PERIÓDICO TCHÊ QUÍMICA
ARTIGO ORIGINAL

AVALIAÇÃO CITOTÓXICA BASEADA EM MTT DE SEIVA DO TRONCO DE BANANA AMBONESE (*Musa paradisiaca* var*. sapientum* (L.) Kuntze) EM FIBROBLASTOS

MTT-BASED CYTOTOXIC EVALUATION OF AMBONESE BANANA STEM SAP (*Musa paradisiaca* var*. sapientum* (L.) Kuntze) ON FIBROBLAST CELLS

UJI SITOTOKSISITAS GETAH BATANG PISANG AMBON PADA KULTUR SEL FIBROBLAS BERBASIS MTT

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RESUMO

Os remédios fitoterápicos tradicionais são substâncias derivadas de plantas de ocorrência natural, sem processamento químico ou limitado, e têm sido usados nas tradições locais ou nacionais de cura para tratar doenças. Nos debates globais sobre saúde, os medicamentos fitoterápicos tradicionais estão recebendo considerável atenção. Muitos esperam que novas pesquisas em fitoterapia tenham um papel vital na saúde global. Países como China, Índia, Nigéria, EUA e também a Organização Mundial da Saúde (OMS) fizeram grandes investimentos em medicamentos fitoterápicos antigos. Atualmente, o uso de plantas da Indonésia melhorou dramaticamente o campo da medicina e odontologia. Os cuidados dentários e bucais muitas vezes estão relacionados a feridas e o caule de banana ambonense tem se mostrado um tratamento eficaz para essas lesões. Este estudo teve como objetivo avaliar a eficácia e segurança da seiva do caule da banana ambonesa por meio de testes de citotoxicidade em cultura de fibroblastos de Baby Hamster Kidney-21 (BHK-21).Este estudo foi realizado em três culturas de fibroblastos BHK-21, a saber, o meio e o controle celular, e a seiva do caule da banana Ambonese com uma concentração de 10%, 20%, 30%, 40%, 50%, 60%, 70 %, 80%, 90% e 100% incubados por 24 horas a 37ºC e 5% CO_2. Em seguida, o MTT foi disperso uniformemente no meio a fim de obter o valor de densidade óptica preciso. Todos os dados quantitativos foram analisados estatisticamente por meio de ANOVA de uma via e do Teste HSD de Tukey. O resultado mostrou diferenças significativas nos valores de densidade óptica entre os grupos com p = 0,000 (p <0,05). Não houve diferença significativa entre o controle celular e o grupo de seiva do caule de banana ambonense com concentrações de 90%, 80%, 70%, 60%, 50%, 30%, 20% e 10%. Observou-se também que a seiva do caule da banana ambonesa não é tóxica para os fibroblastos, pois seu valor de viabilidade foi superior a 60%.

Palavras-chave: *fitoterapia, biocompatibilidade, BHK-21, cultura de células de fibroblastos, densidade óptica*

ABSTRACT

Traditional herbal remedies are naturally occurring, plant-derived substances with limited to no chemical processing and have been used in local or national healing traditions to treat illness. In global health debates, traditional herbal medicines are gaining considerable attention. Many hope new research into herbal medicine will play a vital role in global health. Countries like China, India, Nigeria, USA, and also the World Health Organization (WHO) made large investments in ancient herbal medicines. Currently, the use of the Indonesian plant has dramatically improved the medical and dentistry field. The dental and oral care is often related to wounds, and the Ambonese banana stem has been proven as an effective treatment for these injuries. This study aimed to evaluate the effectiveness and safety of the Ambonese banana stem sap through cytotoxicity tests on the fibroblast cell culture of *Baby Hamster Kidney-21* (BHK-21). This study was carried out on three BHK-21 fibroblast cell culture, namely, the media and cell control, and the Ambonese banana stem sap with a concentration of 10%, 20%, 30%, 40%, 50%, 60 %, 70%, 80%, 90%, and 100% incubated for 24 hours at 37ºC and 5% CO_2. Then, MTT was evenly dispersed on the media to obtain accurate optical density value. All quantitative data were statistically analyzed using one-way ANOVA and Tukey's HSD Test. The result showed significant differences in optical density values between groups with p = 0.000 (p <0.05). There was no significant difference between the cell

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control and the Ambonese banana stem sap group with concentrations of 90%, 80%, 70%, 60%, 50%, 30%, 20%, and 10%. It was also observed that the Ambonese banana stem sap is nontoxic to fibroblast cells since its viability value was more than 60%.

