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Research Paper

In Vitro hair growth and hair tanning activities of mangosteen pericarp extract on hair dermal papilla cells

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ABSTRACT

Premature hair graying and hair loss in men and women can cause a serious aesthetic problem. However, not much effective treatment is available, especially from natural sources. In this study, the efficacy of mangosteen pericarp water extract in promoting hair growth and tanning activities on hair dermal papilla cells was studied by using *in vitro* assays. The proliferation and cytotoxicity study on hair dermal papilla cells treated with mangosteen pericarp extract were measured by 3-(4,5-dimethylthiazol-2- yl)− 2,5-diphenyltetrazolium bromide (MTT) and Sulforhodamine B assay. The degree of hair dermal papilla cells proliferation increased with increasing concentration of the extract up to 500 μg/ml of extract treatment. The cell proliferation significantly increased by 157.56 % compared to the control with 500 μg/ml of extract treatment. The mangosteen pericarp extract was found to stimulate melanin synthesis and tyrosinase activity of hair dermal papilla cells in a concentration-dependent manner. The melanin synthesis in hair dermal papilla cells was upregulated more than four-fold with mangosteen pericarp extract as compared to the control group. The mangosteen pericarp extract was more potent and better in comparison with known effective melanogenic agents such as α-melanocyte stimulating hormone and forskolin in inducing the melanogenic effects of hair dermal papilla cells.

1. Introduction

Garcinia mangostana Linn. known as mangosteen, is a tropical fruit classified to the Guttiferae family (Zarena and Sankar, 2011). The pericarp of mangosteen has a long history of medicinal use in both Chinese and Ayurvedic medicine (Shibata et al., 2013). People have used this waste pericarp as a traditional medicine for the treatment of abdominal pain, diarrhea and dysentery; and to heal wound infections, suppuration and chronic ulcers (Zhou et al., 2011). The pericarp contains a considerable amount of biologically active compounds that have been reported beneficial to human health, such as xanthones, which have relatively strong antifungal and antibacterial activities (Obolskiy et al., 2009); tannins that assure astringency to discourage infestation by insects, fungi, plant viruses, bacteria and animal predation (Akao et al., 2008); anthocyanin like cyanidin-3-sophoroside which have high antioxidant properties (Chaovanalikit et al., 2012); and phenols which exhibited strong pH-dependent bacteriostatic and bactericidal effects against gram-positive bacteria (Palakawong et al., 2013). Furthermore, phenolic compounds such as afzelechin, epiafzelechin, catechin, epicatechin, gallocatechin and epigallocatechin which possess oxygen radical scavenging capacities are also found in mangosteen pericarp (Karim and Azlan, 2012; Zadernowski et al., 2009). However, there is currently no report specifically addressing the effect of mangosteen pericarp extract on hair-growth promoting activity and hair tanning in hair dermal papilla cells.

The hair follicle, is a complicated organ, consisting of many different epidermal (epithelial cells) and dermal (mesenchymal cells) components, which are mesenchymally derived fibroblasts at the base of the hair follicles (Sun et al., 2013; Yoon et al., 2014). The hair follicle is subject to fixed turnover in a hair growth cycle through three phases known as anagen (growth phase), catagen (regressing or involuting phase) and telogen (quiescent phase); and the cycle involves rapid remodeling of both the epithelial and dermal components of hair follicles (Rho et al., 2005; Trüeb, 2002; Yoo et al., 2010). The role of hair

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dermal papilla cells in hair growth is very important. It carries nutrients to maintain and enhance new hair growth, possesses receptors for androgens and other diverse hormones, and induces the anagen phase (Bak et al., 2013; Yoon et al., 2014). Furthermore, a reduction in the secretion of stem cell factor by hair follicle dermal papilla associated with pigmentation losing in miniaturizing anagen follicles of androgenetic alopecia (Tobin and Paus, 2001). Isolated dermal papilla cells are frequently used for studies of hair growth regulation. The size of dermal papilla is well correlated with the hair growth cycle and the cell number of dermal papilla is increased in the anagen phase (Rho et al., 2005). Moreover, the number of dermal papillae dominates the size and shape of the hair, and degeneration of the dermal papilla population causes different types of hair thinning and loss (Yoon et al., 2014).

Melanin is an amorphous, insoluble, heterogeneous dark, high molecular weight polymer that is found in many pigments of biological origin, including those giving the characteristic colors of skin, hair, feathers, scales, eyes and some internal membranes (Ghiani et al., 2008; Tobin, 2008). Melanins are formed as an end-product in cytoplasmic organelles called melanosomes produced by neural-crest derived pigment cells called melanocytes and are the product of a complex, phylogenetically ancient, biochemical pathway called melanogenesis (eu- and pheomelanogenic pathways) (Tobin and Paus, 2001). Melanogenesis is a biochemical production of melanin that converts the amino acid tyrosine into dihydroxyphenylalanine and subsequently into the brown-black pigment eumelanin and the yellow-red pigment pheomelanin (McDonough and Schwartz, 2012). Derivation from different relative amounts of brown-black eumelanin and yellow-red pheomelanin causes the dramatic range of human hair colors (Tobin and Paus, 2001). Tyrosinase is the main enzyme in melanogenesis that catalyzes the hydroxylation of L-tyrosine to L-3,4-di-hydroxyphenylalanine (L-dopa), which is followed by oxidation to dopaquinone. The reduction of L-dopaquinone back to L-dopa, or a direct hydroxylation of tyrosine by tyrosine hydroxylase isoform I have described the alternative mechanism for L-dopa formation (Slominski et al., 2005). There are two separate pathways, which include several intermediate steps, lead to the formation of eumelanin and pheomelanin after the generation of L-dopaquinone (Schmid et al., 2007). Melanogenesis can proceed further through oxido-reduction reactions and intramolecular transformations happening spontaneously once L-dopa is formed, the rate determined by local concentrations of hydrogen ion, metal cations, thiols and other reducing agents, oxygen, and hydrogen peroxide (Slominski et al., 2005).

