiration. Cells suspension (1 x 10^6) were poured into petri dishes containing 2 ml of modified Dulbecco's Modified Eagle's Media (mDMEM) and incubated overnight at 37 °C in a 5% CO_2 incubator and microscopically observed. In quadriplicate, MSC were cultured in mDMEM containing *D. regia* flower extract of 0.0 (control), 0.4, 0.6, 0.8, and 1.0 mg/ml and incubated at 37 °C for 9 days. Population doubling time (PDT) and differentiated cell type were microscopically observed using HE staining on day 1 and 10. Data obtained were analyzed by ANOVA and Tukey test. The results showed that the addition of *D. regia* flowers extracts 0.8 and 1.0 mg/ml significantly reduced PDT compared to that of 0.4, 0.6 and control. The extract, at 0.4 and 0.6 mg/ml, were able to induce MSC differentiation into fibroblast-like and nerve-like cells. In conclusion, *D. regia* flower extracts of 0.6, 0.8 and 1.0 mg/ml were able to stimulate MSC proliferation, but optimum dose for neurogenic differentiation was 0.6 mg/ml. This is the first to show potential of *D. regia* flower extract as neurogenic differentiatian inducer on mice MSC. These findings can be used as preliminary information for using the extract as cellular differentian inducer in basic and applicative reseach using stem cells.

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✉ Correspondence Author:
Jl. Syech Abdur Rauf No. 3, Banda Aceh 23111, Aceh, Indonesia
E-mail: kartini_eriani@unsyiah.ac.id

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INTRODUCTION

Degenerative diseases that occur each year continue to increase due to changes in people's lifestyle. These diseases have become one of the major causes of death in the world. According to the World Health Organization (WHO), degenerative diseases have led to nearly 17 million people die every year. Degenerative disease is a type of non-communicable diseases caused by decreased organ function in certain individual. The disease is becoming a problem for the health of the world in addition to communicable diseases and non-infectious diseases (Handajani *et al*. 2010). Today, many countries including Indonesia have doubled their efforts to overcome health problems related to degenerative diseases.

Degenerative diseases can be cured with stem cell (Wobus and Boheler 2005). Mesenchymal stem cells (MSCs) can differentiate into osteocytes, chondrocytes, adipocytes, and various types of connective tissue compositions (Halim *et al*. 2010). Kang *et al*. (2005) also reported that MSCs could differentiate into neuronal and brain cells, cardiomyocytes, and hepatocytes like-cells. Mesenchymal stem cells are also easy to be identified and isolated from bone marrow.

Stem cell differentiation is influenced by both internal and external cell factors. Internal cell factors include genetic and epigenetic, while the external factors include cell environment, growth factors, or the requirements of tissue and organ (Halim *et al.* 2010). Among growth factors that have the ability to enhance proliferation and differentiation of stem cell are phytochemicals from plants extract (Van den Broek *et. al*. 2004). This will expand the growing use of natural material for medicinal purposes since more elements in the community believe that natural drug or medicine result in less side effects that synthetic drugs (Lestari & Susanti 2015).

One type of plants that has been used as an alternative medicine is flamboyant *Delonix regia (Boj. Ex Hook.) Raf*. which flowers contain phytochemical compounds beneficial for health such as the flavonoids, phenolics (Shanmuka *et al*. 2011), carotenoids and antocyanins (Veigas *et al*. 2012). Carotenoids act as a cell-communication controller, growth controller, immune responses provider, neoplastic transformations inducer, and enzyme regulator that act as carcinogen detoxifiers. However, the potential of *D. regia* flowers in stem cell technology has not been investigated. Therefore, the objectives of the study were to determine the potential and optimal dose of *D. regia* flower extracts as an inducer for proliferation and differentiation of mice bone marrow mesenchymal-like stem cells. These findings can be used as preliminary information for using the extract as cellular differentian inducer in basic and applicative reseach using stem cells. Data obtained also can be used as refference to see potential local medicinal plants in stem cell application.