Keywords: *herbal medicine, biocompatibility, BHK-21, fibroblast cell culture, optical density*

ABSTRAK

Pengobatan tradisional menggunakan herbal merupakan pengobatan alami, zat yang terkandung dari tumbuhan dengan tanpa pemrosesan kimiawi telah digunakan dalam tradisi penyembuhan secara lokal atau nasional untuk mengobati suatu penyakit. Dalam perdebatan kesehatan global, obat-obatan herbal tradisional mendapatkan perhatian yang cukup besar. Banyak yang berharap penelitian baru tentang herbal akan memainkan peran penting dalam kesehatan global. Cina, India, Nigeria, AS, dan WHO semuanya melakukan investasi besar dalam obat-obatan herbal tradisional. Saat ini pemanfaatan tumbuhan Indonesia telah meningkat pesat dalam bidang kedokteran dan kedokteran gigi. Perawatan gigi dan mulut sering dikaitkan dengan luka, bahkan batang pisang ambon terbukti efektif mengobati luka tersebut. Penelitian ini bertujuan untuk membuktikan efektivitas dan keamanan getah batang pisang ambon melalui uji sitotoksisitas pada kultur sel fibroblas *Baby Hamster Kidney*-21 (BHK-21). Penelitian ini dilakukan pada tiga kultur sel fibroblas BHK-21 yaitu media dan kontrol sel, serta getah batang pisang ambon dengan konsentrasi 10%, 20%, 30%, 40%, 50%, 60%, 70 %, 80%, 90%, dan 100% diinkubasi selama 24 jam pada suhu 37ºC dan 5% CO_2. Kemudian MTT diberikan secara merata pada media untuk mendapatkan nilai optical density yang akurat. Semua data kuantitatif dianalisis secara statistik menggunakan ANOVA satu arah dan Uji HSD Tukey. Hasil penelitian menunjukkan bahwa terdapat perbedaan yang signifikan nilai densitas optik antar kelompok dengan p = 0,000 (p <0,05). Tidak ada perbedaan yang nyata antara kontrol sel dan kelompok getah batang pisang ambon dengan konsentrasi 90%, 80%, 70%, 60%, 50%, 30%, 20% dan 10%. Getah batang pisang ambon tidak toksik bagi sel fibroblas, karena nilai viabilitasnya lebih dari 60%.

Kata kunci: *tanaman obat, biokompatibilitas, BHK-21, kultur sel fibroblast, densitas optik*

1. INTRODUCTION:

Traditional herbal medicine has yielded a vast archive of treatments against many health conditions of complex chemical structures and bioactivities throughout history. A typical herbal medicine issue is restricting information about their pharmacological activities and their active constituents. Countries like China, India, Nigeria, the USA, and the World Health Organization (WHO) made large investments in ancient herbal medicines (Tilburt and Kaptchuk, 2008). Using herbal medicine has historically been based on scientific diagnosis and passed. Plant materials have been consistently used in the health sector for preventive, curative, and rehabilitative purposes (Sofowora *et al.,* 2013). Therefore, the use of medicinal plants for treatments has dramatically improved both the medicine and dentistry field (Ekor, 2014; Martínez *et al.,* 2017).

Dental disease is also regarded as a chronic public health problem and a significant drain on health care systems around the world (Hollist, 2004). In people with diabetes, periodontal disease impairs glycemic regulation, and poorly regulated diabetes may worsen periodontal disease (Preshaw *et al.,* 2012). Acacia catechu, Aloe vera, Azadirachta indica, Glycyrrhiza glabra, Cinnamomum zeylanicum, Allium sativum, Propolis, Mikania laevigata, Mikania glomerate, Droserapeltata, Helichrysumitalicum, Coptidis rhizome, Piper cubeba, Azadirachta indica, Syzygium Aromaticum, and Tea tree oil are the frequently tested herbs used for the treatment of periodontitis. Many other herbal products are also undergoing clinical trials in addition to the aforementioned herbal remedies (Shama *et al*., 2014).

Moreover, plant utilization for treatments needs more in-depth exploration, especially on Indonesian vegetative resources. Indonesia is one of ten member states of the Association of Southeast Asian Nations (ASEAN)'s economically and politically diverse national organization. Southeast Asia contains four of the world's 25 biodiversity hotspots, three of the 17 regional megadiverse countries (Indonesia, Malaysia, and the Philippines), and the world's most abundant coral reefs. Biodiversity, e.g., importance in traditional medicine and Indonesian society agriculture, is deep-rooted. In addition to environmental policies, modern biodiversity pathways provide new applications in technology, pharmacy, and the economy (Von Rintelen *et al.,* 2017). Correct identification of source plant species and selecting appropriate parts for use in herbal medicines are necessary and essential

steps for ensuring herbal medicines' safety, quality, and efficacy. Hence, herbal medicines' safety and quality at every stage of the production process have become a significant concern to health authorities, health care providers, the herbal industries, and the public (Kunle *et al*., 2012). Currently, dentistry utilizes natural compounds as clinical and laboratory materials (Palombo, 2011; Kumar *et al.,* 2013). The bone grafting procedure is a technique often used to repair either bone defects or ridge augmentation. However, the failure of the process has been reported with different results. Graft materials used should have an osteoconductive ability that can potentially stimulate the growth of new bone. The development of new material is necessary to promote or accelerate bone growth activity. *Aloe vera* and *Musa paradisiaca* are natural materials known as a biogenic stimulator and hormonal activity modulator during wound healing (Kresnoadi *et al*., 2017; Kapadia *et al.*, 2015). Furthermore, dental and oral health care is often related to injuries that cause infection when not appropriately handled. Therefore, drugs are needed to prevent this occurrence.

