Original Article

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Comparative analysis of mangosteen peel extract and vitamin E treatment on human gingival fibroblasts

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Objective: This study evaluated the effect of mangosteen peel extract compared with vitamin E supplements on human gingival fibroblast (HGFs) behavior.

Materials and Methods: Mangosteen peel was extracted using different ratios of ethanol to distilled water (60:40) and 50:50 (% v/v). Mangosteen peel extracts can be used as food preservatives and have antimicrobial effects. HGFs were treated with mangosteen peel extracts. Total antioxidant capacity (TAC) was determined using an Oxiselect Total Antioxidation Capacity Assay Kit. We investigated cell migration using xCELLigence real-time cell analysis (RTCA).

Results: Ethanol 60% v/v showed the highest percentage of extraction yield. Mangosteen peel (500 µg/ml) generated a higher cell viability was compared with DMEM (-FBS). Cell viability was compared with the other tested conditions. This result demonstrated that mangosteen peel extract had minimal effects on cell viability and could be potentially considered an anti-aging agent and antioxidant. The TAC from HGFs treated with 500 µg/ml mangosteen peel had the highest uric acid concentrations compared with the other tested conditions. The HGFs were used for investigating cell migration. HGFs migration was mangosteen peel concentration-dependent.

Conclusion: These findings demonstrated that mangosteen peel extracts exhibited potent antioxidant activities, similar to vitamin E supplements and mangosteen peel extract exhibited potent antioxidant activities similar to vitamin E supplements. The migration concentration MGE (50 µg/ml) was the same as for vitamin E (positive control).

Keywords: antioxidation, human gingival fibroblast (HGFs), mangosteen peel extract, MTT Cytotoxicity assay

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Introduction

Skin aging is a complex natural process characterised by a gradual diminishing of the structural integrity and physiological imbalance of the skin tissue. The phytochemistry [1] of mangosteen peel has also been widely studied [2]. The mangosteen peel is a rich source of polyphenolic compounds that include ellagitannins and phenolic acids [3], flavonoids, anthocyanins, catechins and other complex flavonoids [4], as well as punicalin, pedunculagin, punicalagin, gallic acid, and uric acid esters of glucose that account for 92% of their antioxidant activities [5]. The antioxidant activity of mangosteen peel extract is comparable to vitamin supplements [6]. It also increases human gingival fibroblast (HGFs) migration [7]. Fibroblasts play an important role in skin aging, and antioxidants are pivotal in maintaining skin health. HGFs are a specific type of fibroblasts derived from the oral gingival tissue. Fibroblasts are connective tissue cells found throughout the body, responsible for producing the extracellular matrix components, e.g., collagen [8], elastin, and glycosaminoglycans, which provide structural support and elasticity to tissues.

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Although fibroblasts affect skin aging, HGFs derived from gingival tissue may not be directly applicable; however, how fibroblasts grow and function remains relevant. Research on various types of fibroblasts, including HGFs, contributes to our understanding of tissue regeneration and potential therapeutic strategies for maintaining skin health and combating the aging processes. Aging is a risk factor for oral mucosal diseases, such as periodontitis, and the occurrence of periodontitis increases with age [9]. Periodontitis is a chronic inflammatory disease, which destroys tooth-supporting structures, leading to tooth loss. Lipopolysaccharide (LPS), the component of gram-negative bacteria membrane induces the release of many inflammatory mediators, interleukins and reactive oxygen species (ROS) [10,11]. The ROS in periodontal tissue by periodontitis bacteria may promote gingival aging. The antioxidant defense system can inhibit the damage caused by free radicals or nonradical reactive species [12].

Fibroblasts have an important role in tissue regeneration [13] and maintaining skin health through various mechanisms [14], including growth factor production and extracellular matrix (ECM) synthesis. Fibroblasts are responsible for synthesizing and remodeling the ECM [15], which provides structural support and biochemical cues for cells. This matrix is essential for tissue organization and function. Fibroblasts produce growth factors, such as fibroblast growth factors, transforming growth factor-beta (TGF-β), and hepatocyte growth factor [16]. These growth factors stimulate cell proliferation, differentiation, and migration, which are crucial for tissue repair and regeneration [17].