METHODS

Sampling plant

Red *D. regia* flowers were collected from Banda Aceh, Indonesia. The flowers were dried at room temperature for three days, weighed as many as 500 grams and grounded into a rough simplicia.

Flower extraction

Flower extracts were prepared by maceration using ethanol 96% as a solvent. Briefly, simplicia powders (500 grams) were soaked in 1.5 liters of ethanol 96% for three days. Filtrate was obtained by filtering macerate using a filter paper and stored in a bottle container at room temperature. The entire filtrate was concentrated using a rotary vacuum evaporator at 60 °C and 80-90 rpm. Subsequently, the extracts were weighed and stored in a sealed container.

Phytochemical Analysis

The extracts of *D. regia* flowers were phytochemically tested on their secondary metabolites by standard method (Azab and Daim 2013).

Experimental animal

Animals used in this study were male Balb-C mice aged two months old, with an average body weight of 30 g. All mice were kept in cages sized 48 cm x 36 cm x 13 cm containing dry rice husk. Food and drink were given *ad libitum*.

Preparation of modified Dulbecco's modified eagles's medium (mDMEM)

The mDMEM culture medium stock was prepared by diluting 1 gram of mDMEM powder in 100 ml of aquadest in a beaker glass. The solution was added with 0.37 g of $NaHCO_3$, 100 µl of AANE, 100 µl of ITS, 125 µl of gentamycin and 1 ml of NBCS. All ingredients are mixed by stirring. The mixture was then sterilized by filtration using a 0.22 µm pore-sized microfilter and stored in a sterile, sealed bottle.

Preparation of cultures bone marrow mesenchymal stem cells

Petri dishes (35 mm diameters) were coated with gelatin 0.1% for one hour at room temperature. Petri dishes were cleaned from gelatin by rinsing with a modified phosphate buffered saline (mPBS) solution and dried for 5 minutes. Each petri dish was then filled with 2 ml of mDMEM and incubated at 37 °C in a 5% CO_2 incubator for hour.

Isolation and primary culture of bone marrow mesenchymal stem cells

The mice were anesthetized using a combination of 10 mg/KgBB *ketamine* (*Troy Laboratories PTY Limited*) and 1 mg/KgBB xylazine (*Troy Laboratories PTY Limited*). All mice were dissected for femur and tibia collection. Under sterile conditions, the bones were cleaned from muscles, washed in mPBS, and cut on both the ends. The bone marrows were then flushed out from bone cavity with mPBS using the syringe. Bone marrow suspensions were collected in a petri dish before repetitive pipetting and centrifugation at 3000 rpm for 10 minutes. Cell pellets were then washed four times with mPBS and one time with mDMEM. The suspension of bone marrow cells at a concentration of 1 x 10^6 was poured into 12 petri dishes containing 2 ml of mDMEM each. After one-day incubation, the media in all petri dishes were replaced. Cells that were adherent to plastic were then considered as mesenchymal stem cell (MSC) (Dominici *et al*. 2006; Halim *et al*. 2010). This referred to the criteria outlined by the International Society for Cellular Therapy (ISCT), which are (1) adherence to plastic, (2) surface expression of MSC specific marker (CD105, CD73, and CD90) but negative expression of hematopoietic markers (CD19, CD14, CD34, and CD45) as well as HLADR, and (3) ability to differentiate into osteoblasts, adipocytes, and chondroblasts (Dominici *et al*. 2006. Sung *et al*. 2008). On the second day of incubation, the media were replaced with new ones. Cell pellets were harvested from 2 petri dishes and counted. In duplicate, a total of 0.0 mg/ml (control), 0.4 mg/ml, 0.6 mg/ml, 0.8 mg/ml and 1.0 mg/ml of *D. regia* extracts were added to the media as treatments. The cells were then incubated at 37 °C in a 5% CO_2 incubator for nine days with mDMEM replacement every two days.