One of the natural ingredients known to be efficacious in healing wounds is banana stem sap (*Musa paradisiaca*). Its extract is characterized by increased hydroxyproline levels, hexuronic acid, hexosamine, superoxide dismutase, and decreased glutathione in granulation tissue. Also, lipid peroxidation has been proven to be useful for ulcer treatment (Agarwal *et al.,* 2009). Ambonese banana stem sap has also been used to accelerate wound healing (Budi *et al*., 2009), due to the presence of saponins (antibiotics), anthraquinones (painkiller), and the lectin content for stimulating skin cells growth (Priosoeryanto *et al*., 2007).

Banana sap advantages include providing aesthetic effects by improving damaged skin structure, accelerating the re-epithelialization of epidermal tissue, forming new blood vessels (neocapilerization), and generating connective tissue infiltrating of inflammatory cells in the wound area (Budi *et al.,* 2017). Banana stem sap is known to be a wound medicine (Prasetyo, 2007) since it accelerates the healing process and increases connective tissue growth (Amutha and Selvakumari, 2016).

However, treatment using natural ingredients should be scientifically justified, both in terms of benefits and safety (Peacock *et al*., 2019). Drug safety test is carried out using cell culture, while BHK-21 cells from kidney fibroblasts are more widely used in testing the cytotoxicity of materials and drugs (Freshney, 2000; Stefanowicz and Ochocka, 2020). Fibroblast cells are the most essential and largest component of the pulp, periodontal ligament, and gingiva used in a culture system with various advantages such as the ability to control the environmental (pH, temperature, osmotic pressure, O2, and CO2), and physiological conditions (Huang *et al*., 2009; Mitry and Hughes, 2012; Han *et al.*, 2014).

The MTT *(3- (4-5-dymethylthiazol-2-yl) - 2,5-diphenyl tetrazolium bromide),* is used in microplate analysis for measuring cell proliferation and cytotoxicity. This test is based on cells' ability to reduce yellow and soluble MTT salts to blue-purple and insoluble formazan (Mosmann, 1983). Furthermore, tetrazolium salts reduction occurs intracellularly involving the succinic enzyme dehydrogenase from the mitochondria and endoplasmic reticulum (Berridge and Tan, 1993; Marshall *et al.*, 1995; Berridge *et al*., 1996).

Therefore, this study aimed to evaluate the effectiveness and safety of the Ambonese banana stem sap through cytotoxicity tests on the fibroblast cell culture of *Baby Hamster Kidney-21* (BHK-21).

2. MATERIALS AND METHODS:

2.1. Sample preparation

This study was carried out at the Farma Veterinaria Center in Surabaya, Indonesia, using the BHK-21 Clone-13 (BHK-21/C13) fibroblast cell culture (ECACC-85011433, Sigma), while the number of samples was obtained using a sample size of the Lemeshow's formulation (1990) :

$$n = 2\sigma^2 \text{ x } \frac{(Z(1-\alpha) + Z(1-\beta)^2}{\mu_0 - \mu_1}$$

Description :

n: sample size
σ: standard deviation
Z: value of Z table
α: significance level of 95%
β: power of test (80-90%)

2.2. Preparation of Ambonese banana (*Musa paradisiaca var. sapientum (L.) Kuntze*) stem sap concentration

Banana plants were obtained from the Purwodadi Botanical Garden Plant Conservation Center and the Indonesian Institute of Sciences (LIPI). The features of the banana plants used were age 12-13 months, the height of 2.5-3 m,

stem diameter of 17.3-18.9 cm, and were obtained shortly after fruiting. Ambonese banana sap was extracted by cutting the lower end of the stem and washed to remove dirt. The determination was made to prove that the banana tree species were Ambonese banana (Figure 1). Stems cut and made into small pieces of 200 grams were added into 200 mL of sterile distilled water and blended until it forms a smooth substance. The mixture was filtered using a Buchner funnel connected with a vacuum pump (Gast, USA) and placed into Whatman filter paper number 1. The resulting 375 mL filtrate was stored in a dark bottle, closed to reduce oxidation, and dried with a freeze dryer.

For the standard solution preparation, 1000 mg of Ambonese banana stem extract was dissolved with 1 L of sterile distilled water. The 100% concentration was prepared by taking 1 mL of the standard solution and adding sterile distilled water to 10 mL. Meanwhile, 90% concentration was obtained by taking 0.9 mL of standard solution, then adding sterile distilled water to 10 mL. The same preparation methods to obtained 80%, 70%, 60%, 50%, 40%, 30%, 20% and 10% concentration.