The use of chemicals and synthetic ingredients in hair products, particularly hair dye has been reported to be associated with bladder cancer (Huncharek and Kupelnick, 2005) and non-Hodgkin lymphoma (Zhang et al., 2008). Hence, it is important to discover natural resources for the development of new hair treatment products to treat hair graying and promote hair growth. Rho et al. (2005) reported that *Asiasari radix* extract shows the most potent hair growth stimulation among other 45 plant extracts examined. The extract was able to significantly upregulate the expression of VEGF in human dermal papilla cells and enhanced proliferation of human dermal papilla cells and immortalized human keratinocyte cell line (HaCaT). Furthermore, *Asiasari radix* extract gives remarkable hair growth stimulation on *in vivo* mice experiments and increases protein synthesis in vibrissae follicle cultures. Other plant extracts such as *Sophora flavescens* extract (Roh et al., 2002), red ginseng extract (Park et al., 2015), *Cinnamomum osmophloeum* Kanehira leaf extract (Wen et al., 2018), *Erica multiflora* extract (Kawano et al., 2009), *Zizyphus jujuba* essential oil (Yoon et al., 2010), *Persea americana*, flowers of *Althaea officinalis*, *Chamaemelum nobile*, *Thymus vulgaris*, leaves of *Rosmarinus officinalis*, and *Urtica dioica* (Rastegar et al., 2015) were reported to significantly increase hair dermal papilla cells proliferation and enhance hair growth. However, not much has been reported on the hair tanning effect of plant extract. In this study, the potency of mangosteen pericarp water extract as hair loss and hair greying treatment were studied.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals and solvents used were of analytical grade, which include methanol (Merck), ethanol (Merck), 3-(4,5- Dimethylthiazol-2-yl)− 2,5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), α-Melanocyte Stimulating Hormone (α-MSH), forskolin (FK), Bovine Serum Albumin (BSA) Fraction V, glucose, Folin-Ciocalteau reagent were purchased from Sigma-Aldrich (St. Louis, USA). Human follicle dermal papilla cell growth basal medium and follicle dermal papilla cell growth medium supplement mix were purchased from PromoCell (Germany). The final supplement concentration included 0.04 ml/ml fetal calf serum, 0.004 ml/ml bovine pituitary extract, 1 ng/ml basic fibroblast growth factor and 5 μg/ml insulin.

2.2. Extraction of mangosteen pericarp

Fresh mangosteens were purchased from Segamat, Johor, Malaysia in August 2014. The voucher specimen (KLU49850) was deposited in the Herbarium of the University of Malaya. The mangosteen fruits were cleaned with water and inspected to remove damaged, diseased or pest-infested fruits. The edible aerial parts were removed. The mangosteen pericarps were washed, cut into small pieces and underwent a drying process in an oven at 45 °C for 48 h. The tray-dried samples were ground into powder of 100 meshes fineness using a stainless steel powder grinder. The samples were stored at room temperature under aseptic conditions.

The extraction of mangosteen pericarp was carried out by using an in-house turbo extractor. 10 kg of dried mangosteen pericarp were extracted for 2 h in 80 litres of boiling water. The extracted solution was separated from the solids by filtration. The filtrate was spray-dried by a pilot spray dryer with inlet temperature 200 °C, outlet temperature 110 °C and 7 L/h feed capacity. The mangosteen pericarp crude extract was yielded at an average of 2 % (w/w) of the total raw material. The resultant powder of mangosteen pericarp extract was used to treat hair dermal papilla cells for hair-growth and promote hair melanogenesis activity. Mangosteen pericarp extract working solution was prepared as follow: dried powder was dissolved in water to 1 % (w/v) final concentration and sterilized through filtration. The resulted filtrates were stored at − 20 °C.

2.3. Hair dermal papilla cell culture

Human follicle dermal papilla cells were obtained from PromoCell (Germany) and cultured in a basal medium supplemented with SupplementMix (PromoCell, Germany) that contains 0.04 ml/ml fetal calf serum, 0.004 ml/ml bovine pituitary extract, 1 ng/ml basic fibroblast growth factor, and 5 μg/ml insulin. The cells were cultured at 37 °C in a humidified atmosphere with 5 % CO_2. The human follicle dermal papilla cells were cultured according to the instruction manual of PromoCell.

2.4. α-MSH and mangosteen pericarp extract treatment

Human dermal papilla cells were seeded at a density of 1.5×10^5 cells/well for 6-well plates and 2×10^3 cells/well for 96-well plates that consisted of medium supplemented with fetal calf serum, bovine pituitary extract, basic fibroblast growth factor and insulin. After 24 h, the medium was replaced by a fresh medium supplemented with 10 nM of α-MSH, 10 μM of FK, or various concentrations of mangosteen pericarp extract (62.5, 125, 250, 500 or 1000 μg/ml) and further incubated for 48 h. Cells untreated with α-MSH were used as a negative control and 10 μM FK was used as positive controls to compare with the results of various concentrations of mangosteen pericarp extract (Hamid et al., 2012).