In contrast, when the antioxidant system is not working correctly, the outcome will be an increase in oxidative damage and its downstream consequences. The literature is rich in data concerning different antioxidants [18]. Vitamin E is an essential nutrient that has antioxidant and anti-inflammatory properties. Studies using in vitro experiments demonstrated that mangosteen peel extract and its active compounds also have antioxidant and antimutagenic effects. The objective for this study was to evaluate the effect of mangosteen peel extract compared with vitamin E supplements on HGFs behavior. The observed higher cell viability when exposed to mangosteen peel extract compared with a nutrient-deprived medium (DMEM without FBS) suggests that the extract's components may have antioxidant effects and protect skin cells. This finding could be particularly relevant for applications in cell culture studies or in exploring the potential health benefits of mangosteen peel extract.

Materials and Methods

Natural mangosteen and vitamin E supplement

Mangosteen peels were weighed to 100 grams. Mangosteen peel extracts were obtained using a mixing ratio (50:50, and 60:40 % volume/volume) of ethanol and distilled water for 24 hours. The phenolic compounds were isolated from the mangosteen extract [19]. The solvents were evaporated via lyophilization, to obtain the mangosteen peel extract powder. Lyophilization was performed using the following conditions: temperature at -50 °C and pressure at 0.140 mbar for 72 hours. The dried product extraction concentrations (50 and 500 ug/ml (weight/volume)) were obtained by varying the percentage of extract to ethanol. The vitamin E (d-alpha Tocopheryl) was obtained from Mega Lifesciences PTY, Company Limited, Thailand.

Cell culture

HGFs (ATCC PCS-201-018 Human Gingival Fibroblasts; HGFs) were prepared in 10 ml Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, California, USA), 100 UT/ml penicillin,

100 µg/ml streptomycin, and 2 mmol/L glutamine (Gibco, Grand Island, New York, USA) in complete DMEM in T 75 cm^3 flasks. The cell lines were maintained at 37 °C in a humidified atmosphere of 5% CO_2. Ten thousand cells were cultured in 96-well plates. For the control experiment, HGFs (10,000 cells/ml) in control media (DMEM containing antibiotics/antimycotics) were plated in 96-well plates, in a humidified atmosphere.

MTT cytotoxicity assay

The same density of cells treated with complete DMEM was used as a control. After 24 hours of incubation, the cells were treated with the following conditions: HGFs in control DMEM (control), HGFs treated with 500 µg/ml mangosteen peel (MGE 500 µg/ml), HGFs treated with 50 µg/ml mangosteen peel (MGE 50 µg/ml), HGFs treated with 50 µg/ml vitamin E (positive control), and HGFs in DMEM (-FBS, negative control). The treated cells were incubated for 24 hours. The HGFs were evaluated for cell viability using the colorimetric tetrazolium assay (MTT assay) [20]. Following the manufacturer's protocol, 500 µl of diluted MTT solution (0.5 mg/ml) (Sigma-Aldrich, USA) in serum-free DMEM culture medium was incubated with the treated cells for 2 h at 37 °C. Next, 100 µl dimethyl sulfoxide (DMSO) (LGC, Brazil) was added and incubated for 15 min at room temperature. After crystal solubilization, 200 µl of the solution from each well was transferred into 96-well plates and the absorbance (test wavelength of 570 nm and a reference wavelength of 650 nm) was determined using a microplate reader (Biotek Instruments, CA, USA).

Antioxidant effect

An OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit (Cell Biolabs, Inc) [21] was used on a microplate reader to measure the absorbance at 490 nm. An OxiSelect™ TAC Assay Kit was used to measure the total antioxidant capacity of biomolecules from a variety of samples via a set mechanism. The TAC Assay is based on the reduction of copper (II) to copper (I) by antioxidants, such as uric acid. Upon reduction, the copper (I) ion reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm [22, 23].

Aliquots of the supernatant were stored at -80 ºC. The absorbance of the supernatant was determined after using the TAC assay. The treatment groups were the following: HGFs in control DMEM (control), HGFs treated with 500 µg/ml mangosteen peel (MGE 500 µg/ml), HGFs treated with 50 µg/ml mangosteen peel (MGE 50 µg/ml), HGFs treated with 50 µg/ml vitamin E (positive control), and HGFs in DMEM (-FBS, negative control). The concentrations of the uric acid antioxidant were obtained from a standard curve.

