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Original Research Article

Effects of Nanoparticle of *Saussurea lappa* on Stem Cell from Human Exfoliated Deciduous Teeth (SHED) Proliferation and Cancer Cell Inhibition

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ABSTRACT

Saussurea lappa, a medicinal plant, is known for its anti-inflammatory, and anticancer properties. This study explored the effects of nanoparticle of *Saussurea lappa* on stem cells from human exfoliated deciduous teeth (SHED) proliferation at various concentrations, aiming to assess its potential in promoting stem cell growth. *The preparation of nanoparticles from Saussurea lappa was accomplished through an encapsulation method utilizing Eudragit RS 100*.Simultaneously, the cytotoxic effects of nanoparticle of *Saussurea lappa* on HeLa cells were evaluated by determining its IC_{50}, providing insights into its anticancer properties. The dual focus on enhancing stem cell proliferation and inhibiting cancer cell growth highlights the therapeutic versatility of nanoparticle of *Saussurea lappa*. SHED proliferation was assessed at concentrations of 12.5, 25, 50, 100, and 200 µg/ml after exposure for 24, 48, and 72 hours. Cell viability was measured using absorbance assays. Additionally, the inhibitory effects of nanoparticle of *Saussurea lappa* on HeLa cells were evaluated to determine the IC_{50} value. The nanoparticle *Saussurea lappa* demonstrates a concentration- and time-dependent effect on cell proliferation. At high concentrations (100–800 µg/mL), it significantly reduces cancer cell viability, indicating strong cytotoxic potential. For SHED cells, proliferation decreases notably at 12.5 µg/mL ($p < 0.0001$) across 24, 48, and 72 hours, with inhibitory effects diminishing at higher concentrations. Nanoparticle of *Saussurea lappa* shows promising dual effects as a stimulant of SHED proliferation and an inhibitor of cancer cell growth. These findings suggest its potential therapeutic applications in regenerative medicine and cancer treatment, warranting further investigation into its underlying mechanisms and clinical efficacy.

Keywords: *Saussurea lappa,* stem cells, cancer, regenerative medicine, nanoparticles, deciduous teeth

Introduction

Saussurea lappa, a medicinal plant utilized in Ayurvedic and traditional Chinese medicine, has garnered significant attention for its potential therapeutic applications in promoting tissue regeneration and inhibiting cancer cell growth.[1].This plant is renowned for its diverse pharmacological properties, which encompass anti-inflammatory,[2] antimicrobial,[3] and anticancer effects.[4] The therapeutic potential of *Saussurea lappa* can be attributed to its rich composition of bioactive compounds, including sesquiterpene lactones, flavonoids, alkaloids, triterpenes, and essential oils.[5,6] Among these compounds, sesquiterpene lactones such as costunolide and dehydrocostus lactone have been shown to exhibit notable anticancer effects by inducing apoptosis in various cancer cell lines, while also possessing anti-inflammatory properties that facilitate tissue repair.[1,7,8]

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Flavonoids, particularly quercetin and kaempferol, are recognized for their potent antioxidant activities, which help mitigate oxidative stress and inflammation, thereby enhancing cellular healing and exhibiting additional anticancer properties.[9,10] Alkaloids, including saussureamine A, further bolster the plant's anticancer effects by inhibiting the proliferation of malignant cells.[11] Furthermore, triterpenes found in *Saussurea lappa* are associated with both anti-inflammatory and anticancer activities, enhancing tissue regeneration through the modulation of signaling pathways involved in cell growth and survival.[12] The essential oils derived from the plant contain compounds that demonstrate antimicrobial and wound-healing properties, which are essential for effective tissue regeneration. Additionally, the presence of phenolic compounds and vital nutrients, such as vitamins A, C, and E, supports cellular integrity, immune function, and healing processes.[13,14] Despite these promising attributes, the specific mechanisms through which *Saussurea lappa* modulates cellular processes, particularly in stem cells and cancer cells, remain an area of active investigation.
Stem cells from human exfoliated deciduous teeth (SHED) present a promising avenue in regenerative medicine due to their remarkable capacity for proliferation and differentiation into various cell types.[15] Enhancing SHED proliferation is a critical objective in tissue engineering and regenerative therapies and identifying natural compounds from sources like *Saussurea lappa* that stimulate stem cell growth could lead to novel therapeutic strategies for tissue repair and regeneration.