2.5. Cell viability (MTT) assay

The MTT assay is a colorimetric assay that analyzes cell viability and cell proliferation. It can also be used to determine the cytotoxicity of a compound. It measures the mitochondrial activity of enzymes that reduce MTT to purple color formazan dye. Human dermal papilla cells were treated as described in Section 2.4. After 48 h, the culture medium was eliminated and incubated with the MTT solution for 90 mins at 37 °C. 0.04 N HCl-isopropyl alcohol solution was used to substitute the solution and undergo further incubation for half an hour at room temperature. The harvested solution was centrifuged for 5 mins at 13,000 rpm. The absorbance of the supernatant was measured at 570 nm by a microplate reader (Mosmann, 1983).

2.6. Cell cytotoxicity (SRB) assay

The Sulforhodamine B assay, SRB is used to determine the cytotoxic effects of the unknown material by counting viable cells after staining with a vital dye. In this study, SRB assay was carried out according to Sigma-Aldrich *in vitro* toxicology assay kit Sulforhodamine B based (TOX6) protocol. The dermal papilla cells were plated at 2×10^3 cells/well in 96-well plates and left it to attach for 24 h before treatment. After 48 h of treatment, the cells were fixed with 1⁄4 vol of cold 50 % (w/v) trichloroacetic acids (TCA) solution on top of growth medium and incubated for 1 h at 4 °C. and then were rinsed with water four times to remove the TCA solution. The cells were stained with 0.4 % SRB solution for 30 mins. The stain was removed and rinsed quickly with wash solution (1 % acetic acid) and the incorporated dye was then solubilized in solubilization solution (10 nM Tris) for 5 mins at room temperature. The absorbance was measured at 565 nm by using a microplate reader.

2.7. Cell proliferation (WST-1) assay

The proliferation of human dermal papilla cells was evaluated by measuring their metabolic activities using a 2-(2-methoxy-4-nitrophenyl)− 3-(4-nitrophenyl)− 5- (2,4-disulfophenyl)− 2 H-tetrazolium (WST-1) assay. In this study, WST- 1 assay was carried out by using Cell Proliferation Reagent WST-1 (Roche Applied Science, Germany). The cell proliferation reagent WST-1 is used for the non-radioactive, spectrophotometric quantification of cell proliferation, viability, growth, and chemosensitivity in cell populations using 96- well plate format. The dermal papilla cells were seeded at a concentration of 2×10^3 cells/well of a 96-well microplate in 100 µl culture medium. After 48 h of treatment, 10 µl of WST-1 was added into each well and the cells underwent incubation for 4 h at 37 °C. The absorbance was measured at 440 nm by using microplate reader.

2.8. Melanin assay

2.8.1. Secreted melanin assay

In this study, secreted melanin assay was carried out according to the method reported by Rosenthal et al. (1973). After 48 h of α-MSH and mangosteen pericarp extract treatment, the culture medium was harvested and centrifuged for 10 mins at 10,000 rpm. The absorbance was measured at 405 nm by a microplate reader. The ratio formulation of secreted melanin was calculated by using Eq. (1).

$$\text{Secreted melanin } (\%) = (\text{C-A})/\text{B} \times 100 \quad (1)$$

In this formula, all absorbance value was obtained from measurements at 405 nm. A = absorbance value of the medium supplemented with extract before incubation, B = absorbance value of the untreated medium which harvested from incubation after 48 h, C = absorbance value of the medium supplemented with extract which harvested from incubation after 48 h.

2.8.2. Intracellular melanin assay

Intracellular melanin assay was carried out according to the method reported by Rosenthal et al. (1973). After 48 h of α-MSH and mangosteen pericarp extract treatment, the cells were washed with PBS and harvested. Cell pellets were obtained from centrifugation for 10 min at 1000 rpm and lysed with 1 ml of (ratio of water: 1 N NaOH: DMSO is 7: 2: 1) solution for 24 h at 37 °C. The absorbance of supernatant was measured at 405 nm by using microplate reader.

2.8.3. Intracellular tyrosinase activity assay

After 48 h of α-MSH and mangosteen pericarp extract treatment, the cultured cells were washed using PBS. The cells were harvested in RIPA cell lysis buffer supplemented with a protease inhibitor. The cells underwent centrifugation for 15 mins at 13,000 rpm. The cell lysate was collected. The protein amount was determined by Bradford assay, with BSA (bovine serum albumin) as standard. Each cell lysate was adjusted with lysis buffer to achieve the same concentration of protein among all lysate samples. 10 µl L-DOPA in 10 nM sodium phosphate buffer was added and incubated at room temperature for 1 h. The absorbance was measured at 475 nm by a microplate reader (Rosenthal et al., 1973).

2.9. Statistical analysis

Statistical significances were assessed using the Student's t-test. All results were presented as the mean ± SD of the combined data obtained from replicate experiments.

3. Results and discussions

3.1. Yield of mangosteen pericarp extract

The yield of the mangosteen pericarp crude extract was obtained at 2 % (w/w) of the total raw material. Chemical constituents extracted depend on the solvent used. Yoshimura et al. (2015) extracted 600 kg of powdered dried mangosteen pericarps with 5600 L of hot water for 3 h to yield 92.1 kg of spray-dried powder with the addition of dextrin (to 25 %). In other words, the above reported extraction yield of mangosteen pericarp crude extract was 0.15 % (w/w) of the total raw material with an addition of 25 % dextrin. In this study, the hot water extraction using a turbor extractor has a better crude extraction yield compare to normal hot water decoction.