The xCELLigence RTCA (real-time cell analysis)

The xCELLigence real-time cell analysis (RTCA) [24] provided a platform for label free and operator independent investigation of the migration of cells. The rate of cell migration was observed in real-time using CIM-plates (ACEA Biosciences Inc., San Diego, CA, USA) using the xCELLigence system. Ten thousand (10,000) cells in CIM-plates (200 µl/well) were treated the same as the MTT assay for 24 hours. Once the CIM-Plate had equilibrated, it was placed in the RTCA DP Station, and the background cell-index values were measured (Figure 1). The CIM-Plate was then removed from the RTCA DP Station, and the cells were added to the top chamber at the desired density. The CIM-Plate was placed back in the RTCA DP Station. The migration was monitored every two minutes for 24 hours. The treated cells were further incubated for 24 hours in the xCELLigence RTCA and compared with the controls. The CIM-Plate provided a kinetic cell-response profile throughout the experiment, detailing the onset and rate of migration. Different densities of HGFs cells were analyzed.

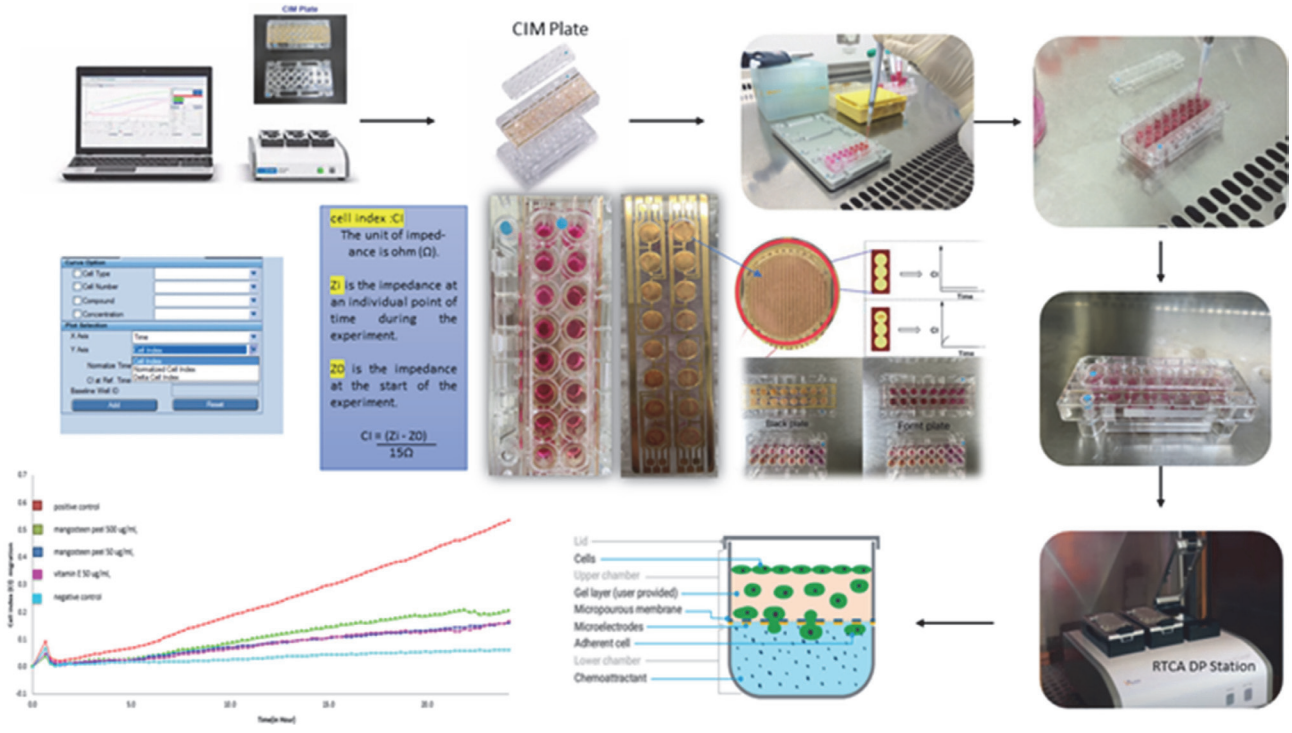

Figure 1 The xCELLigence Real-Time Cell Analysis of concentration treatment of HGFs 24 hours. Measured electrical impedance was translated as a dimensionless parameter, the Cell Index (CI) was calculated based on the formula; CI = (Zi - Z0) / 15 Ω. When cells are not present or adhered, the CI value is zero. When there are no cells on the electrode surface, the impedance describes only the background. In contrast, CI values increase progressively and proportionally as cells become attached to the electrodes. The CI variations are displayed in a real-time plot by the software

The cell analysis covered many measurement modalities, including energy metabolism and real-time cell migration. The RTCA read the entire CIM-plates in 15 seconds. At each time point, the impedance was measured (Zi). The cell index (CI) [25] was then calculated using the formula, CI = (Zi - Z0) / 15 Ω. This was done to investigate the relative change in the cell index values between the different test groups over time.

