# Mangosteen Skin *(Gracinia mangostana L)* as Stem Cell Growth Factor

Ira Arundina[1]*, Ketut Suardita[2], Indeswati Diyatri[1], Meircurius Dwi C.S[3]

1. Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Department of Conservative Dentistry, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
3. Master Program in Dental Health Science, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

**Abstract**

Stem cells gave a new way to accelerate healing progress because the stem cells can be utilized for the treatment of various degenerative diseases including periodontitis. The number of stem cells is limited; therefore, a growth factor is needed to increase stem cell proliferation. Growth factor that has been used is still expensive and difficult to obtain so it is necessary to develop an alternative use of growth factors from natural materials that potentially accelerate wound healing. Mangosteen (*Gracinia mangostana* L) contains xanthones which are flavonoid compounds that can activate kinase protein, as growth factor stem cells.

To discover the potential effect of mangosteen skin extract (*Gracinia mangostana* L) as growth factor of stem cells.

Proliferative ability test of mangosteen skin (*Gracinia mangostana* L) as a growth factor for mesenchymal stem cell (MSC) using MTT assay with FGF comparator. Test of osteogenic differentiation using Alizarin Red coloring.

Viability cells in MSC combined with higher mangosteen skin compared with MSC combined with FGF. After stem cells and mangosteen skin extracts were added the osteogenic induction medium, a presence of mineralization indicating differentiation into osteoblasts.

The addition of mangosteen skin extract on MSC can increase the ability of proliferation and differentiation into osteoblasts from MSC.

**Experimental article (J Int Dent Med Res 2018; 11(3): 765-769)**

**Keywords:** Mangosteen skin extract, Growth factor, Stem cell.

**Received date:** 14 October 2017 **Accept date:** 08 November 2017

## Introduction

Repair of periodontal tissue damage can be done through various surgical procedures, namely the use of grafting material and growth factors. In dentistry, the use of mesenchymal stem cells (MSC) has shown satisfactory results that can be used as a new strategy for regenerative periodontal therapy. But, this is constrained by the number of stem cells is limited so that required growth factor to increase stem cell proliferation. Growth factor that has been used such as Fibroblast Growth Factor (FGF) is still expensive and difficult to obtain.[1] Therefore, an alternative is needed to developed use of growth factor from natural materials

***Corresponding author:***
**Ira Arundina**
Department of Oral Biology,
Faculty of Dental Medicine, Universitas Airlangga,
Surabaya, Indonesia.
E-mail: arundinafkg@yahoo.com

The skin of the mangosteen fruit (*Gracinia mangostana* L) contains alkaloids, saponins, triterpenoids, tannins, phenols, flavonoids, glycosides, steroids, benzophenone and xanthones which are the main classes of phenols in plants.[2] Xanthones contain compounds including mangostin, mangostenol, mangostinone A, mangostenon B, trapezifolixanthone, tovophyllin B, α-mangostin, β-mangostin, garcinon B, mangostanol, flavonoid epicatechin and gartanin. The main compound or phytochemical of Mangosteen's skin (*Garcinia mangostana* L.) is α-mangostin [3] α-mangostin is a natural xanthone found as a dominant compound on the skin of the mangosteen fruit. [4]

The Xanthone has very high antioxidant content, even the antioxidant content in xanthone substances is higher in comparison with the existing antioxidants in vitamin C and E.[2] The mangosteen skin is expected to increase the ability of stem cell proliferation so it is expected to function as a natural growth factor for stem cell growth. This study aims to determine the ability

of mangosteen skin extract as a natural growth factor for stem cells.