Other reported extractions of mangosteen pericarp used ethanol or methanol as extraction solvent to extract xanthones such as α- and γ-mangostin in the pericarp of *Garicinia mangostana*. According to Pothitirat et al. (2010), the content of α-mangostin in mangosteen pericarp extract from different solvent extraction was in the following order: dichloromethane extract (46.21 % w/w), ethanol extract (18.03 % w/w), and hexane extract (17.21 % w/w), respectively. These results showed that α-mangostin is soluble in alcohol, ether, acetone, chloroform and ethyl acetate because of the moderate polarity of the chemical structure of α-mangostin but not soluble in water. In addition, according to Widowati et al. (2014), ethanol extract from mangosteen pericarp is comprised of 75 %− 85 % α-mangostin and 5 %− 15 % γ-mangostin. α-mangostin was undetectable in water extract of mangosteen pericarp (Ngawhirunpat et al., 2010).

In the previous study, Yoshimura et al. (2015) also reported that α-mangostin and other xanthones which known as representative bioactive components of mangosteen were not observed in the hot water extract of the pericarp. These prenylated xanthones were effectively extracted with ethanol from the mangosteen pericarp. In traditional medicine, mangosteen pericarp is mainly decocted or macerated in water, thus preserving the bioactive properties of the polar compounds such as polyphenols and condensed tannins. Yoshimura et al. (2015) found three new polyphenols and 14 other known compounds isolated from the hot water extract of mangosteen pericarp. The major polyphenolic compounds detected using HPLC in the hot water extract of

mangosteen pericarp were epicatechin and procyanidin B2.

3.2. Cell viability (MTT) assay

The cellular mitochondria activity of cultured hair dermal papilla cells was measured by MTT assay. Fig. 1 shows the viability of hair dermal papilla cells by using mangosteen pericarp crude extract.

As shown in Fig. 1, 10 nM α-MSH and 10 μM forskolin increased cellular mitochondrial activity of hair dermal papilla cells in comparison to negative control with no sign of adverse effects on the cell viability of dermal papilla cells. The concentration of 10 nM α-MSH and 10 μM forskolin were used in this study as positive control. The treatment with mangosteen pericarp extract showed no detectable adverse effect on hair dermal papilla cellular mitochondrial activity at concentrations between 62.5 μg/ml to 500 μg/ml over 48 h treatment. The concentrations of 62.5, 125, 250 and 500 μg/ml extract resulted in increases in cell viability with 95.69 %, 98.95 %, 107.33 % and 123.05 %, respectively. Rho et al. (2005) reported that the optimum increase in proliferation of dermal hair papilla cells treated with 0.00001 % of *Asiasari radix* extract was 115.6 %. Park et al. (2011) reported that *Fructus Panax ginseng* extract significantly increased the proliferation of human hair dermal papilla cells by 128 – 135 % with 0.8–100 μg/ml treatment concentration. These plant extracts reported have comparable results with mangosteen pericarp extract in promoting cell proliferation in hair dermal papilla cells by using MTT assay.

3.3. Cell cytotoxocity (SRB) assay

The effect of mangosteen pericarp extract on cell survival of hair dermal papilla cells was determined through SRB assay method (Fig. 2). Mangosteen pericarp extract showed no cytotoxicity effect between 62.5 μg/ml and 500 μg/ml concentrations. The cell survival of hair dermal papilla cells increased from 95.57 %, 97.56 %, 98.54–99.5 % with increases in the extract concentration from 62.5, 125, 250–500 μg/ml, respectively.

MTT assay and SRB assay resulted in an increasing degree of cell survival and cell viability of dermal papilla cells with increasing concentration of magosteen pericarp extract from 62.5 to 500 μg/ml. The extract within this range of concentration did not possess any significant toxic effect on the hair dermal papilla cells as the cell viability was above 90 % of those untreated control. Ngawhirunpat et al. (2010) reported that the water extract of mangosteen pericarp has no significant cytotoxicity to keratinocyte cells under 50 μg/ml up to 500 μg/ml concentration range. Sattayasai et al. (2013) uses up to 800 μg/ml of mangosteen pericarp ethanolic extract treated on SK-N-SH human neuroblastoma cells and do not show cytotoxicity to the cells. Moongkarndi et al. (2014) reported that the water extract of mangosteen pericarp does not show cytotoxicity towards breast cancer cell (SKBR3) with 50 % cancer cell killing effect (ED50) for the water extract of mangosteen pericarp were 160.50 μg/ml, however, the phenolic constituents (α-mangosteen) from the ethanolic of mangosteen pericarp extract recorded ED50 of 8.21 μg/ml on the same cancer cell. Therefore, from various reports, the water extract of mangosteen pericarp does not show significant cytotoxicity to other cells.

3.4. Cell proliferation (WST-1) assay

The proliferation of hair dermal papilla cells was determined based on the WST-1 assay. As shown in Fig. 3, treatment with 62.5, 125, 250, and 500 μg/ml of extract significantly increased cell proliferation by 114.53 %, 136.78 %, 142.47 % and 157.56 % compared with untreated control respectively. The degree of hair dermal papilla cells proliferation increased with increasing concentrations of extract and this indicated that dermal papilla cells treated with a higher concentration of mangosteen pericarp extract showed better efficacy as compared to lower concentration. Therefore, it was concluded that mangosteen pericarp extract concentrations of 125, 250, and 500 μg/ml have maximum growth-promoting effect on hair dermal papilla cells, and these concentrations were used in further experiments. The highest degree of proliferation was achieved when treated with 500 μg/ml of mangosteen pericarp extract.