Statistical analysis

The data are presented as the mean ± standard deviation. Differences among multiple groups were assessed by one-way analysis of variance followed by Scheffe's multiple range testing. Differences were considered statistically significant at $p<0.05$.

Results

The concentrated dried mangosteen extracts were 50 and 500 µg/ml (weight/volume). Table 1 shows that 60% ethanol and 40% distilled water (volume/volume) yielded a higher mangosteen peel extract, 2.33 ± 0.01%. Figure 2 shows the percentage cell viability after the various HGFs treatment conditions as evaluated by the MTT assay. DMEM (-FBS) demonstrated the lowest cell viability, and HGFs treated with 500 µg/

ml mangosteen peel extract (MGE 500 µg/ml) had the highest cell viability. These results demonstrated that the cells were healthy and alive in all tested conditions. Figure 3 shows that HGFs treated with MGE 500 µg/ml had the highest uric acid concentration. Figure 4 shows that HGFs treated with MGE 500 µg/ml had a cell index that was comparable to the HGFs control. The migration of the HGFs was higher in MGE 500 µg/ml than in MGE 50 µg/ml. The migration in MGE 50 µg/ml was the same as vitamin E 50 µg/ml (positive control).

Table 1 The %(w/w) yield of mangosteen peel extraction

Extraction mangosteen peel (v/v)	Fresh peel weight (g)	Net weight (g)	%Yield
ethanol 50: distilled water50	100	1.98	1.98
ethanol 60: distilled water40	100	2.33	2.33

w/w = weight/weight, v/v = volume/volume

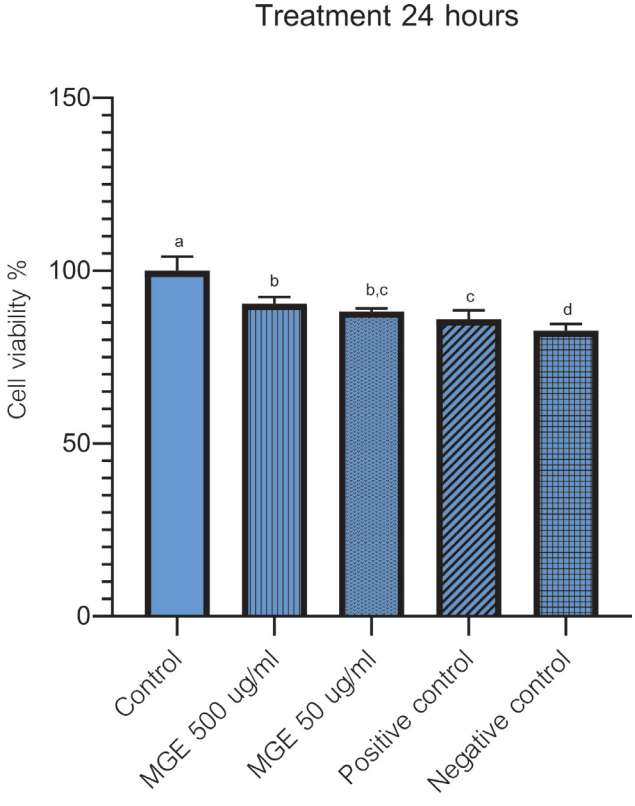

Figure 2 % Cell viability in various HGFs treatment conditions. Data are expressed as the mean ± SD. Error bars indicate an SD of at least three independent determinations. (n=3, $p<0.05$, a, b, c and d indicate a significant difference compared with the others).