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Conversely, *Saussurea lappa*'s role in inhibiting the growth of cancer cells, such as HeLa cells—a widely used model for studying cervical cancer—presents significant implications for oncology. Targeting cancer cell proliferation while preserving healthy cells remains a fundamental challenge in cancer treatment, and natural products represent a promising reservoir of potential anticancer agents.[16] This study aim to provide an analysis of the effects of *Saussurea lappa* on SHED proliferation and its inhibitory activity on HeLa cells, thereby contributing to a broader understanding of its biomedical potential and paving the way for its application in regenerative medicine and cancer therapy.

Materials and Methods

Cell Culture

HeLa cells (human cervical cancer cell line) were obtained from a cell bank and cultured in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained in a humidified incubator at 37°C with 5% CO_2. Prior to experimentation, cells were passaged and allowed to reach approximately 70-80% confluency.

Plant Material and Extraction

The Saussurea lappa used in this study was sourced from Indonesia (-7.966122, 112.637823). The plant was thoroughly cleaned to remove any foreign matter and impurities. It was then washed and air-dried in the shade to avoid direct sunlight, which could degrade its active compounds. Once fully dried, the plant material was ground into a fine powder using a mechanical grinder and sieved through a standardized sieve to achieve uniformity in particle size.

The extraction of bioactive compounds from Saussurea lappa was performed using a maceration method. A specified weight of the powdered plant material was immersed in 70% ethanol in a closed container for 24 hours (maceration). The maceration process continued until thin-layer chromatography (TLC) analysis indicated that no significant amounts of compounds remained in the plant material. The collected filtrate was then concentrated using a rotary evaporator at low pressure until the solvent had evaporated completely, yielding a viscous extract. The remaining solvent was further removed in a fume hood, resulting in the dry ethanol extract.

Preparation of nanoparticle of Saussurea lappa

The preparation of nanoparticles from Saussurea lappa was accomplished through an encapsulation method utilizing Eudragit RS 100. During the process the encapsulation was stirred using a magnetic stirrer at a speed of 200 rpm for 30 minutes at room temperature. The supernatant was discarded, and the nanoparticles were collected by vacuum filtration using Whatman No. 2 filter paper. The collected nanoparticles were heated in a water bath at 50°C for 15 minutes, followed by homogenization using a rotor-stator homogenizer at a speed of 5,200 rpm for 2.5 minutes to ensure uniformity in particle size. The characteristic of nanoparticles of Saussurea lappa including particle size, polydispersity index, and zeta potential was analyzed using a Malvern Particle Size Analyzer (PSA). This result showed that the nanoparticle size was 119.7 nm. After obtaining the nanoparticle, it was dissolved in dimethyl sulfoxide (DMSO) to create a stock solution of 200 mg/mL. This stock solution was further diluted to achieve the following final concentrations: 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL. A control group was included, which consisted of cells treated with DMSO only, without the extract. HeLa cells or SHED were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere overnight. Following this, the cells were treated with the prepared concentrations of *Saussurea lappa* extract (12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, and 200 µg/mL) along with the control group. The treatment was performed in eight for each concentration. The cells were then incubated for three different time periods: 24, 48, and 72 hours.

Proliferation and inhibition assay

After the respective incubation periods, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for an additional 4 hours at 37°C.[17] The MTT solution was then removed, and 150 µL of DMSO was added to each well to dissolve the formazan crystals formed. The absorbance was measured at 570 nm using a microplate reader (GloMax Explorer System, Promega, USA) with a reference wavelength of 630 nm. IC_{50} Determination and the percentage of cell viability was calculated using the following formula:

$$Cell\ viability\ (\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} x\ 100\%$$

Ethical approval

The protocol of the study on this research has registered and approved by Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia with registration number 1081/HRECC.FODM/XI/2024.

Statistical analysis

The data were plotted as a dose-response curve using the concentrations of nanoparticle of *Saussurea lappa* extract on the x-axis and the corresponding cell viability percentages on the y-axis. The IC_{50} value, defined as the concentration of extract that inhibits 50% of cell viability, was determined using non-linear regression analysis. The statistical analysis was performed using PRISM 9 for macOS.

Results and Discussion

The inhibitory effect of nanoparticle of *Saussurea lappa* on HeLa cell showed different viability in a dose-dependent manner. At higher concentrations (800 µg/mL and 400 µg/mL), there is a significant reduction in cell viability compared to the control group, as evidenced by a decrease to approximately 50% and 60% viability. This indicates a potent cytotoxic effect at these doses. Conversely, at lower concentrations (200 µg/mL and below), the cell viability remains relatively stable, with values exceeding 80%, suggesting that lower doses do not exert a significant toxic effect on HeLa cells (Figure 1). These results showed that nanoparticle of *Saussurea lappa* has a pronounced inhibitory effect on HeLa cell viability at higher concentrations, highlighting its potential as an anticancer agent.