Dermal papilla cells play an important role in hair growth regulation. The morphology of dermal papilla cells can be altered through the hair growth cycle, being maximal in volume in the growing phase (anagen) and least in the resting phase (telogen) (Kang et al., 2013). Previous studies have shown that the size of the dermal papilla cells correlates

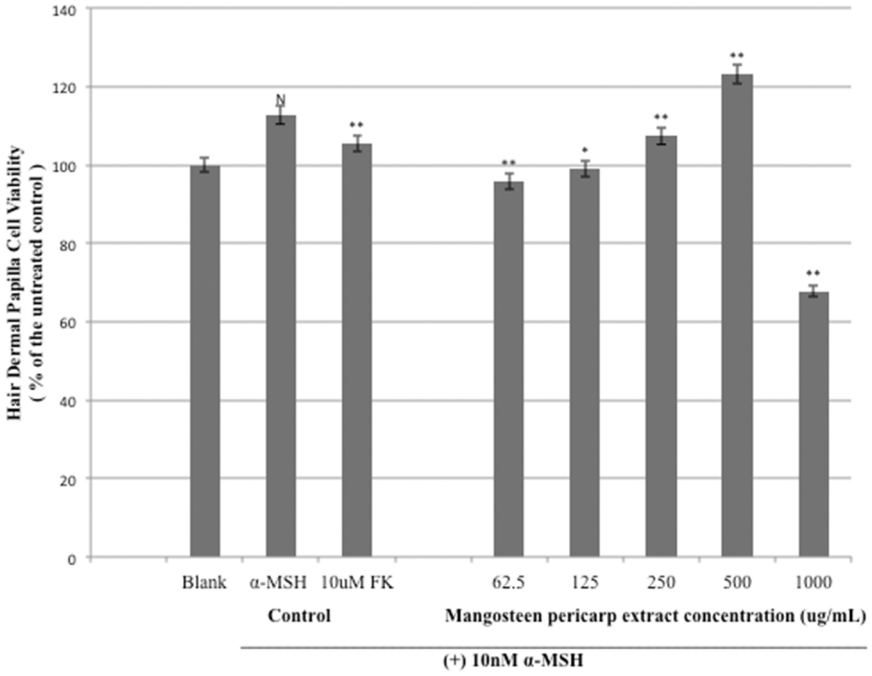

Fig. 1. Cell viability of hair dermal papilla cells treated by mangosteen pericarp crude extract at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive α- MSH control.

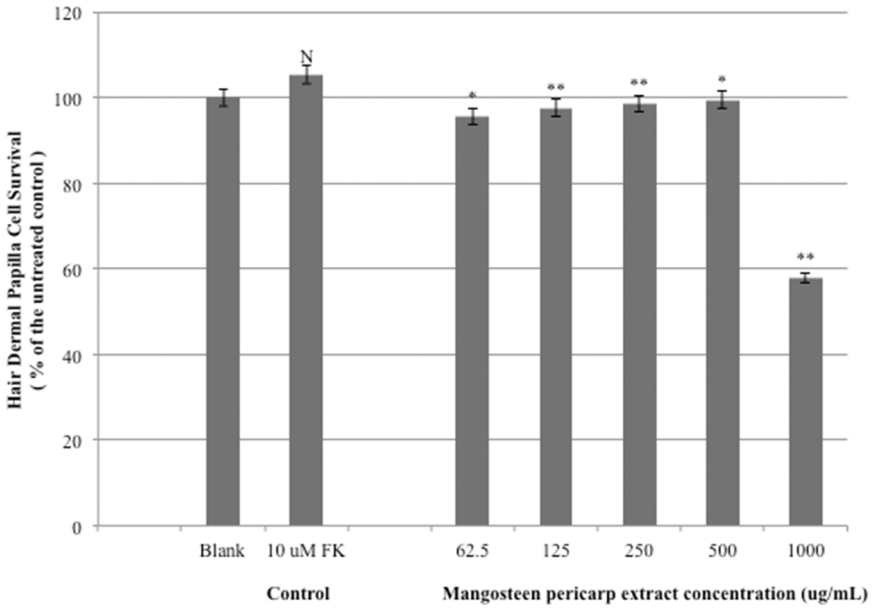

Fig. 2. Cell survival of hair dermal papilla cells treated by mangosteen pericarp crude extract at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive forskolin control.