2.3. Preparation of BHK-21 fibroblast cell culture

Seed cell culture frozen in sterile distilled water at 37ºC was thawed and centrifuged at 500 RPM for 5 mins. The cells were suspended into 36 mL Eagles media and 4 mL fetal bovine serum; therefore, the resulting 40 mL suspension was deposited in a sterile Roux bottle and incubated at 37ºC and 5% CO^2 until a monolayer cell was formed (± two days, seen under a microscope). The large Roux bottle containing the BHK-21 cells was then discarded and washed with PBS 15 mL for 3-5 times, and filled with 1 mL of versene trypsin. The result showed that the cells were clustered and homogenized with Eagles media. The homogeneous cells were placed in a microplate of the density of 2×10^5 cells/mL, then incubated for 24 hours at 37ºC and 5% CO_2 (Freshney, 2000).

2.4. Cytotoxicity test of Ambonese banana stem sap through MTT method

The microplate containing fibroblasts was observed under a light microscope to ensure the cells confluently in 96 wells plate, divided into 12 groups. A total of 10 of 12 groups were treatment groups containing Ambonese banana stem sap with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%. Two of 12 were control groups as control media and control cells. Therefore, 25 µL media was deposited in each dish and incubated for 24 hours at 37ºC and 5% CO^2; after this, the media was replaced with 10 µL MTT (M2003, Sigma), covered with aluminum foil paper, and incubated again for 4 hours. Lastly, each dish was deposited with 50 µL DMSO, shook vigorously, and inserted into the Elisa Reader at a wavelength of 620 nm, and then the absorbance level was measured (Freshney, 2000).

3. RESULTS AND DISCUSSION:

The cytotoxicity test results of Ambonese banana stem sap deposited in a BHK-21 fibroblast cell culture showed differences in the formazan formed in each group. Purplish crystal formation was observed in the reaction of MTT with succinic dehydrogenase enzyme found in mitochondria (Figure 2). Formazan formed in each dish was observed using Elisa Reader at a wavelength of 650 nm.

There was a significant difference in optical density values between groups with $p = 0.000$ ($p < 0.05$). The results of the one-way ANOVA and HSD test showed that there were significant differences between the media and the cell control group. The optical density value showed a significant difference in the cell control group with $p = 0.044$ and $p = 0.034$ ($p < 0.05$). However, there was no significant difference between the cell control groups with the concentration of 90%, 80%, 70%, 60%, 50%, 30%, 20% and 10% (Table 1).

Ambonese banana stem sap is widely used for accelerating wound healing. However, drug safety level needs to be considered before administration, which is carried out on BHK-21 fibroblast cell culture before using experimental animals and humans (NRC, 2004; Greaves, 2011). According to Freshney (2000), safety testing is conducted using the cell culture system. The basic principle for growing cells is to design in vitro culture system similar to their initial medium. The cells to be investigated are removed from their original tissue and placed in the in vitro culture system to obtain adequate growth and nutrition at 37ºC, pH 7.4-7.7, and a gas environment of 95% CO_2 / 95% air.

This research used BHK-21 cell culture from hamster kidney fibroblasts since it was widely used to test the cytotoxicity of materials and drugs in dentistry (Basuony *et al*., 2018). The cytotoxicity testing method adopted the MTT assay (3- (4-5-dymethylthiazol-2-yl) -2,5-diphenyl tetrazolium bromide), for measuring cell proliferation and cytotoxicity. This was based on the living cells'

ability to reduce yellow and soluble MTT salts to blue-purple and insoluble formazene. In contrast, the tetrazolium salts reduction occurred intracellularly by involving a small amount of succinic dehydrogenase enzyme from mitochondria and endoplasmic reticulum enzyme. The MTT test was used due to its accurate measurement, sensitivity, ability to detects changes in cell metabolism, equipment availability, ability to save time and energy, and radioisotope disuse (Riss *et al*., 2004). Spectrophotometric tool was used to test for absorbance level. Therefore, the more concentrated the color produced, the higher the absorbance value, and the more the number of cells (Mohler *et al.,* 1996; Santos-Ballardo *et al*., 2015).

Previous studies had shown that the content of Ambonese banana stem sap, such as lectins, saponins, flavonoids, saponins, and tannins, played roles in the wound healing process (Atun et al., 2010; Amutha and Selvakumari, 2016; Budi *et al*., 2017). Meanwhile, saponins and lectins caused hemostatic and antibacterial effects. Saponins and lectins in banana stem sap modulated the immune response by increasing T cell lymphocytes with CD3 + CD4 + CD8 and the hematopoietic system to minimize infection (Swanson, 2010). Therefore, the T cells activated the B cell lymphocytes, NK cells, and macrophages in the presence of antigens (Alberts *et al*., 2002; Uzhachenko and Shanker, 2019). The tannin content acted as the hemostatic (Song *et al.,* 2019) and antibacterial through the mechanism of protein precipitation in blood cells and bacteria, which resulted in coagulation (Budi and Astuti, 2019). Furthermore, tannins and anthraquinones also acted as donors of free radicals and Reactive Oxygen Species (ROS). Free radicals triggered lipid peroxidation and malondialdehyde (MDA) compound, which caused damages to protein and DNA cells (Kaimal *et al*., 2010; Benmehdi *et al.,* 2017). The flavonoid contents such as leucocyanidin and anthocyanin acted as anti-inflammatory enzymes by inhibiting cyclooxygenase enzyme and prostaglandin synthesis (Sumathy and Vijayakumar, 2015; Pandey *et al.,* 2016).