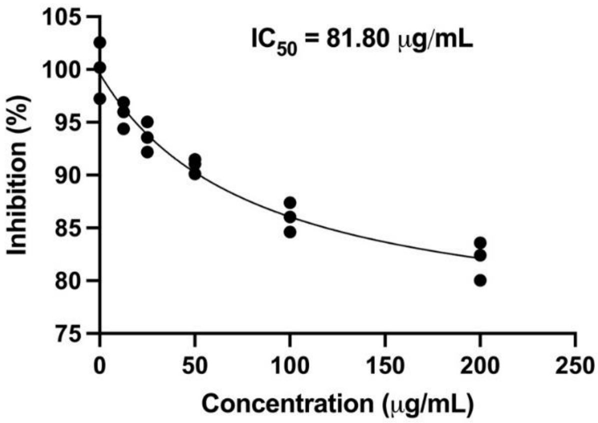

Figure 1: The inhibition response of HeLa cells to various concentrations of *Saussurea lappa* nanoparticle. IC_{50} value of 81.80 µg/mL. At the highest concentration, 800 and 400 µg/mL, cell growth inhibition reached 50%.

SHED proliferation at 24 hours significantly decreased at a concentration of 12.5 µg/mL compared to the control group and the 25 µg/mL concentration, reflecting a considerable difference. At higher concentrations (50, 100, and 200 µg/mL), there was a significant but lower inhibition of proliferation (Figure 2A)

After 48 hours of exposure, the significant decrease in SHED proliferation was observed at concentrations of 12.5 µg/mL and 25 µg/mL, reflecting a highly substantial difference. At a concentration of 50 µg/mL, a significant decrease occurred, indicating a small but still significant difference. At higher concentrations (100 and 200 µg/mL), a significant decrease was observed, indicating a greater inhibitory

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effect compared to the control (Figure 2B).
At 72 hours, the concentrations of 12.5 µg/mL and 25 µg/mL, SHED proliferation significantly decreased compared to the control, confirming a highly substantial difference. At a concentration of 50 µg/mL, a significant decrease was observed, indicating a small yet significant inhibitory effect. Meanwhile, at concentrations of 100 and 200 µg/mL, a similar pattern to the 48-hour results was observed, showing significant inhibition, reflecting a greater difference compared to the control (Figure 2C).

The data indicate that nanoparticles of *Saussurea lappa* inhibits SHED proliferation in a concentration- and time-dependent manner. Lower concentrations (12.5 µg/mL and 25 µg/mL) show a stronger initial inhibitory effect, while higher concentrations (100 µg/mL and 200 µg/mL) demonstrate a cumulative inhibitory impact over longer exposure times. This suggests that nanoparticles of *Saussurea lappa* inhibitory activity is influenced by both dose and duration, emphasizing its potential as a modulator of cell proliferation with implications for therapeutic or cytotoxic applications.