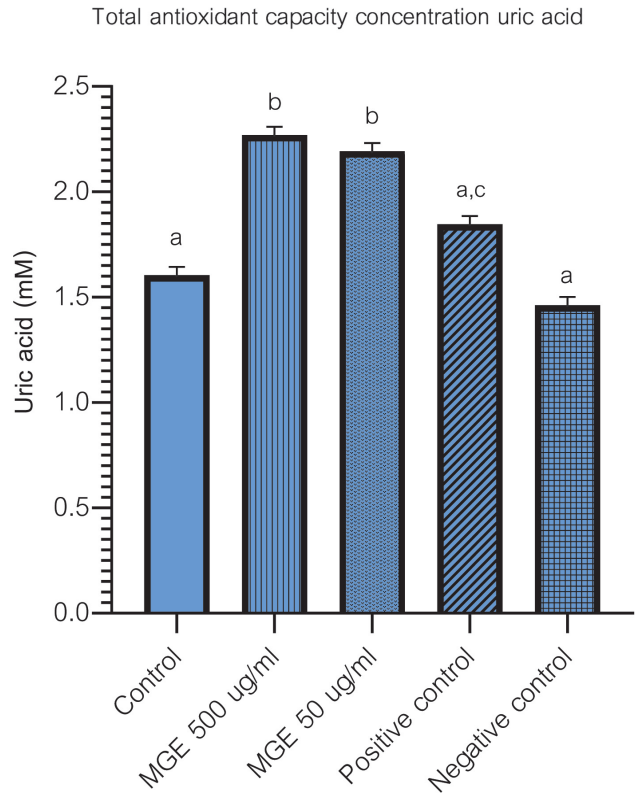

Figure 3 Total antioxidant capacity (TAC) concentration of uric acid. Data are expressed as the mean ± SD. Error bars indicate SD of at least three independent determinations. (n=3, $p>0.05$ a, b, and c indicate a significant difference compared with the others)