Hydroxyproline is an amino acid which acts to improve the stability of collagen. One aspect of the wound healing process Is the measurement of rates of hydroxyproline. The higher the hydroxyproline level, it means an improvement in collagen synthesis, which is closely linked to the acceleration of the wound healing process (Shoulders and Raines, 2009, Agarwal *et al.,* 2009). The glycosaminoglycans are known to stabilize the collagen fibers and likely regulate their ultimate orientation and characteristic size by improving electrostatic and ionic interactions with it. Concentrations of hexuronic acid and hexosamine, which are glycosaminoglycans' components, have risen dramatically in wound healing (Shetty *et al*., 2008). Superoxide is dismutated by superoxide dismutase (SOD) into hydrogen peroxide (H_2O_2) and an oxygen atom, thus preventing extremely deleterious ROS, such as peroxynitrite (ONOO−) or hydroxyl radicals (*OH). Low H_2O_2 levels can act as a signaling molecule that modulates specific signaling pathways that control blood coagulation, thrombosis, replication, proliferation, fibrosis, angiogenesis, and so on, in the process of hemostasis, proliferation, maturation and remodeling (Kurahashi and Fujii, 2015).

Cytotoxicity testing showed that the Ambonese banana stem sap was not toxic to fibroblast cells; the most connective tissue coir in a cell body. The use of 10%-100% concentration of the Ambonese banana stem sap showed the presence of more than 60% fibroblasts (Figure 3). Therefore, the higher the concentration used, the less the number of living cells, while the higher the active compounds in the banana stem sap, the more the effect of fibroblast cell death. The drug was considered safe when it has a broad therapeutic index or small effective dose and large toxic doses.

The result indicated that the Ambonese banana stem sap with a 10% concentration to 100% did not cause a toxic effect on fibroblast cells. This was evidenced by the fact that the percentage of living cells was more than 60% at all concentrations. However, when less than 60%, the material became toxic.

4. CONCLUSIONS:

Medicinal plants are beneficial in fulfilling human life's needs. Medicinal plants in the pharmaceutical world are a source of raw materials for both modern and traditional medicines. Nowadays, there is a trend for people to consume conventional drugs, owing to improvements in lifestyle returning to nature and the high cost of modern medicines that have raised the demand for medicinal plants, not just in Indonesia but also worldwide. If this medicinal plant can be developed as the Herbal Medicine Standard (OHT) and Fitofarmaka would have a higher sales value and greater competition in both domestic and foreign markets. This study has

proven that the use of Ambonese banana stem sap from a 10% concentration to 100% does not cause a toxic effect on fibroblast cells using the MTT assay method. This work was intended to offer additional scientific information about the advantages and disadvantages of using the Ambonese banana plant, which could help make decisions for the industry and government world and guide community medicinal plants.

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6. REFERENCES:

1. Agarwal, P. K, Singh, A., Gaurav, K., Goel, S., Khanna, H., Goel, R. (2009). Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats. *Indian Journal of Experimental Biology*, 47, 32-40.

2. Alberts, B., Johnson, A., Lewis, J., Morgan, D., Raff, M., Roberts, K., Walter, P. (2002). *Molecular biology of the cell* (6th ed.). New York, Garland Science.

3. Amutha, K., and Selvakumari, U. (2016). Wound healing activity of methanolic stem extract of Musa paradisiaca Linn. (Banana) in Wistar albino rats. *International wound journal*, *13*(5), 763–767.

4. Atun, S., Arianingrum, R., Handayani, S., Rudyansah, R., Garson, M. (2010). Identification and antioxidant activity test of some compounds from methanol extract peel of banana (Musa paradisiaca Linn.). *Indonesian Journal of Chemistry*, 7(1), 83-87

5. Basuony, A. E., Hossary, E. N., Amin, R. N. (2018). Apoptosis inducing effects of chlorhexidine and essential oil mouthwashes on BHK-21 fibroblast cell line: An in vitro study. *F1000 Research,* 7, 1703.

6. Benmehdi, H., Behilil, A., Memmou, F., Amrouche, A. (2017). Free radical scavenging activity, kinetic behaviour and phytochemical constituents of Aristolochia clematitis L. roots. *Arabian Journal of Chemistry*, 10(Suppl 1), S1402-S1408.

7. Berridge, M., Tan, A., McCoy, K., Wang, R. (1996). The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. *Biochemical Journal,* 4, 14–19.

8. Berridge, M. V., and Tan, A. S. (1993). Characterization of the cellular reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT): Subcellular localization, substrate dependence, and involvement of mitochondrial electron transport in MTT reduction. *Archives of Biochemistry and Biophysics,* 303(2), 474–482.

9. Budi, H. S, Kriswandini, I. L, Sudjarwo, S. A. (2016). Ambonese banana stem sap (Musa paradisiaca var. sapientum) effect on PDGF-BB expressions and fibroblast proliferation in socket wound healing. *International Journal of ChemTech Research,* 9(12), 558-564.