Level of Proliferation Based Population Doubling Time (PDT)

Cell proliferation rate was determined based on the population doubling time (PDT), the time required by cell population to double number. PDT was calculated using the formula as follow:

PDT = T ln2/ln (Xe/Xb)

with T is the incubation time in any units, Xb is the cell number at the beginning of the incubation time, and Xe is the cell number at the end of the incubation time (Davis 2011).

Identification of cell differentiation

Cell differentiation was determined based on morphological changes recorded from microscopical examination according to the protocol of Djuwita *et al*. (2012). In brief, cultures on the tenth day were fixed in 4% paraformaldehyde for 24 hours and stored in alcohol 50% for two hours. The cells were then incubated in alcohol 50% for three minutes and in distilled water for five minutes. Furthermore, the cells were stained with hematoxylin staining for four minutes, rinsed with distilled water, stained with eosin for 2 minutes, and rinsed again with distilled water. The cells were then processed with increased alcohol gradients (70%, 80%, 90%, 96%, the absolute I and II) for 10 minutes each. After clearing process in *xylol* the cells were mounted onto glass objects and fixed. Cells were then observed using a microscope (Olympus) with a magnification of 10x10 for identifying morphological changes indicating differentiation (shape, extension, and nucleus).

Ethics

This research used the protocols approved by the Research Ethics Committee of the Faculty of Veterinary Medicine Syiah Kuala University.

Experimental design

This research used a completely randomized design (CRD) method consisting of five treatments and four replications each. The treatments were P0 (untreated control) and those given increasing doses of ethanolic extract of *D. regia* flowers namely 0.4 mg/ml (P1), 0.6 mg/ml (P2), 0.8 mg/ml (P3), and 1.0 mg/ml (P4).

Data analysis

The data of the cell differentiation were analyzed descriptively. The data of PDT scores and cell numbers statistically were analyzed using analysis of variance (ANOVA) at the level significancy of 95% continued by Tukey test with 5% confidence interval (Hanafiah 1997).

RESULTS AND DISCUSSION

Bioactive Compounds

Phytochemical analysis indicated that methanolic extract of *D. regia* flowers contained alka-

loids, terpenoids, saponins, and phenolics.

Level of Proliferation

Cell proliferation rate was calculated by comparing the numbers of cells at the initial and final (day 10) stages of incubation (-).

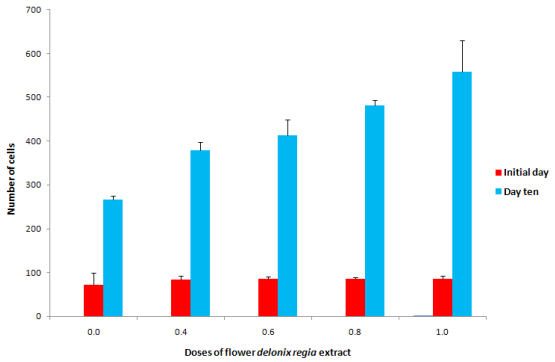

Figure 1. Average cell numbers in the cultur media contain different concentration of *D. regia* extract at the initial and final (day 10) incubation time

Data in Figure 1 indicates that there were differences in the average numbers of mice bone marrow stem cells growth in the mDMEM media supplemented with *D. regia* flower extract ranged from 0.0 – 1.0 mg/ml cells between the initial and final days of incubation. Average cell numbers at the final incubation time were higher than that at the initial incubation time. The highest and lowest cell numbers were found in media contained 1.0 mg/ml and 0.0 mg/mL of *D. regia* flower extract.

The rates of cell proliferation were determined based on the values of Population Doubling Time (PDT) (Table 1).