## Materials and methods

The material for the research is Mangosteen Skin (*Garcinia mangostana* L.) obtained and determined in Purwodadi Botanical Garden, Pasuruan conservation hall, Indonesia. The skin is cleaned from other plants and dirt and then dried in the open air without direct sunlight exposure. The dried material is smoothed with a grinding machine and sifted with a powder sieve. The obtained powder is stored in a sealed container. The powder is then given a solvent to make it easier to contact the plant's active ingredients so that the extraction is more perfect.[5]

The preparation of extracts from Mangosteen skin (*Garcinia mangostana* L.) is made by maceration, soaking the powder of Mangosteen skin (*Garcinia mangostana* L.) in a methanol solvent for 24 hours in a closed vessel left at room temperature while stirring frequently. The Mangosteen skin is then filtered with a Buchner filter and the filtrate obtained is accommodated. The obtained pellet is macerated again with new solvent. The maceration is discontinued if the xanton content seen using Thin Layer Chromatography (TLC) using the methanol mobile phase (eluent) with AlCl3 marker does not show a brownish red color on the TLC plate. The result of maceration is collected, evaporated by rotary evaporator at low pressure (rotary evaporator) until it cannot evaporate again to obtain the mass of viscous extract. The residual solvent in the viscous extract evaporated in the resulting acid cabinet is called dry methanol extract.[6]

Isolation of Mesenchymal Stem Cells (MSC) derived from Wistar rats' femur bone marrow. The proliferative ability test of MSC was performed by MTT assay method. In this study, the stem cells were divided into three groups: MSC group given mangosteen skin, MSC group given FGF and control group. Cells were cultured in 96/well plates in medium DME. The addition of mangosteen skin was done as much as 1 µg/ml and the addition of FGF was given as much as 1 µg/ml then cells were cultured for 4 days. MTT test results are read with Elisa reader at wavelength 550 nm.[7]

Osteogenic differentiation ability test is done by using Alizarin Red coloring. The test was divided into three groups: control group, MSC group and MSC group given mangosteen skin extract. The MSC group was bred for 28 days in DME medium with 10 mM beta-glycerophosphate, 100 nM dexamethasone, and 50 µg/ml ascorbic acid-2-phosphates (medium osteogenic). The control group was not given an osteogenic medium but was bred only in DME medium. Then, a coloring is done with Alizarin red staining.[7]

## Results

The visible fibroblastic MSC appears to be attached to the bottom of the dish. Substitution of culture media is done every three days. On microscopic examination, the number of MSCs seen is increasing (Figure 1).

In the microscopic examination results, MSC group proliferation combined with mangosteen skin (Figure 1C) was higher when compared with MSC proliferation combined with FGF (Figure 1B). The cell vitality (%) was the amount of MSC present in the media. MTT test results can be seen in Table 1.

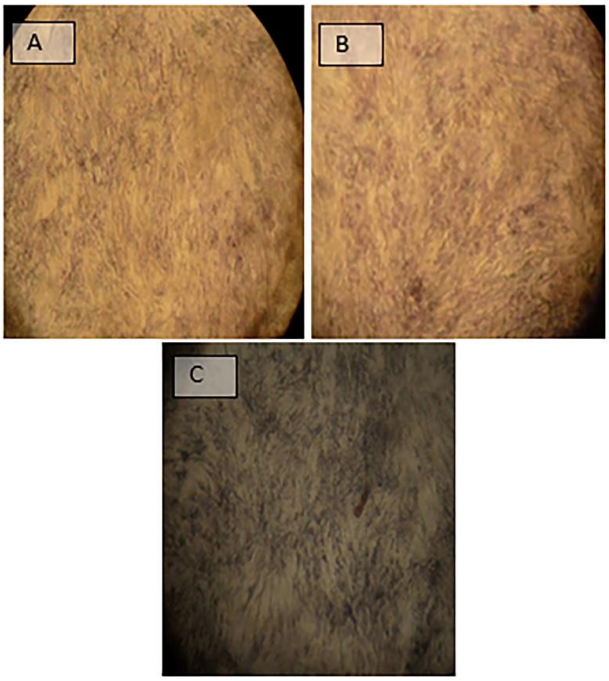


**Figure 1.** A description of MSC fibroblastic proliferation in the media. (A) control group. (B) MSC + FGF. (C) MSC + mangosteen skin extract.

| OD media | OD Cell control | OD MSC+FGF | OD MSC+mangosteen skin extract | Viability cell MSC+FGF (%) | Viability cellMSC+ mangosteen skin extract s (%) |
|---|---|---|---|---|---|
| 0.07 | 0.356 | 0.448 | 0.634 | 132.17 | 197.20 |
| 0.074 | 0.352 | 0.444 | 0.634 | 133.09 | 201.44 |
| 0.074 | 0.376 | 0.446 | 0.586 | 123.17 | 169.54 |
| | | | **Average** | **129.48** | **189.39** |