10. Budi, H. S, Soesilowati, P, Imanina, Z. (2017). Gambaran histopatologi penyembuhkan luka pencabutan gigi pada makrofag dan neovaskular dengan pemberian getah batang pisang ambon. *Majalah Kedokteran Gigi Indonesia*, 3(3), 3-9.

11. Budi, H. S., and Astuti, E. R. (2019). The MMP-2, MMP-9 expression and collagen density of the ambonese banana stem sap administration on wound healing. *Journal of International Dental and Medical Research*, 12(2), 492-449.

12. Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*, 4,177.

13. Freshney, R. I. 2000. *Culture of animals cell: a manual of basic technique* (4th ed.) Newyork, Wiley.

14. Greaves, P. (2011). Preclinical testing. In: Schwab M. (eds) *Encyclopedia of Cancer*. Springer, Berlin, Heidelberg.

15. Han, J., Menicanin, D., Gronthos, S., Bartold, P. M. (2014). Stem cells, tissue

engineering and periodontal regeneration. *Australian Dental Journal,* 59(1 Suppl), 117–130.

16. Hollist, N. A. (2004). *Collection of traditional Yoruba oral and dental medicaments*. Nigeria, Olubena Printers. Ibadan.

17. Huang, G. T., Gronthos, S, Shi, S. (2009). Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine. *Journal of Dental Research,* 88(9), 792-806.

18. Kaimal, S., Sujatha, K. S., George, S. (2010). Hypoglicemic and antioxidant effect of fruits of musa AAA (chenkadali) in alloxan induced diabetic rats. *Indian Journal of Experimental Biology*, 48, 165-173.

19. Kapadia, S. P., Pudakalkatti, P. S., Shivanaikar, S. (2015). Detection of antimicrobial activity of banana peel (Musa paradisiaca L.) on Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans: An in vitro study. *Contemporary Clinical Dentistry,* 6(4), 496–499.

20. Kresnoadi, U., Rahayu, R. P., Rubianto, M., Sudarmo, S. M. Budi, H. S. (2017). TLR2 signaling pathway in alveolar bone osteogenesis induced by Aloe vera and xenograft (XCB). *Brazilian Dental Journal,* 28(3), 281-286.

21. Kumar, G., Jalaluddin, M., Rout, P., Mohanty, R., Dileep, C. L. (2013). Emerging trends of herbal care in dentistry. *Journal of Clinical and Diagnostic Research,* 7(8), 1827-1829.

22. Kunle, O. F., Egharevba, H. O., Ahmadu, P. O. (2012). Standardization of herbal medicines - A review. *International Journal of Biodiversity and Conservation,* 4(3), 101-112.

23. Kurahashi, T., and Fujii, J. (2015). Roles of antioxidative enzymes in wound healing. *Journal of Developmental Biology,* 3, 57-70.

24. Lemeshow, S., Hosmer, D, W., Klar, J., Lwanga, S. K., World Health Organization. (1990). *Adequacy of sample size in health studies*. Chichester, Wiley.

25. Marshall, N. J., Goodwin, C. J., Holt, S. J. (1995). A critical assessment of the use of microculture tetrazolium assays to measure cell growth and function. *Growth Regulation,* 5(2), 69–84.

26. Martínez, C. C., Gómez, M. D., Sook Oh, M. (2017). Use of traditional herbal medicine as an alternative in dental treatment in Mexican dentistry: A review. *Pharmaceutical Biology*, 55(1), 1992-1998.

27. Mitry, R. R., and Hughes, R. D. (2012). Introduction to cell culture. In C. Philippeos, R. D. Hughes, A. Dhawan, R. R. Mitry (eds), *Human cell culture protocols, methods in molecular biology (3rd ed.)* (pp. 1-13). London, UK, Humana.

28. Mohler, W. A., Charlton, C. A., Blau, H. M. (1996). Spectrophotometric quantitation of tissue culture cell number in any medium. *Biotechniques*, 21, 260-266.

29. Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods,* 65, 55–63.

30. National Research Council (US) Committee to Update Science, Medicine, and Animals. Science, Medicine, and Animals. (2004). *Safety Testing*. Washington (DC), National Academies Press (US).

31. Palombo, E. A. (2011). Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. *Evidence-Based Complementary and Alternative Medicine,* 2011, 680354.

32. Pandey, A., Alok, A., Lakhwani, D., Singh, J., Asif, M. H., Trivedi, P. K. (2016). Genome-wide expression analysis and metabolite profiling elucidate transcriptional regulation of flavonoid biosynthesis and modulation under abiotic stresses in banana. *Scientific Reports,* 6, 31361.

33. Peacock, M., Badea, M., Bruno, F, Timotijevic, L, Laccisaglia, M, Hodgkins, C, Raats, M, Egan, B. (2019). Herbal supplements in the print media: communicating benefits and risks. *BMC Complementary Medicine and Therapies,* 19, 196.

34. Preshaw, P. M., Alba, A. L., Herrera, D., Jepsen, S., Konstantinidis, A., Makrilakis, K., Taylor, R. (2012). Periodontitis and diabetes: a two-way relationship. *Diabetologia*, 55(1), 21–31.