Table 1. Value of Population Doubling Time (PDT) of mice bone marrow mesenchymal stem cells treated by flamboyant flower extract (*D. regia*)

Treatment (mg/mL)	PDT (Day)
0.0	$0.24^{a} \pm 0.04^{a}$
0.4	$0.23^{a} \pm 0.03^{a}$
0.6	$0.22^{a} \pm 0.02^{a}$
0.8	$0.08^{b} \pm 0.00^{b}$
1.0	$0.06^{b} \pm 0.00^{b}$

Note: The numbers followed by different letters indicate significant differences at 5% level.

Table 1 shows that the administrations of flamboyant flower (*D. regia*) extract affected PDT scores. At the dosage of 0.8 mg/ml and 1.0 mg/ml flamboyant flower extracts had significant effects on PDT compared to the control treatment and to the dose of 0.4 mg/ml and 0.6 mg/ml ($P < 0.05$). Two other treatments (*D. regia* flower extracts of 0.4 mg/mL and 0.6 mg/mL), however, did not show significant effects on PDT versus control. Thus, *D. regia* flower extracts at dosages of 0.8 mg/ml and 1.0 mg/ml were optimum to enhance proliferation.

Cell proliferation aims to double the number of cells to become more numerous (Pellegrini *et al*. 2008). In this study the rate of cell proliferation was determined based on the difference in cell numbers at the initial and end of incubation days as well as based on the Population Doubling Time (PDT). While increased cell numbers showed that the addition of *D. regia* flower extracts ranged from 0.4 – 1.0 mg/ml was able to maintain MSC cells proliferation until the end of incubation (day 10) (Figure 2), data of PDT values (Table 1) suggested that *D. regia* flower extracts might signifiantly increase ($p<0.05$) PDT if they were given in the dosages of 0.8 – 1.0 mg/ml.

Differentiation of Mesenchymal-like Stem Cell

Microscopical examination on the morphological changes of mice-bone marrow mesenchymal stem cells in the culture media indicated that mice mesenchymal stem-like cells (Figure 2A), could differentiate into nerve-like cells (Figure 2B) and unidentified type of cells (Figure 3). The first was observed in the mice mesenchymal stem-like cells grown in media contained 0.6 mg/ml and the latter was found in the cells growth in media supplemented with 0.8 and 1.0 mg/ml of *D. regia* extract.

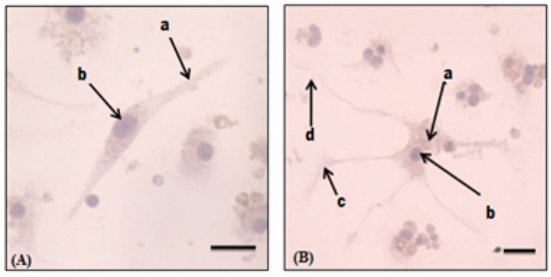

Figure 2. Morphology of mesenchymal-like stem cells (A) and neuronal-like cells (B). Note: a: Cytoplasm; b: Nucleus; c: Dendrite; d: Axon. Bar: 20 µm.

The addition of *D. regia* flower extract into mDMEM growth media not only resulted in different proliferation rate of mice mesecnhymal stem-like cells but also their differentiation potential. If it was given 0.6 mg/ml *D. regia* flower extract stimulated MSC differentiation into nerve-

like cells (Fig. 2B), but if the extract was given in a dose of 0.8 mg/ml and 1.0 mg/ml it induce MSC diffrentation into unidentified cells (Fig. 3).

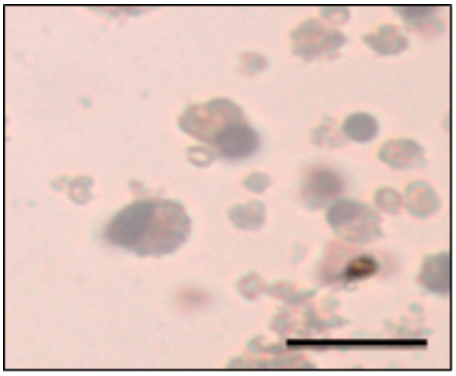

Figure 3. Morphology of unidentified cells in culture medium. Bar: 20 µm.