OD : optical density

**Table 1.** Result of MTT Assay.

| | N | X | SD | Levene test (sig) | Independent t test (sig) |
|---|---|---|---|---|---|
| MSC + FGF | 3 | 129.48 | 0.055 | 0.071 | 0.019 |
| MSC + mangosteen skin extract | 3 | 189.39 | 0.173 | | |

**Table 2.** Test Results of Levene Test and Independent T Test Viability Cell.

Table 1 shows that the viability cell of 189.39% in MSC combined with higher moss skin preparation when compared with MSC combined with FGF of 129.48%. The data were then tested using Levene test and T-Test test.

The statistical test using Levene test shows that the data is homogeneous data $P$=0.071 ($P$>0.05). Different test using independent t-test showed a significant difference of viability cell between MSC combined with mangosteen skin with MSC combined with FGF $P$=0.019 ($P$<0.05) (Table 2). The MSC group and mangosteen skin extracts are grown on osteogenic induction mediums have seen mineralization showing differentiation to osteoblasts (Figure 2).

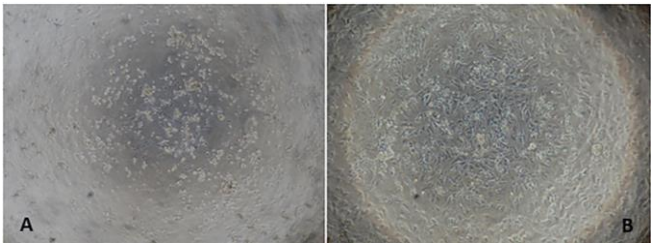


**Figure 2.** Differentiation of osteoblasts in MSC cultures combined with mangosteen skin (A) and MSC cultures (B).

## Discussion

MSC can be found in a variety of adult tissues, such as adipose tissue, periosteum, synovial membrane, muscle, dermis, pericyte, blood, trabecular bone and bone marrow. [8] Bone marrow is the largest and most accessible source of MSC. The number of MSCs in the bone marrow is only 0.01 to 0.0001% of the total nucleated cell bone marrow.[9] Growth factor plays an important role in increasing the number of MSC. A growth factor is a material that can help the process of proliferation and differentiation of stem cell. A sufficient number of stem cells can be obtained from outside the body then the cells can be applied in the body by using the appropriate scaffold.[10] In MSC, several growth factors were found. Growth factor in stem cells has several roles to secrete VEGF (vascular endothelial growth factor), FGF (Fibroblast Growth Factor), TGF (transforming growth factor) -β, hepatocyte growth factor IL-10 and 13, and cytokines and other growth factors (paracrine signal), which helps remodeling the extracellular and neovascular matrices.[11]

An attempt to increase the number of stem cells should not eliminate the ability of these cells to differentiate. In addition to attempting to multiply the number of cells, attempts to regulate the MSC differentiation process can also be performed. In-vitroally, the MSC can be directed to differentiate according to our purpose e.g. to differentiate into osteoblasts, MSC should be cultured in osteogenic medium containing beta-glycerophosphate, ascorbic acid, and dexamethasone.[12]

Mangosteen's skin *Garcinia mangostana* contains bioactive compounds such as xanthones, flavonoids, triterpenoids and benzophenone. The main compounds of the skin content of mangosteen fruit that can have an anti-oxidant effects, antiaging and anti-inflammatory is the xanthone group. Xanthones are natural chemical substances belonging to flavonoid compounds, commonly found in plants that are red, purple, blue, or yellow. The xanthone classification is oxygenated xanthone, xanthone glycoside, prenylanted xanthone, xanthonolignoid, and miscellaneous xanthone. The most important xanthone content is Alfa-mangostin.[13] Garcinia mangostana has the highest xanthone content with more than 50 xanthone compounds such as α-, β-, ɣ-mangostin, Garcinone E, 8-deoxygartanin, and Gartanin. The mangosteen skin has the highest xanthone content compared to the other mangosteen parts.[14]