35. Priosoeryanto, B. P, Putriyanda, N, Listyanti, A. R, Juniantito, V, Wientarsih, I, Prasetyo, B. F, Tiuria, R. (2007). The effect of ambon banana stem sap (Musa paradisiaca sapientum L.) on the acceleration of wound healing process in mice (Mus musculus albinus). *Journal of Agriculture and Rural Development in the Tropics and Subtropics*, 35-49.

36. Riss, T. L., Moravec, R. A., Niles, A. L., Duellman, S., Benink, H. A., Worzella, T. J. (2004). Cell viability assays. In: Markossian, S., Sittampalam, G. S., Grossman, A., Brimacombe, K., Arkin, M., Auld, D., Austin, C. P., et al. *Assay Guidance Manual.* Bethesda (MD), Eli Lilly and Company and the National Center for Advancing Translational Sciences.

37. Santos-Ballardo, D. U., Rossi, R., Hernández, V., Gómez, R. V., Rendón-Unceta, M. C., Caro-Corrales, J., Valdez-Ortiz, A. (2015). A simple spectrophotometric method for biomass measurement of important microalgae species in aquaculture. *Aquaculture,* 448, 87-92.

38. Shama, N. S., Prasanna, K. R., Joshna, A., Lakshmi, S. T. (2014). Effect of herbs on periodontitis – a serious gum infection. *International Journal of Pharmacological Research,* 4(1), 17–22.

39. Shetty, S., Udupa, S., Udupa, L. (2008) Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of Ocimum sanctum Linn in rats. *Evidence-Based Complementary and Alternative Medicine*, 5(1), 95–101.

40. Shoulders, M. D. and Raines, R. T. (2009). Collagen structure and stability. *Annual Review of Biochemistry*, 78, 929–958.

41. Sofowora, A., Ogunbodede, E., Onayade, A. (2013). The role and place of medicinal plants in the strategies for disease prevention. *African Journal of Traditional Complementary and Alternative Medicines*, 10(5), 210-229.

42. Song, B., Yang, L., Han, L., Jia, L. (2019). Metal ion-chelated tannic acid coating for hemostatic dressing. *Materials,* 12(11), 1803.

43. Stefanowicz-Hajduk, J., and Ochocka, J. R. (2020). Real-time cell analysis system in cytotoxicity applications: Usefulness and comparison with tetrazolium salt assays. *Toxicol Reports,* 7, 335-344.

44. Sumathy, C., and Vijayakumar, N. (2015). Review on antiulcerogenic activity of Musa sapientum on experimental peptic ulcers in rats. *World Journal of Pharmaceutical Research,* 4(5), 832-846.

45. Swanson, M. D. (2010). Molecular engineering of a banana lectin that inhibits hiv-1 replication. *The University of Michigan, Dissertation,* p. 16.

46. Tilburt, J. C., and Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization*. 86(8), 577-656.

47. Uzhachenko, R. V., and Anil, S. (2019). CD8+ T lymphocyte and NK cell network: Circuitry in the cytotoxic domain of immunity. *Frontiers in Immunology,* 10, 1906.

48. Von Rintelen, K., Arida, E., Hauser, C. (2017). A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. *Research Ideas and Outcomes*, 3, e20860.

Table 1. *The average optical density of BHK-21 fibroblast cells in Elisa Reader and the percentage of living cells*

Groups	Optical Density $\bar{X}$ ± SD	Living cells (%)	P-value
Media control	0.099 ± 0.012	0*	
Cell control	0.295 ± 0.077	100	
K 100	0.225 ± 0.051	82.23*	
K 90	0.241 ± 0.017	83.29	P = 0.000
K 80	0.254 ± 0.024	85.59	
K 70	0.234 ± 0.029	84.52	
K 60	0.230 ± 0.032	83.5	
K 50	0.231 ± 0.026	83.76	
K 40	0.223 ± 0.021	81.73*	
K 30	0.263 ± 0.039	91.88	
K 20	0.309 ± 0.039	103.55	
K 10	0.269 ± 0.032	93.40	

*: Significant p-value<0.05. *One-way Anova* compare to control cell.