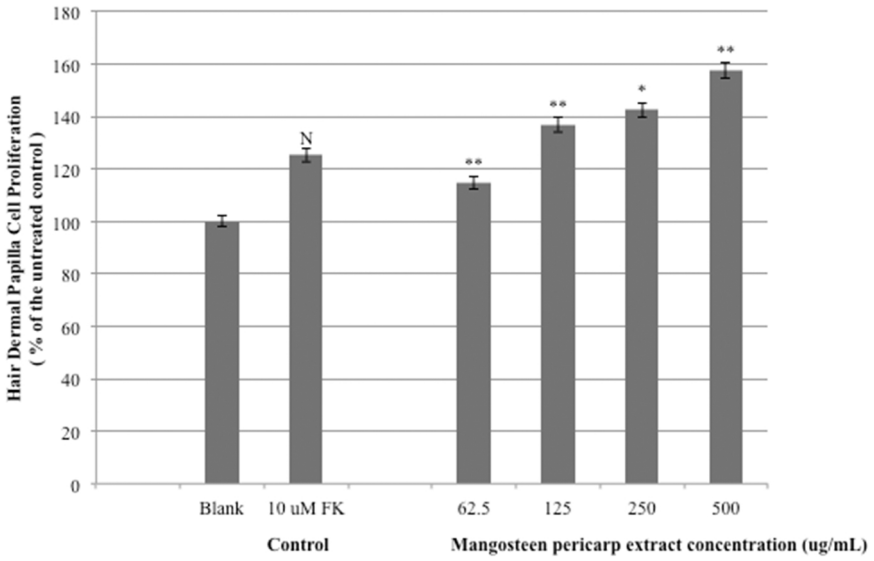

Fig. 3. Cell proliferation of hair dermal papilla cells treated by mangosteen pericarp crude extract at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive forskolin control.

well with hair growth and the number of the dermal papilla cells was increased in the growing phase of the hair cycle (Sun et al., 2013).

The increment of dermal papilla cells proliferation seems to result in an increase of hair-fiber length (Kang et al., 2013). Mangosteen pericarp extract treatment increases the proliferation of hair dermal papilla cells as compared with the control group (Fig. 3). Forskolin, a positive control, enhanced the proliferation of dermal papilla cells by 125.2 % at the concentration of 10 μM compared with the untreated control. The results indicated that the mangosteen pericarp extract exhibit hair growth promoting effect *via* the proliferation of dermal papilla cells. The extract has the substantial potential of acting as a hair growth active ingredient.

3.5. Melanin assay

3.5.1. Secreted melanin assay

The secreted melanin assay was carried out using different concentrations of mangosteen pericarp extract on hair dermal papilla cells. Both α-MSH (alpha- melanocyte stimulating hormone) and forskolin (FK) have been proven as effective melanogenic agents, so they were used as positive controls. Furthermore, both α-MSH and FK are known as cAMP-elevating agents, as cAMP pathway is one of the most pivotal signaling pathways in melanogenesis (Hamid et al., 2012). In this study, dermal papilla cells were treated with mangosteen pericarp extract in concentration ranges of 2-fold series dilutions (62.5, 125, 250 and 500 μg/ml), 10 nM α- MSH, and 10 μM FK for 48 h, and the amount of melanin secreted into the culture medium and present inside the cells was analyzed.

After 48 h of treatment with the mangosteen pericarp extract, the secreted melanin content increased with increasing concentration of mangosteen pericarp extract treatment from 62.5 μg/ml to 500 μg/ml as shown in Fig. 4. Forskolin showed a significant 3-fold increase in melanin secretion, proving its powerful melanogenic activity; whereas

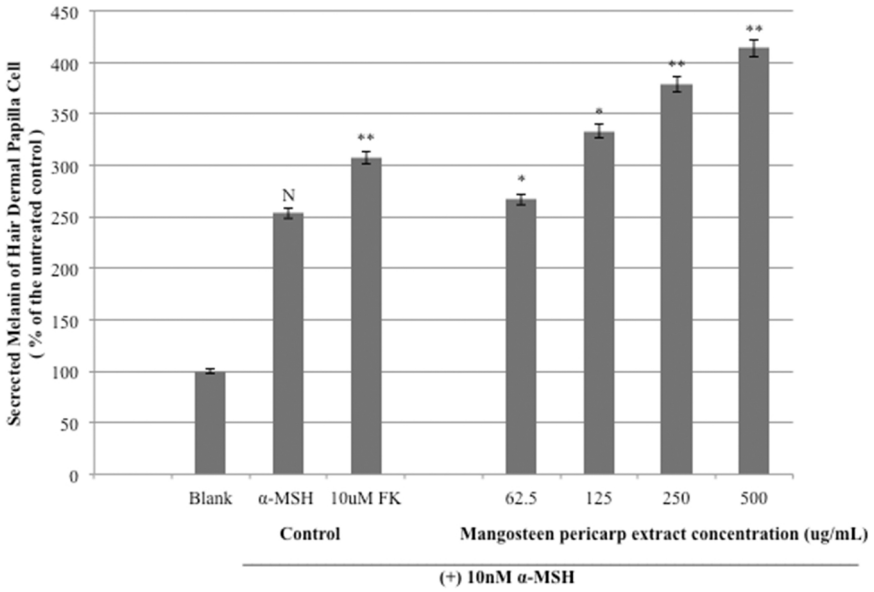

Fig. 4. Effects of mangosteen pericarp extract on melanin secretion of hair dermal papilla cells at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive α- MSH control.

α-MSH increased the secreted melanin to the level of 2.5-fold of the untreated control. However, mangosteen pericarp extract showed stronger melanogenic effects in the secretion of melanin than forskolin and α-MSH under these experimental conditions. At 62.5 μg/ml treatment concentration, the melanin secretion increased 267.11 %, which had a similar melanogenic effect to α-MSH at 253.63 %. The effect of 125 μg/ml and 250 μg/ml mangosteen pericarp were comparable to the α-MSH-treated control and were 3.3-fold and 3.8-fold higher as compared to the negative control, respectively. The melanin secretion of hair dermal papilla cells increased in a concentration-dependent manner up to the highest extract concentration of 500 μg/ml. The result of 500 μg/ml was more efficient than the α-MSH-treated and forskolin control, which was 4-fold higher compared to the negative control. The secreted melanin assay shows that the mangosteen pericarp extract strongly increases melanin synthesis in hair dermal papilla cells and was more effective than 10 nM of α- MSH and 10 μM of forskolin.