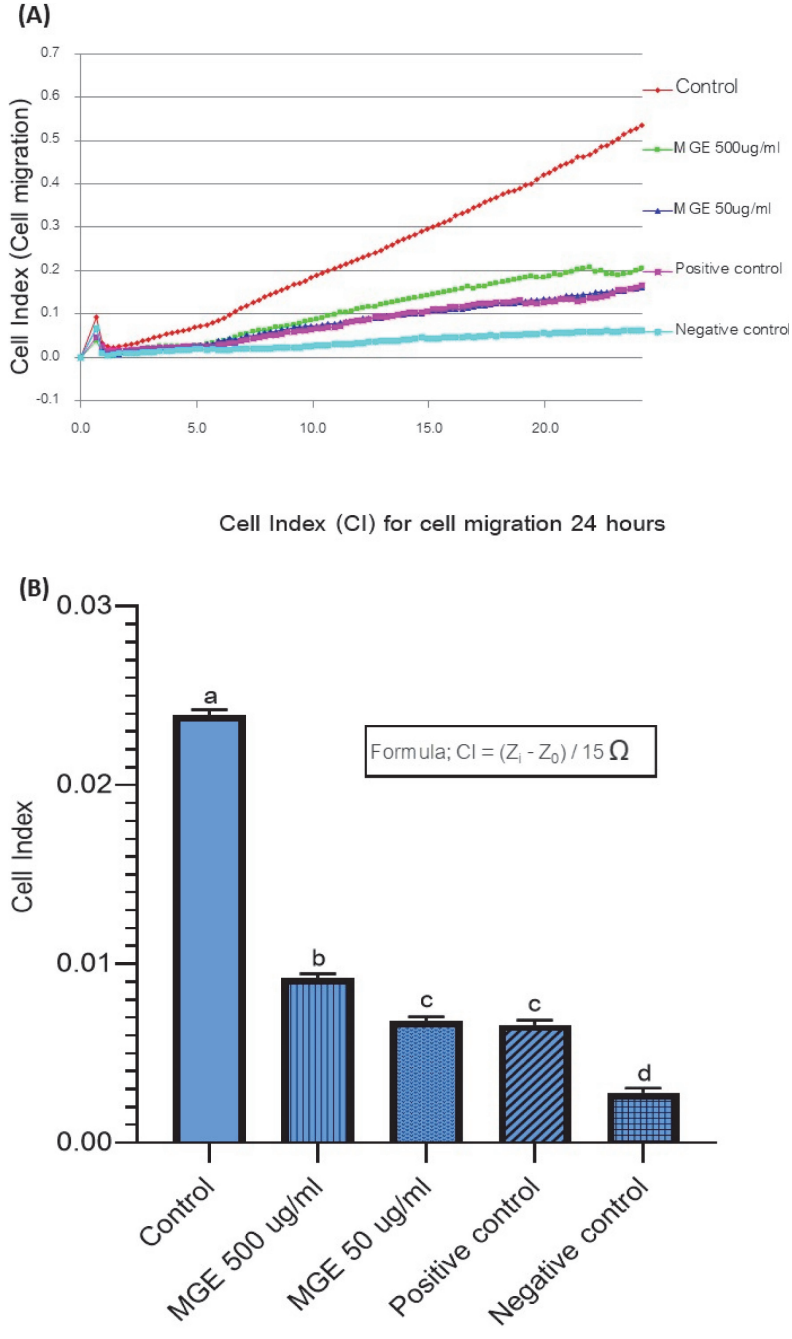

Figure 4 The migration of HGFs after different treatments for 24 hours. (A) Cells were grown in a cell migration (CIM) plate and DMEM control, mangosteen peel extraction 500 ug/ml (MGE 500 ug/ml), mangosteen peel extraction 50 ug/ml (MGE 50 ug/ml), vitamin E (Positive control) and negative control. (B) The rate of cell migration was determined by calculating Cell Index (CI) the slope of the line between 2 time points. The different superscript letters (a, b, c, and d) indicate a significant difference (p>0.05) compared with the others (n=3).