Figure 1. *Freeze drying process of Ambonese banana stem sap.*

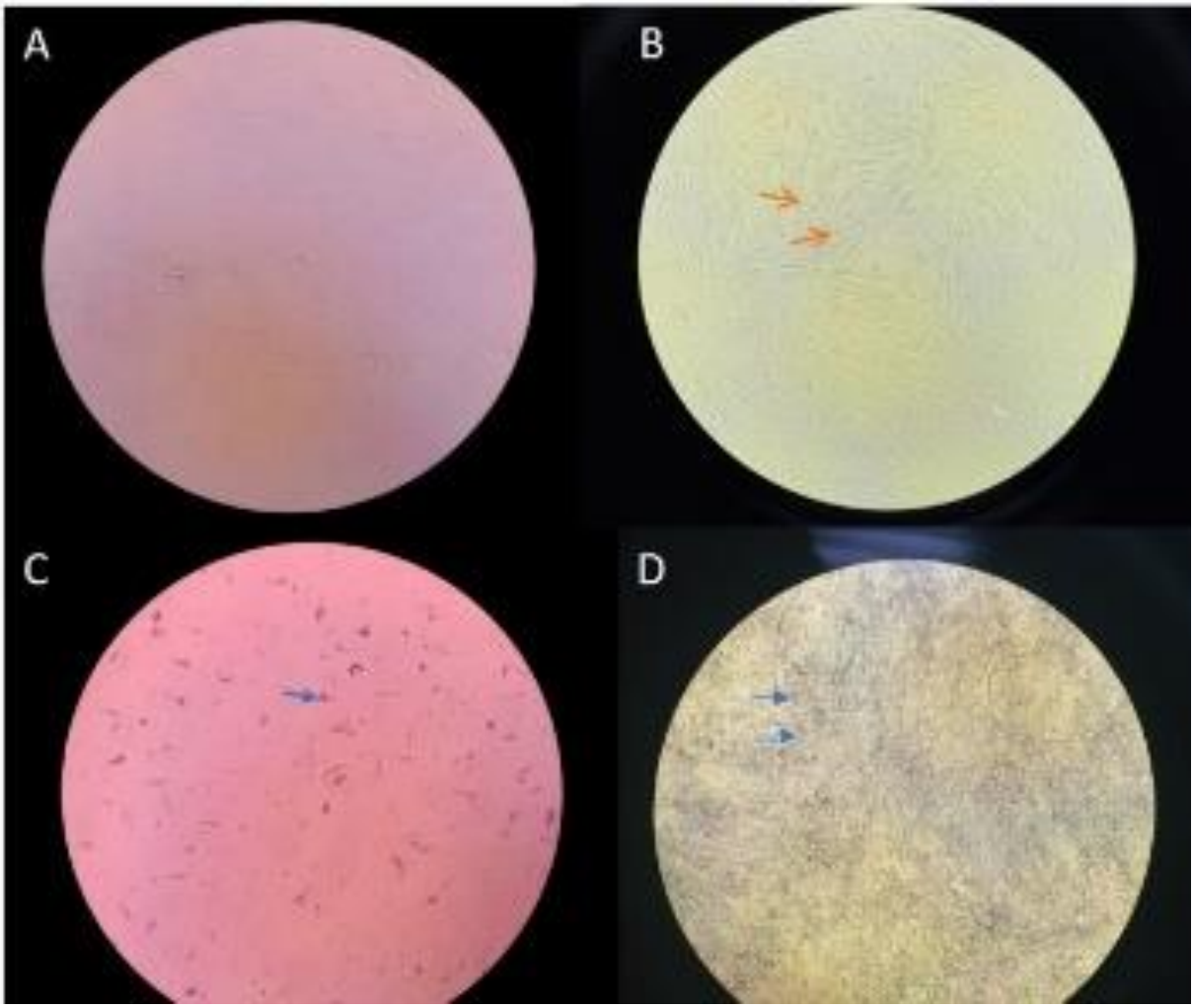

Figure 2. *BHK-21 fibroblast cells at 40X magnification microscope observation. A) Media control, B) Cell control, C) Media control after MTT, D) Cell control after MTT. Red arrows were fibroblasts, while the blues were formazan.*

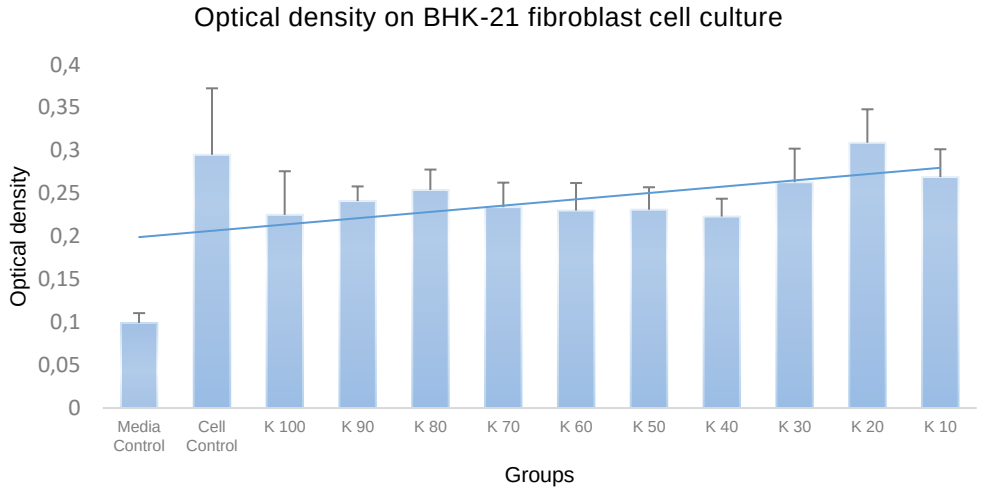

Figure 3. *The relationship between the concentration of Ambonese banana stem sap and living cells percentage*