3.6. Intracellular melanin assay

Fig. 5 shows the intracellular melanin content of hair dermal papilla cells. The results of the intracellular melanin assay show a similar pattern to the secreted melanin assay, which also demonstrate a concentration-dependent increasing trend. Treatment with 125 and 250 μg/ml mangosteen pericarp extract resulted in intracellular melanin contents of 116.77 % and 135.7 %, respectively. Furthermore, for the highest concentration of 500 μg/ml extract treatment, the intracellular melanin content for hair dermal papilla cells was increased by 157.42 %, which showed almost 1.6-fold higher than the negative control. α-MSH treatment shown no significant effect in intracellular melanin assay. α-MSH increased only the secreted melanin significantly, but not the intracellular melanin. However, forskolin treatment increased intracellular melanin content up to 1.2-fold compared to the untreated control, and had a similar effect to 125 μg/ml mangosteen pericarp extract

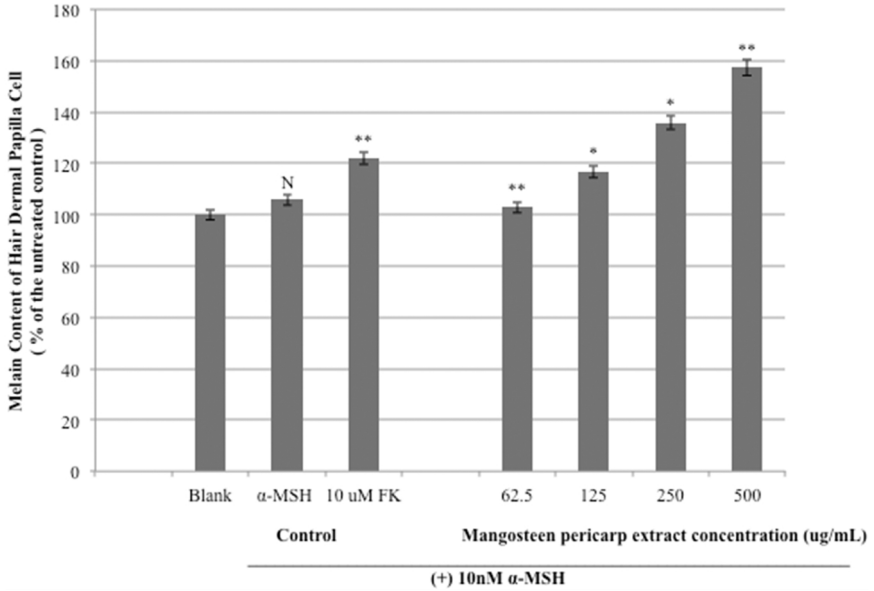

Fig. 5. Effects of mangosteen pericarp extract on intracellular melanin content of hair dermal papilla cells at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive α- MSH control.

treatment. The treatment with 250 and 500 μg/ml of mangosteen pericarp extract showed better melanogenic activity in hair dermal papilla cells with higher intracellular melanin content as compared with the positive control groups, α-MSH and forskolin.

The total melanin content was calculated by adding the percentage of both intracellular melanin assay and secreted melanin assay (Fig. 6). Forskolin had induced total melanin of 429.22 %, which exhibited a very strong melanogenic effects by stimulating melanogenesis activity up to 4.3-fold when compared to untreated control. Although the results obtained from the melanin content assay were not as significant as those obtained by the secreted melanin assay, α-MSH had induced 359.48 % total melanin, and also showed significant effects on stimulating melanogenesis activity by 3.6-fold.

The mangosteen pericarp extract displayed stronger melanogenic activity as compared to forskolin and α-MSH. The total melanin content in hair dermal papilla cells treated with 125, 250 μg/ml and 500 μg/ml extract was 4.5-fold, 5.1-fold and 5.7-fold higher compared to the negative control, respectively. This showed that the mangosteen pericarp extract has a significant melanogenic effect to induce melanogenesis activity. The total melanin content of hair dermal papilla cells increased in a concentration-dependent manner up to the highest concentration of 500 μg/ml extract.

3.7. Intracellular tyrosinase assay

Hair dermal papilla cells were treated with the mangosteen pericarp extract at the same ranges of concentrations with those of the melanin assay to describe the stimulation of melanogenesis mechanism stimulated by the extract. The percentage of tyrosinase activity in the treated hair dermal papilla cells was determined by comparison with the control group. The same amount of cell lysate calibrated with respect to protein concentration was applied to the oxidation reaction with L-DOPA.

The intracellular tyrosinase activity of the dermal papilla cells by treating with α-MSH increased 2.4-fold compared with untreated control as shown in Fig. 7, whereas treatment with forskolin increased the intracellular tyrosinase activity significantly by 3-fold in comparison with a negative control group. Treatment with 125 μg/ml and 250 μg/ml mangosteen pericarp extract resulted in upregulating the intracellular tyrosinase activity of hair dermal papilla cells by 298.67 % and 354.05 %, respectively; which were ~3-fold and 3.5-fold higher than the negative control, respectively. These two treatment concentrations were comparable and better in stimulating the intracellular tyrosinase activity in hair dermal papilla cells than α-MSH and forskolin. Furthermore, the treatment with the highest concentration of 500 μg/ml extract showed a 4-fold increase in intracellular tyrosinase activity.

