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# THE TOXICITY OF METHANOL EXTRACT OF MAULI BANANA STEM (*Musa acuminata*) AGAINST BONE MARROW MESENCHYMAL STEM CELL IN VITRO

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***ABSTRACT***

*The latest regeneration therapy has developed towards the usage of Mesenchymal stem cells (MSC) including for aggressive periodontitis, but limited amounts of MSC need extra growth factor in cells culture process. Growth factor is quite expensive so an alternative source is needed. Mauli banana stem is a proven antioxidant and has the most bioactive tannin. Methanol extract of mauli banana stem is not toxic towards fibroblast cell BHK 21 with 25% concentrate, but there is no research about the toxicity of methanol extract of mauli banana stem against MSC.* ***Purpose:*** *To analyze the toxicity of methanol extract of mauli banana stem against MSC in vitro.* ***Method and source:*** *True experimental using the posttest only control group design. MSC culture with treatment methanol extract of mauli banana stem with dosage 2,5 mg/ml; 5 mg/ml; 7,5 mg/ml; 10 mg/ml. Treatment Con A5 µg/ml is used for positive control group and is not treated as negative control group. Each group is incubated for 24 hours and 48 hours, then it is given reagent MTT and is read with ELISA reader.* ***Result:*** *Kruskal-Wallis and independent-sample T-test result shows that there is a significant difference between treatment group and control group.* ***Conclusion****: Methanol extract of mauli banana stem with dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml is toxic towards MSC in vitro under treatment for 24 hours and 48 hours.*

**Key words** : *Mauli banana stem, Mesenchymal stem cells, Toxicity*

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## INTRODUCTION

Aggressive periodontitis is a destructive periodontal disease and grown rapidly in younger patients, who are being treated using bone graft. Bone graft can only regenerate part of periodontal tissues so the latest therapy is being developed to the usage of stem cells.[1,2] Mesenchymal stem cells (MSC) can be differentiated into cementum and periodontal ligament, so MSC can be applied to aggressive periodontitis therapy. [3,4] The amounts of MSC are very limited, there is only 1 MSC in every 10.000nucleated cells.[5] As people are getting older, the amounts of MSC are also decreased, therefore before it is applied to MSC patients, it needs to be checked using in vitro culture first.[6] Growth factor is needed as one of culture cells media supplement to reach the correct amounts of cells, but growth factor is used as a supplement is quite expensive so alternative source, with affordable price, is needed. Phytochemical exogenous elements in herbal plants extract can be an affordable alternative source to increase the amounts of MSC, one of them is using mauli banana stem.

Methanol extract of mauli banana stem is proven to have antibacterial against Streptococcus mutants, antifungal against Cadidaalbicans, antioxidant, and can fasten the recovery time

mucous incision wound in buccal oral cavity clinically.[7,8,9,10] Methanol extract of mauli banana stem has 67.5% bioactive tannins compounds, which are anti-oxidant, cardio protective, anti-tumor, anti-fungi, anti-plasmin, anti- inflammation, apoptosis and immunomodulatory.[10,11,12]

Methanol extract of mauli banana stem is not toxic towards the fibroblast cell BHK21 at 25% concentrate, however there is no research in the toxicity of mauli banana stem extract towards MSC.[13] The toxicity is an extraction between a substance which generate a toxic and cell, so that the used of different cell may cause a different result in the research.[14] Fibroblast cell is a mature cell, this cell is simply bred on the cell culture and has a regeneration capability against injury, while MSC is an immature cell which yet has no form and a specific function like a mature cell.[15,16] The purpose of this research is for analyzing methanol extract of mauli banana stem toxicity towards MSC in vitro. This research is expected to be used as a dose reference for the development of herbal medicine of methanol extract of mauli banana stem which is used as an alternative way to accelerate the escalation of MSC amount in vitro, so that, in the future, it can be applied as a therapy for damaged alveolar bone on periodontitis aggressive patient.