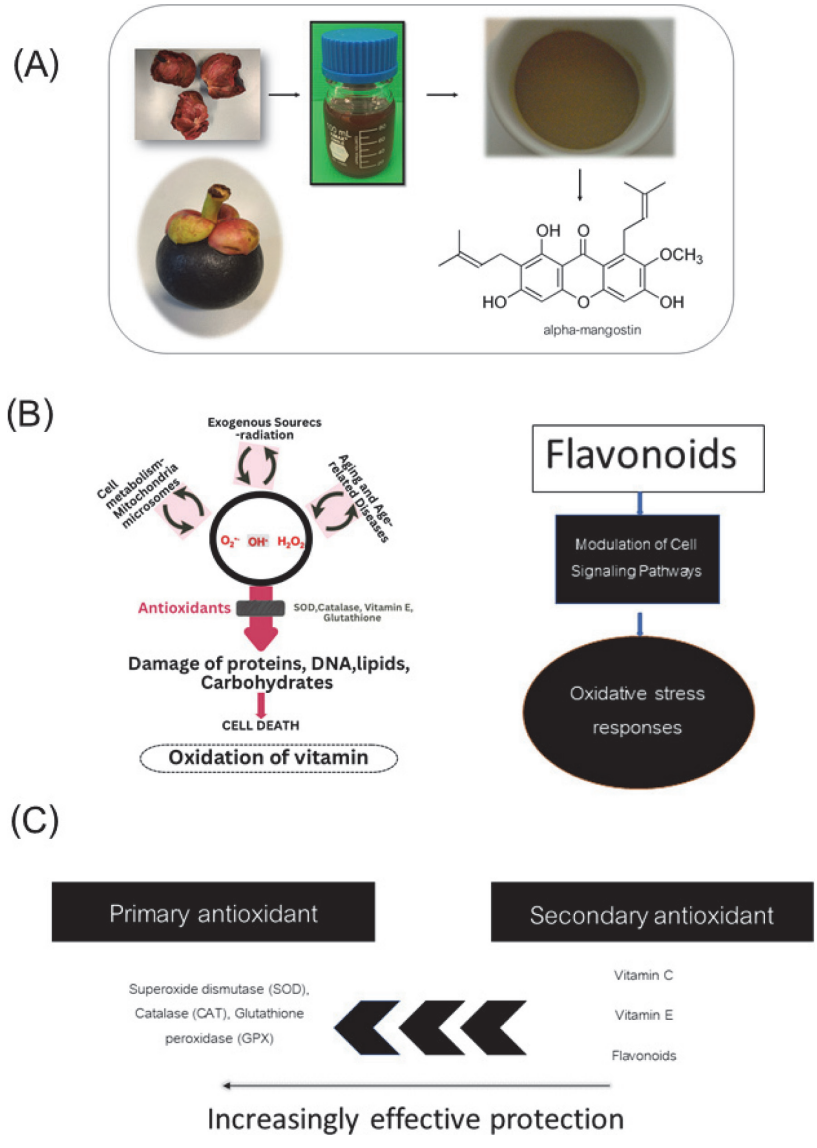

Figure 5 (A). The mangosteen peel extract powder (Garcinia mangostana L.) was obtained using lyophilization. Lyophilization was performed at -50°C and pressure at 0.140 mbar for 72 hours. (B). The oxidation of vitamin and mechanisms of antioxidant effects of flavonoids. Flavonoids may exert their antioxidant effects by preventing generation of ROS, direct scavenging of ROS, or indirectly through enhancement of cellular antioxidant enzymes. In summary, flavonoids exert their antioxidant effects through multiple mechanisms, including scavenging free radicals, chelating metal ions, enhancing antioxidant enzyme activity, regenerating other antioxidants, reducing inflammation, and modulating cellular signaling pathways. (C). Primary antioxidants have high catalytic properties and are involved in the elimination of millions of free radicals. In contrast to primary antioxidants, secondary antioxidants quench only one free radical and are quickly exhausted with no possibility of renewal. Thus, secondary antioxidant reserves can become quickly saturated and the oxidative stress will be uncontrolled.

Discussion

The present study evaluated the effect of mangosteen peel extract compared with vitamin E supplements on HGFs behavior. We found that HGFs treated with MGE 500 µg/ml had the highest cell viability compared with the other tested conditions. This result demonstrated that mangosteen peel extract had minimal cytotoxic effects on cells and could be potentially considered an anti-aging agent and antioxidant. The uric acid in HGFs treated with MGE 500 µg/ml was higher than those treated with MGE 50 µg/ml. Oxidative stress and high levels of ROS [26] can impair cell viability by causing oxidative damage to cell components, e.g., proteins, lipids, and DNA. By scavenging ROS, mangosteen peel extract may help maintain cell integrity and function [27], thus promoting cell viability and survival. Mangosteen peel extract acts similar to hydrogen peroxide in reducing HGFs death by increasing cell viability and decreasing LDH (Lactate Dehydrogenase) release [28].

The HGFs treated with MGE 50 µg/ml had a higher amount of uric acid compared with those treated with the vitamin E supplement. The present study suggested that mangosteen peel extract might be a potential skin healing agent. Mangosteen peel extract contains bioactive compounds, such as xanthones, flavonoids, and phenolic acids, which have antioxidant properties [5]. The extract shows a similar mechanism to hydrogen peroxide (H_2O_2) and other free radicals generated during oxidative stress. By reducing ROS levels, mangosteen extract may protect HGFs from oxidative damage that could otherwise lead to cell death [3]. The mangosteen peel extract, possibly through its antioxidant properties, can protect HGFs from oxidative stress-induced cell death by increasing cell viability and reducing LDH release [29]. This potential therapeutic benefit warrants further research to elucidate the specific bioactive components and mechanisms involved, as well as to explore its application in preventing or treating conditions associated with oxidative stress and cell damage in oral health [30].