The potential of *D. regia* flower extract as proliferation inducer was probably related to its bioactive compounds contents. Based on phytochemical analysis, *D. regia* flower extract contained the alkaloid, terpenoid, saponin, and phenolic compounds. The compounds synthesized in plants could act as inducers of cell proliferation *in vitro* (Punturee *et al*. 2005). Some of bioactive compounds act as proliferation inducers are carotenoids and flavonoids (Omayma *et al*. 2013; Polya 2003). Proliferation occurs due to transmission of cellular signaling (Kim *et al*. 1997) as a response to any stimulus in the environment followed by chemical reactions within cells (Forgacs *et al*. 2004). Bioactive compounds might provide environmental stimuli for molecular signaling enhances proliferation reactions such as mitogenesis, cell interaction, and molecules adhesion.

The mesenchymal-like stem cells used for this study characteristically have a great core, oval-shape and purple color with a cytoplasm extension in the opposite directions (Figure 2A). This is consistent with a previous study demonstrating that the mesenchymal-like stem cell morphology is characterized by an elongated shape cell with cytoplasm extension and dark, elliptical nucleus (Goodpaster *et al*. 2008). Morphological observations showed that mesenchymal-like stem cell growth in culture media supplemented with *D. regia* flower extracts might differentiate into nerve-like and unidentified cells (Figure 2B and 3).

The nerve-like cells found in the study showed characteristics of a great core and rough cytoplasm extends in all directions (Figure 2B). This is in agreement with the results of previous study suggested that nerve cells characteristically have a large, centrally located nucleus and cytoplasm extension consisting of dendrites and axons (Eroschenco, 2003). In addition, the rough cytoplasm indicates the presence of endoplasmic reticulum granular of neurons required for neuronal cells differentiation. The image obtained showed developmental stages of nerve-like cells followed by maturation. Development of nerve cells *in vitro* begins with proliferation of round of cells and followed by their differentiation to form axon in the second incubation day and dendrites formation on the following day (Chotimah *et al*. 2014).

The unidentified cells were suspected as osteoblasts-like cell due to the characteristics of rounded cell shapes and the presence of dark colored, marginally located nucleus (Fig 3). These cell types were only present in the mesenchymal stem-like cells cultures treated *D. regia* flower extracts of 0.8 mg/ml and 1.0 mg/ml. Since these cells could not be accurately identified further research are required to confirm potential of the extract to promote osteoblast differentiation from mice mesenchymal-like stem cell.

The numbers of nerve-like cells found in each petri dish ranged from 15 to 20 cells. The process of mesenchymal stem cell differentiation into nerve like cells might be related to the occurrence of phytochemical compounds in the extract that are able to activate transcription factors (Aveline *et al*. 2011). We assumed that bioactive compounds of *D. regia* flower extract were able to induce cells differentiation into specific cell via the same or different pathways that need to identify in further study.

This study found that ethanolic extract of *D. regia* flower contained alkaloids, terpenoids, saponins, and phenolics, bioactive compounds have been mentioned their biological effects beneficial for health and metabolic processes including cell proliferation and differentiation. Aksara *et al.* (2013) reported that the compounds played an important role in triggering the development and death of nerve cells are alkaloids. A previous study (Omar *et al*. 2011) demonstrated that *triterpenoids*, *flavonoids*, and fatty acids in Gotu kola plants (*Centella asiatica*) also play an important role in the development of nerve cells *in vitro*. Additionally, in the Gotu kola plant a dose of 1-50 mg/mL was able to properly initiate nerve cells development (Lu *et al.* 2004). Specific compounds that exactly induce of mesenchymal stem cells differentiation into nerve like cells, however, remained unknown.