## METHOD AND SOURCE

This study is an experimental research laboratory with post test only control group design. The research material used in this study is the banana stems mauli, 70% methanol, α-MEM, fetal calf serum (FCS), trypsin 0.25%, phosphate buffered saline (PBS), penicillin 100 IU, fungizone, petridish, disposable tubes, alcohol 70%, distilled water, disposable filter, yellow tip 0-20µl, blue tip 1000µl, dimethyl sulfoxide (DMSO), EDTA, ficoll isopaque gradient 0,177g / ml, culture mesenchymal stem cells, α-MEM, versene trypsine, Fetal Bovine serum (FBS) 10%, Phosphate Buffer Saline, MTT, Dimethyl sulfoxide (DMSO), concanavalin A (Con A). The tools used in this study is a knife, oven, blender, scales, measuring cups, water bath, sifter, 2000 ml beaker glass, Buchner funnel, filter paper, tube, Proline 20-20 mL micropipette, 200µl conical tube, a small glass tube, CO2 incubator, CO2 tank, CO2 regulators, laminar flow BSL II, sentrifuse refrigerator adjustable, automatic pipette, glass pipettes, pipette 0-200 mL eppendorf, inverted microscope, refrigerator 4°C, frezeer -20°C, 96-well microplate Falcon 3072, Roux bottles NUN C, biological safety cabinet, ELISA reader, vari shaker.

Mauli banana stem used as the sample was originated from SMK-PP Banjarbaru. Mauli banana stem was taken 10 cm from the root nodules, washed, cut, and then put into an oven with 60$^{o}$C temperature. Dried mauli banana stem was mashed with a blender and sifted with a mesh 25 sized. Sifted mauli banana stem was marinated in 70% methanol on 1 cm above sample surface for 3x24 hours, stirred for a while. It was strained every day and the result was steamed with vacuum rotary evaporator under 40-50$^{o}$C temperature, therefore the result was thick extract. Then it was tested with methanol-free test using spectra gas chromatography.

Thick mauli banana stem was marinated in α-MEM solution and kept for 24 hours, then it was centrifuged and strained into supernatant. Supernatant was given α-MEM solution to become mauli banana stem methanol extract with dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml.

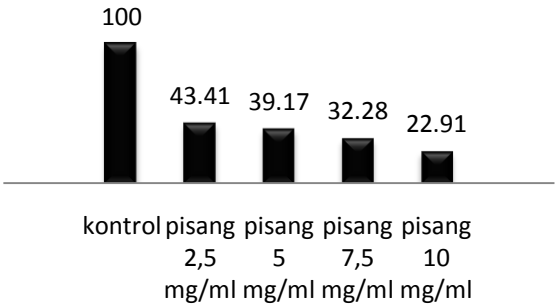
80% confluent mesenchym stem cell was distributed in microplate 96 wheel, which was divided into 7 treatment groups. Group 1-4 were MSC with mauli banana stem extract dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml. group 5 was given Con A 5µg/ml (positive control). Group 6 was not given any treatment (negative control). Finally group 7 was media group which was replicated 10 times from each group. Microplate was incubated for 24 and 48 hours. After 24 and 48 hours, the cell was washed twice with PBS, then given MTT reagent to each wheel as much 25 µln and incubated for 4 hours under 37$^{o}$C temperature. After 4 hours, the medium in mircroplate was disposed and replaced with DMSO 200 µL/wheel. Then it was read with ELISA reader under 595 nm wavelength. The data was analyzed with viability cell formula:[17]

$$\text{Viability cell (\%)} = \frac{\text{OD treatment - OD media control}}{\text{OD cell control - OD media control}} \times 100\ \%$$