Mangosteen peel extract and vitamin E exhibit potent antioxidant activities [31]; however, the specific mechanisms and spectrum of action may differ slightly due to their different chemical compositions and modes of action. Vitamin E, particularly alpha-tocopherol, is well-absorbed and utilized by the body. Mangosteen peel extract's bioavailability can vary depending on the form and formulation used [32]. Although both have antioxidant properties, mangosteen peel extract has also been evaluated for its potential anti-inflammatory effects beyond its antioxidant activity [5].

Uric acid is an antioxidant only in a hydrophilic environment, which is a major limitation of the antioxidant function of uric acid and can affect the total antioxidation capacity within a sample. The assay kit containing uric acid is extensively used to monitor the antioxidants biological effect, thus, hydrophilic and lipophilic samples are compatible with the assay. Therefore, mangosteen peel extract shows protective effects on HGFs in the sample. The degeneration of cells from hydrogen peroxide, at least part of the mechanism of action, may be the result of antioxidant properties found in mangosteen peel extract. This degeneration makes it possible to reduce the occurrence of pathologies because degeneration reduces HGFs death. If the mangosteen peel extract assay has a high amount of uric acid, that indicates that the extract can have a high antioxidant effect. Despite the reaction of uric acid with oxidants [33], it is important to highlight that

these reactions can lead to the production of ROS and other radicals [34]. These reactive species have the potential to initiate radical chain reactions within biological systems, which can result in oxidative damage to cells and tissues. Uric acid can undergo oxidation in the presence of oxidants, such as hydrogen peroxide, leading to the formation of radicals, such as urate free radicals and other reactive intermediates. These radicals can then react with biomolecules, e.g., lipids, proteins, and DNA, causing oxidative modifications that can disrupt cell function and lead to various pathologies. Most of the potentially harmful effects of oxygen are due to the formation and activity of various chemical compounds, i.e., ROS, which tend to donate oxygen to other substances. Free radicals and antioxidants have become commonly used terms in modern discussions of disease mechanisms [35]. Exogenous ROS are considered the most important factor in ROS generation. In particular, xenobiotic exposure induces oxidative stress via metabolic reactions in the liver and it can also damage biomolecules, such as lipids, proteins, and DNA.

The cell index plot, including the specific time points corresponding to the wound healing status, is found in Figure 4. The result demonstrated that mangosteen peel extracts promoted wound healing compared with vitamin E supplements.

HGFs can migrate in a substance [36], and their migration was mangosteen peel extract concentration-dependent. The results demonstrated an inverse line indicating a decrease in migration as the concentration decreased. However, in cancer research [37], the skin cancer cell migration was not affected by higher concentration extract. The HGFs cell migration increased in a concentration-dependent manner.

HGFs migrated more in MGE 500 µg/ml than in MGE 50 µg/ml. The migration at MGE 50 µg/ml was similar to vitamin E 50 µg/ml (positive control). Migrating human gingival fibroblasts refers to human gingival fibroblast cells that are actively moving from one location to another. Gingival fibroblasts are a type of cells found in the gingiva that play a crucial role in maintaining its integrity. HGFs are the main cells in the gingiva. Wound healing properties contribute to reducing oxidative stress and modulating inflammatory responses. Wound healing with mangosteen peel extract [38] can reduce inflammation [39]. Reduced redness, swelling, warmth, and pain, along with improved wound closure and tissue regeneration, suggest the beneficial effects of mangosteen peel extract in promoting the healing process similar to vitamin E. In our next study, we plan to clarify the roles of various physical parameters in collective cell migration based on the formulating biophysical model. This complex phenomenon is discussed in the model systems, such as the movement of cell clusters on the invasion method [40].

Conclusions

The HGFs remained viable after being treated with mangosteen peel extracts and had comparable cell viability values as vitamin E supplements. The mangosteen peel extracts yielded a higher level of uric acid antioxidant compared with vitamin E supplements, and had a comparable cell index. The cell index plot comprising specific time points, evaluated cell migration. The result demonstrated that higher mangosteen peel extract concentration induced higher HGFs migration compared with vitamin E supplements. This study may be useful for the development of oral drugs and the current trend in dental products using natural products.

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