Many studies have also showed that mesenchymal stem cells can mediate tissue repair through modulating local environment, affecting

immune responses, sustaining angiogenesis and establishing co-operative effects with the resident cells. The mechanisms primarily depend on the secretion of specific bioactive factors rather than the direct differentiation of the transplanted stem cells in the host tissue, given the low incidence and poor efficiency of their survival and therapeutically relevant level of engraftment (El-Badrawy *et al*. 2016). In this study, *D. regia* flower extracts has demonstrated to be a potential inducer of cell differentiation of mesenchymal-like stem cell into neural-like cells. Further investigations are required to explore potential application of *D. regia* flower extracts as a medium in stem cell therapy in patients with neurodegeneration such as Alzheimer's and Parkinson's.

CONCLUSION

The present study suggested that *D. regia* flower extracts at a dose of 0.6, 0.8 and 1.0 mg/ml were able to stimulate MSC proliferation, but optimum dose for neurogenic differentiation was 0.6 mg/ml. Further investigations, however, are required to determine bioactive compounds are responsible for the effect, their metabolic pathways and level of gene expression during cell proliferation.

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REFERENCES

Aaby, K., Stine, G. & Linda, H. (2013). Extraction of phenolic compounds from bilberry (*Vaccinium myrtillus* L.) press residue: Effects on phenolic composition and cell proliferation. *LWT-Food Sci Technol,* 54, 257-264.

Aksara, R., Musa, W. J. A. & Alia, L. (2013). Identification of alkaloid compounds from methanol extract of mango bark (*Mangifera indica* L). *Entropi*, 3, 514-519.

Aveline, C. P., Bourseguin, I. J. & Rochefort, Y.G. (2011). Mesenchymal stem cells and acquisition of a bone phenotype: An ion channel overview. *Develop. Biol. Tissue. Engineer*, 3(9), 103-18.

Azab, S. S. & Daim, M. A. (2013). Phytochemical, cytotoxic, hepatoprotective, antioxidant, properties of *Delonix regia* leaves extract. *Med. Chem. Res*, 22, 4269-77.

Chotimah, C., Rahayu, M., Ciptadi, G. & Fatchiyah, F. (2014). Optimization of neuron cell maturation and differentiation. *Biotropika*, 2(4), 191-7.

Davis, J. M. (2011). Basic Technique and Media, the Maintenance of Cell Lines and Safety, John MD (Ed). In Animal Cell Culture Essential Methods. John Wiley and Sons Ltd., UK.

Djuwita, I., Pratiwi, I. A., Winarto, A. & Sabri, M. (2012). Proliferation and differentiation of mouse bone cells in *vitro culture* medium containing trunk extract *Cissus quadrangular* Salisb (Sipatah-patah). *Ked. Hewan*, 6(2), 75-80.

Dominici, M., Le Blanc, K., Mueller, I., Slaper-Cortenbach, I., Marini, F. C., Krause, D. S., Deans, R. J., Keating, A., Prockop, D. J. & Horwitz, E. M. (2006). Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*, 8(4), 315-7.

El-Badrawy, M. K., Shalabi, N. M., Mohamed, M. A., Ragab, A. & Abdelwahab, H. W. (2016). Stem cells and lung regeneration. *Int. J. Stem. Cells*, 9, 31-5.

Eroschenco, V. P. (2003). Di Fiore's Atlas of Histology with Functional Correlations, Lippincott Williams and Wilkins Inc., USA.

Forgacs, G., Yook, S. H., Janmey, P. A., Jeong, H. & Burd, C. G. (2004). Role of cytoskeleton in signaling network. *Cell. Sci,* 117, 2769-2775.

Goodpaster, T., Legesse-Miller, A., Hameed, R. M., Seena, C., Aisner, C. S., Randolph-Habecker, J. & Coller, A. H. (2008). An immunohistochemical method for identifying fibroblasts in formalin-fixed, paraffin-embedded tissue. *Histochem. Cytochem*, 56(4), 347-58.