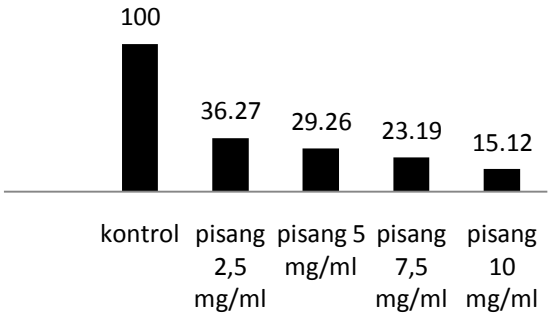
## RESULT

Methanol extract of mauli banana stem test research uses MTT aessay method whichs read by using ELISA reader. ELISA reader works by reading the optical density (OD) through color changes produced from mitochondrial activity becoming blue formazan. The color thickness shows high level of OD in ELISA reader.

Based on the OD level, it can be seen the cell viability percentage. Cell can be assumed non-toxic if the cell viability is over 60%. Based on the OD level reading, methanol extract of mauli banana stem test towards MSC in vitro generates results as follows:




Picture 1 : The mean of MSC viability after getting methanol extract of mauli banana stem treatment for 24 hours




Picture 2 : The mean of MSC viability after getting methanol extract of mauli banana stem treatment for 48 hours

Picture 1 and picture 2 show methanol extract of mauli banana stem dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml with 24 and 48 hours treatment has the mean of MSC viability less than 60%, which implies methanol extract of mauli banana stem is toxic towards MSC in vitro. Kruskall wallis test and independent-sampel T-test result show significant difference between treatment group and control group, where the mean of MSC cell viability decreases as the increased dosage given.

## DISCUSSION

The research result shows methanol extract of mauli banana stem is toxic towards MSC in vitro with dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml with 24 and 48 hours treatment. The research result is in contrast to other research which shows methanol extract of mauli banana stem with 25% concentrate does not cause toxicity towards fibroblast cell. The different cell used might cause the different research result because toxicity is an interaction between toxic substances and cell. This research used stem cell which did not have shape and function alike fibroblast cell. Scientific evident shows that MSC population in mature tissue looks like active cell population, which the function could be seen under specific time and condition. Differentiation potency and MSC proliferation are also limited. This is in contrast to fibroblast cell, which is mature cell that can be easily breed in cell culture and also has the ability to thrive against harm. Methanol extract of mauli banana stem dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml toxicity effect towards MSC in vitro with treatment for 24 and 48 hours might be caused by phytochemical substance activity in it, which is tannins. Tannins in high dosage can cause toxicity in cell.[18] Tannins can also be anti-oxidant and act as prooxidant.[19,20] Excessive anti-oxidant condition can cause anti-oxidant to be considered oxidant, so it can change into prooxidant.[21] Oxidant classified as free radicals can increase reactive oxygen species (ROS).[22] Increased ROS in high concentrate or excessive amounts can cause oxidative damage to fat, protein, and DNA, trigger oncogenic transformation, increase metabolic activity and mitochondrial dysfunction.[23] Increased ROS activity can activate p53 through DNA damage from the exposure from tannins.[19,24] Tannins can increase Erk so that it forces p53 to be apoptosis.[25] When DNA is damage, there will be p53 tumor suppressor gene accumulation, this condition will stop cell cycle (in phase G) to make repairment. If the DNA repairment is not happen, p53 will trigger apoptosis from the transcription increasing of some members of pro-apoptosis Bcl family, especially Bax.[26] Banana stem has proanthocyanidin tannins (condensable tannins).[27] Proanthocyanidin can induce phosphorylation tyrosine in tyrosine kinase insulin receptor on the MSC surface, so it activates MAPK/Erk pathways and PI3K/Akt/mTOR pathways which can regulate apoptosis.[25,28,29,30] Based on the study above, it can be concluded that methanol extract of mauli banana stem with dosage 2.5 mg/ml; 5 mg/ml; 7.5 mg/ml; 10 mg/ml is toxic towards MSC in vitro with under treatment for 24 hours and 48 hours.

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