In this study, mangosteen pericarp extract significantly increased and up-regulated the intracellular tyrosinase activity and melanin synthesis of hair dermal papilla cells in a concentration-dependent manner. Furthermore, the extract at the concentrations as low as 250 μg/ml induced melanogenesis significantly in cultured hair dermal papilla cells and were more potent than those treated by 10 nM α-MSH and 10 μM forskolin. Therefore, the mangosteen pericarp extract can be one of the melanogenic-stimulating agents that stimulate the melanogenesis activity in hair dermal papilla cells.

4. Conclusion

The degree of hair dermal papilla cells proliferation increased by 157.56 % with 500 μg/ml concentration of mangosteen pericarp extract as compared to the untreated control group. The mangosteen pericarp extract is non-toxic and did not affect the hair dermal papilla cell viability up to 500 μg/ml. The mangosteen pericarp extract was found to stimulate melanin synthesis and tyrosinase activity of hair dermal papilla cells in a concentration-dependent manner. This study also showed that mangosteen pericarp extract was more potent and better in comparison with known effective melanogenic agents such as α-melanocyte stimulating hormone and forskolin in inducing the melanogenic effects of dermal papilla cells. The overall results suggest that mangosteen pericarp water extract could potentially be used as a safe active ingredient for the development of hair growth and hair tanning products.

CRediT authorship contribution statement

Ying Fang Tan: Methodology, Validation, Formal analysis, Investigation, Data curation, Resources, Visualisation, Writing – original draft. **Yin Shin Koay:** Methodology, Validation, Formal analysis, Investigation, Data curation, Resources, Visualisation, Writing – review & editing. **Razauden Mohamed Zulkifli:** Conceptualization, Supervision, Resources. **Mariani Abdul Hamid:** Conceptualization, Supervision, Funding acquisition.

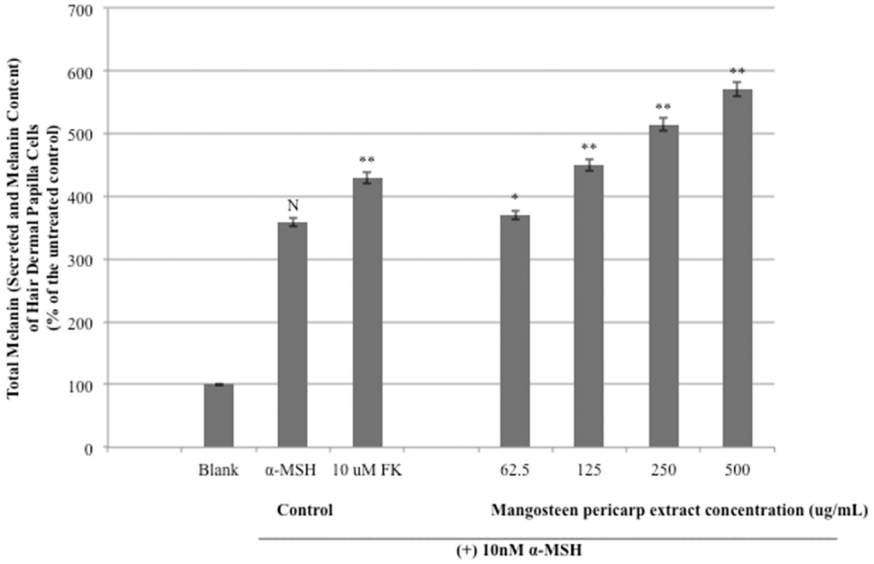

Fig. 6. Effects of mangosteen pericarp extract on total melanin (secreted and melanin content) of hair dermal papilla cells at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive α-MSH control.

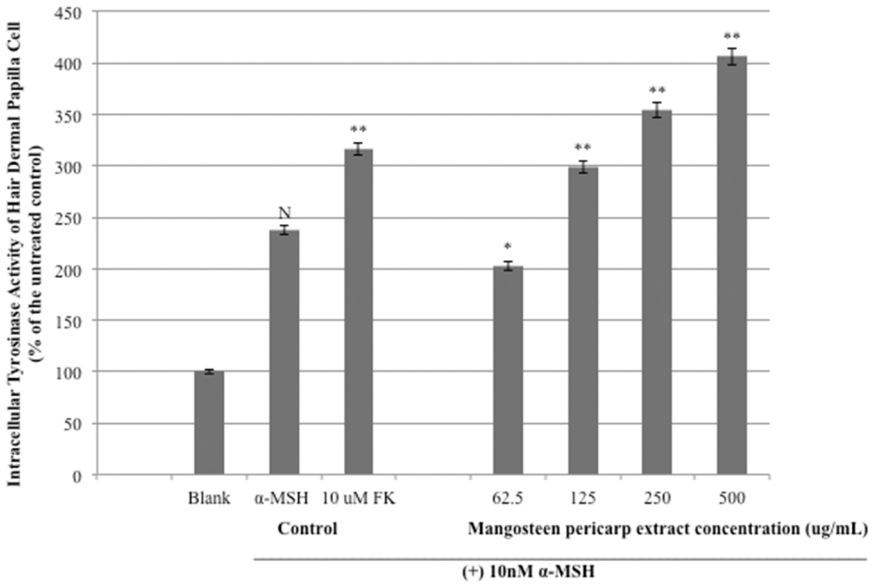

Fig. 7. Effects of mangosteen pericarp extract on intracellular tyrosinase activity of hair dermal papilla cells at different concentration (μg/ml). Results were the average of three independent experiments ± SD. *P < 0.05, **P < 0.01 compared to positive α-MSH control.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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