Halim, D., Murti, H., Sandra, F., Boediono, A., Djuwatono, T., Boenjamin, S. (2010). Stem Cell: Basic Theory and Clinical Application. Erlangga, Jakarta.

Hanafiah, K. A. (1997). The Design of Theory and Application Trials, PT Raja Grafindo Persada, Jakarta.

Kang, X. Q., Zang, W. J., Song, T. S., Xu, X. L., Yu, X. J., Li, D. L., Meng, K. W., Wu, S. L. & Zhao, Z .Y. (2005). Rat bone marrow mesenchymal stem cells differentiate into hepatocytes *in vitro*. *World. J Gastroenterol*. 11(22), 3479-84.

Kim, S. J., Lim, M. H, Chun, I. K. & Won, Y. H. (1997). Effects of flavonoids of *Ginkgo biloba* on proliferation of human skin fibroblast. *Skin. Pharmacol,* 10, 200-205.

Lestari, D.R. & Susanti, R. 2015. Effectivity of pedada fruit (*Sonneratia caseolaris*) extract to the level of SGOT and SGPT in rat treated by paracetamol induction. Biosaintifika 7 (1), 31-38.

Lu, L., Ying, K., Wei, S., Fang, Y., Liu, Y., Lin, H., Ma, L. & Mao, Y. (2004). Asiaticoside induction for cell-cycle progression, proliferation and collagen synthesis in human dermal fibroblasts. *Int. J. Dermatol*, 43, 801-807.

Omar, S. N., Zakaria, C. A. Z., Mian, S. T., Ngah, W.

Z. W. & Mazlan, M. (2011). *Centella asiatica* modulates neuron cell survival by altering caspase-9 pathway. *Med. Plants. Res*, 5(11), 2201-2209.

Omayma, A., Eldashan, Nasser, A. & Singab, B. (2013). Carotenoids. *Pharmacog. Phytochem,* 2(1), 225-234.

Pellegrini, M. P., Pinto, R. C. V. & Castilho, L. D. R. (2008). Animal Cell Technology: From Biopharmaceuticals to Gene Therapy. Tylor and Francis, New York.

Polya, G. M. (2003). Biochemical Targets of Plant Bioactive Compounds: A Pharmacological References Guide to Site of Action and Biological Effects, Tylor and Francis, New York.

Punturee, K., Kasinrerk, W., Wild, P. C. & Vinitket, K. U. (2005). Immunomodulatory effects of Thai medicinal plants on the mitogen-stimulated proliferation of human peripheral blood mononuclear cells *in vitro*. *Chiang. Mai. Med. Bull*, 44(1), 1-12.

Shanmuka, I., Patel, H., Patel, J. & Riyazunnisa. (2011). Quantification of total phenol and flavonoid content of *Delonix regia* flowers. *Int. J. Chem. Tech. Res*, 3(1), 280-300.

Sung, J. H., Yang, H. M., Park, J. B., Choi, G. S., Joh, J. W., Kwon, C. H., Chun, J. M., Lee, S. K. & Kim, S. J. (2008). Isolation and characterization of mouse mesenchymal stem cells. *Transplant.* Proc, 40(8), 2 link: https://www.ncbi.nlm.nih.gov/pubmed/18929828

Vandebroek, I., Calewaert, J., Dejonckheere, S., Sanca, S., Semo, L., Damme, R. V., Puyvelde, L. V. & Kimpe, N. D. (2014). Use of medicinal plants and pharmaceuticals by indigenous communities in the Bolivian Andes and Amazon. *Bull. World. Health. Organ*, 82, 243-250.

Veigas, J. M., Narayan, M. S. & Kotamballi, N. (2007). Antioxidative efficacies of floral petal extracts of *Delonix regia* Rafin. *Int. J. Biomed. Pharm. Sci*, 1(1), 73-82.

Wobus, A. M. & Boheler, K. R. (2005). Embryonic stem cells: Prospects for developmental biology and cell therapy. *Physiol. Rev*, 85, 635–678.