Mangosteen skin contains xanthones that can effectively protect oxidative damage from hydroxyl DNA. One of the techniques of xanthone to protect against oxidative damage of DNA caused by hydroxyl is through the reduction of ROS (Reactive Oxygen Species) which can be

mediated through metal-chelating, and direct radical retrieval, through the administration of hydrogen atoms (H) and electrons (e). Both provide a hydrogen atom (H) and an electron (e) that can produce xanthone oxidation into a stable quinone form.[15]

There are phenolic compounds such as tannins, flavonoids, and xanthones in mangosteen skin extract (Garcinia mangostana L.). Tanin content in mangosteen skin extract has antioxidant ability that can inhibit lipid peroxidation and inhibit the initiation of free radical arrangement. Flavonoids and xanthones have antioxidant ability that can decrease ROS (reactive oxygen species).[16]

The α-mangostin content of mangosteen skin inhibits the release of TNF-α and IL-4 inflammatory markers in macrophage-like cells U937. The α-mangostin compound may stimulate the release of fibroblast chemotactic mediators, i.e. TGF-β which play a role in the proliferation of fibroblasts. The α-mangostin and γ-mangostin compounds in the mangosteen skin also have an anti-inflammatory property by inhibiting the cyclooxygenase pathway and the release of PGE2. Mangosteen skin extract has anti-inflammatory and anti oxidant properties that can stimulate FGF-2.[17]

Mangosteen skin also contains flavonoid compounds. Flavonoids play a role in inducing the release of TGF-β which serves to promote proliferation and migration of fibroblasts to the wound and synthesis of extracellular matrix.[18] Xanthone is a flavonoid compound capable of activating phosphatidylinositol-3 kinase (PI3K) / Akt, protein kinase C and mitogen-activated protein kinase.[19]

Research conducted, showed that the addition of Mangosteen skin extract (*Garcinia mangostana L.*) on MSC can increase MSC proliferation when compared with the addition of fibroblast growth factor (FGF). The process of cell proliferation and differentiation involves activation of MAPK / ERK signaling pathways. FGF is also involved in the process of cell proliferation. The role of FGF in the proliferation process begins through the binding and activation of fibroblast growth factor receptor (FGFR) that activates the RAS/MAP kinase pathway. FGF stimulates the phosphorylation of fibroblast growth factor receptor substrate (FRS), followed by forming the GRB2-SHP2-GAB-1 complex which ultimately results in activation of the RAS-MAPK kinase pathway, resulting in a proliferation process.[20] Increased MSC proliferation after addition of Mangosteen skin extract (Garcinia mangostana L.) is related to the ability of α-mangostin and xanthone to increase the signaling activity of MAPK / ERK transcription factor. Increased signaling activity of transcription factor MAPK/ERK, resulting in increased proliferation and differentiation of MSC.[21]

Mangosteen skin extract (*Garcinia mangostana L.*) also able to increase MSC differentiation into osteoblasts. Based on the results of this study it can be concluded that the addition of Mangosteen skin extract (Garcinia mangostana L.) on MSC can improve the proliferation ability of MSC and higher than FGF.

## Conclusions

The addition of Mangosteen skin extract (Garcinia mangostana L.) on MSC can increase MSC proliferation to differentiate into osteoblasts.

## Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

## References

1. Pejcic A, Kojovic D, Mirkovic D, Minic I. Stem Cell For Periodontal Regeneration. Balkan J Med Genet. 2013; 7-12.
2. Gutierrex-Orozc F, Failla ML. Biological Activities and Bioavailability of Mangosteen Xanthones: A Critical Review of the Current Evidence. Nutrients. 2013; 5: 1-21.
3. Ovalle-Magallanes B, Eugenio-Pérez D, Pedraza-Chaverri J. MedicinalProperties of Mangosteen (*Garcinia mangostana* L.): A Comprehensive Update. Food Chem Toxicol. 2017; 109: 102-122.
4. Jindarat S. Xanthones from Mangosteen (*Garcinia mangostana*): Multi-Targeting Pharmacological Properties. J Med Assoc Thai. 2014; 97: S196-201
5. Padua LS, Bunyapraphatsara N, Lemmens RHMJ. Plant Resources of South East Asia. Medicinal and Poisonous Plant. 1st ed. Bogor Indonesia; 2000: 139-148.
6. Hendiani I, Hadidjah D, Susanto A,Pribadi IMS.The Effectiveness of Mangosteen Rind Extract as Additional Therapy on Chronic Periodontitis (Clinical trials). Padjadjaran J Dent. 2017;29(1): 64-70.
7. Freshney RI. Culture of Animal Cells a Manual of BasicTechnique.4 ed. Canada: Wiley-Blackwell; 2000: 329-343.
8. Caplan AI, Bruder SP. Mesenchymal Stem Cells:Building Blocks for Molecular Medicine in The 21st Century. Trends Mol Med. 2001; 7: 259-264.
9. Barthold PM, Shi S, Gronthos S. Stem Cell and Periodontal Regeneration. Periodontol 2000. 2006; 40:164-172.

10. Pratiwi AR, Yuliati A, Ariani MD. Vascular Endothelial Growth Factor Expression after Induced by Chicken Shank Collagen Scaffold in Bone Regeneration. Journal of International Dental and Medical Research. 2017; 10 (2) : 333-337
11. Kalra K, Tomar PC. Stem Cell: Basics, Classification and Application. American J Phytomedi Clin Ther. 2014; 2(7): 919-930.
12. Arundina I, Suardita K, Setiabudi H, Ariani MD. Golden Sea Cucumbers (Stichopus Hermanii) as Growth Factors of Stem Cells. Journal of International Dental and Medical Research. 2016 ; 9 (3) : 242-248
13. Putri IP. Effectivity of Xanthone of Mangosteen (*Garcinia mangostana* L.) Rind As Anticancer. Med J Lampung University. 2015; 4(1): 33-38.
14. Ramesh S, Priya MK, Prabhu S. Isolation of Garcinone E from Garcinia Mangostana Linn and Its Cytotoxic Effect on Sp2/0 Cell Lines. J Pharmacog Phytochem. 2017; 6 (5): 67-68.
15. Manimekalai I, Sivakumari K, Ashok K, Rajesh S. Antioxidant and Anticancer Potential of Mangosteen Fruit, Garcinia Mangostana Against Hepatocelluler Carcinoma (HePG-2) Cell Line. World J Pharm Pharmaceutical Sci. 2016; 5: 253-293.
16. Yun YR, Won JE,Jeon E,Lee S,Kang W,Jo H, Jang JH,Shin US, Kim HW. Fibroblast Growth Factors: Biology, Function, and Application for Tissue Regeneration. J Tissue Eng. 2010; 2010: 1-18.
17. Aisha AFA,Abu-Salah KM,Ismail Z, Majid AMSA.In Vitro and In Vivo Anti-Colon Cancer Effects of Garcinia MangostanaXanthones Extract. BMC Complement Altern Med. 2012; 12(104): 1-10.
18. Simandjuntak RM, Soesanto R, Permatasari D. The Effect of Mangosteen Rind on Fibroblasts Proliferation in Wound Healing Process Post Tooth Extraction of Rat.Oral Maxillofac Surg J. 2014 ; 3(1): 23-31.
19. Ibrahim MY, Hashim NM, Mariod AA,Mohan S, Abdulla MA, Abdelwahab SI, Arbab IA.α-Mangostin from Garcinia mangostana Linn: An Updated Review Of Its Pharmacological Properties. Arabian J Chem. 2016; 9: 317–329.
20. Liu Q, Li D, Wang A, Dong Z, Yin S, Zhang Q, Ye Y, Li L, Lin L.Nitric Oxide Inhibitory Xanthones From The Pericarps of Garcinia Mangostana. Phytochemistry. 2016; 131: 115-123.
21. Ekuni D, Battino M, Tomofuji T, Putnins EE. Studies on Periodontal Disease Oxidative Stress in Applied Basic Research and Clinical Practice. London: Springer Science & Business Media; 2014: 42.