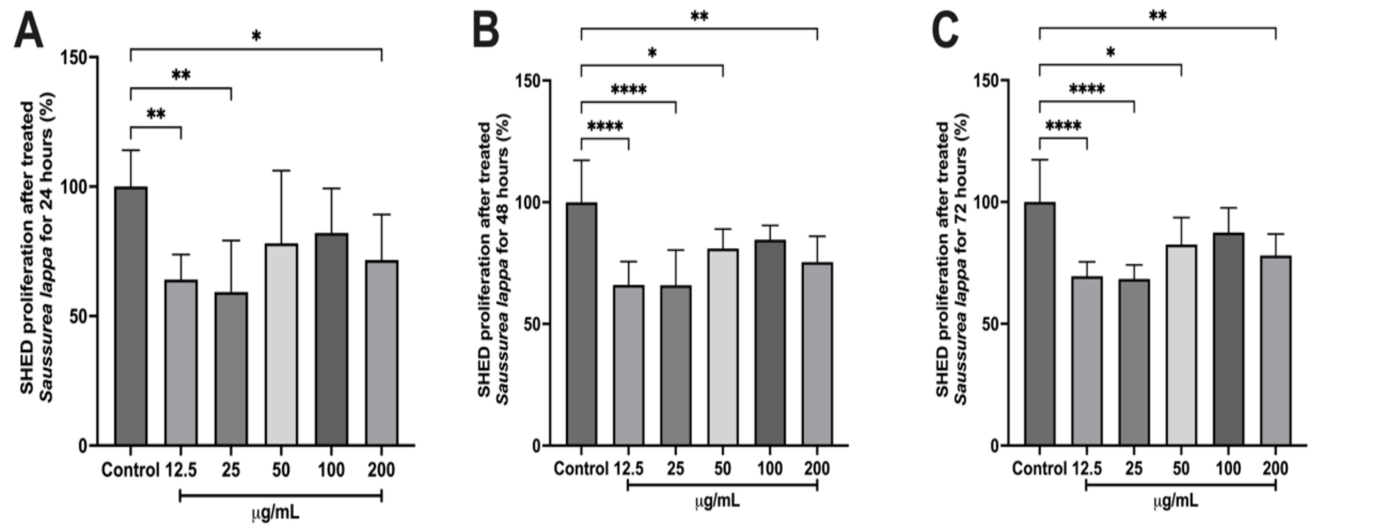

Figure 2: The effect of nanoparticles of *Saussurea lappa* on SHED proliferation at various concentrations after 24 hours (A), 48 hours (B), and 72 hours (C) of treatment. The bars represent the relative proliferation percentage compared to the control group, with significant differences indicated by asterisks: $p < 0.05$ (*), *$p < 0.01$ (**), and *$p < 0.0001$ (****). Data are presented as the mean ± standard deviation.

The dual effects nanoparticles of *Saussurea lappa* on HeLa cells and SHED can be explained by the bioactive components of *Saussurea lappa* and their specific mechanisms of action. *Saussurea lappa* is rich in sesquiterpene lactones, such as costunolide and dehydrocostus lactone, as well as alkaloids, flavonoids, and polyphenols.[1,6] These compounds are well-documented for their cytotoxic, anti-inflammatory, and antioxidant properties. In the case of HeLa cells, the nanoparticles exhibit potent cytotoxic effects, especially at higher concentrations (800 µg/mL and 400 µg/mL). The sesquiterpene lactones are known to induce apoptosis in cancer cells through multiple pathways. For example, they generate reactive oxygen species (ROS), which lead to oxidative stress, mitochondrial dysfunction, and subsequent activation of caspases that drive cell death.[18] Additionally, these compounds inhibit the NF-κB pathway, a critical signaling cascade that supports cancer cell survival and proliferation.[19–21] Since HeLa cells, like many cancer cells, are characterized by higher metabolic activity and reduced antioxidant defenses, they are particularly vulnerable to oxidative stress and apoptotic signaling induced by nanoparticles of *Saussurea lappa*, resulting in the significant decrease in cell viability observed at higher doses.
On the other hand, the inhibitory effects of nanoparticles of *Saussurea lappa* on SHED proliferation appear to be both dose- and time-dependent. While SHED cells are non-cancerous and generally less prone to oxidative stress, the bioactive compounds in the nanoparticles can still disrupt normal cellular processes. At lower concentrations (12.5 µg/mL and 25 µg/mL), the nanoparticles may interfere with cell cycle progression or signaling pathways like Wnt/β-catenin or MAPK, which are essential for cellular growth and proliferation.[22,23] These disruptions could explain the significant decrease in SHED proliferation observed even at low doses. Prolonged exposure to the nanoparticles, as seen in the 48- and 72-hour results, exacerbates these effects. Over time, the accumulation of ROS and sustained modulation of signaling pathways may overwhelm the cell's repair mechanisms, leading to cumulative damage and greater inhibition of proliferation. Higher concentrations (100 µg/mL and 200 µg/mL) amplify these effects further, resulting in pronounced cytotoxicity over extended periods.
The differences in response between HeLa cells and SHED can also be attributed to their inherent biological characteristics. Cancer cells like HeLa are more susceptible to oxidative damage and apoptosis due to their altered metabolic profiles and reliance on proliferative signaling pathways, which are specifically targeted by the bioactive compounds in *Saussurea lappa*. SHED cells, in contrast, have more robust mechanisms to withstand oxidative stress and maintain normal cellular functions, but prolonged exposure or higher doses of nanoparticles can still overwhelm these defenses. This dual behavior highlights the therapeutic potential of nanoparticles of *Saussurea lappa* as an anticancer agent, particularly against HeLa cells, while also emphasizing the need for caution due to their inhibitory effects on normal cells like SHED. To maximize its therapeutic efficacy and minimize off-target effects, further research is required to optimize the dose and duration of treatment, as well as to explore potential delivery systems that can enhance selectivity for cancer cells.

Conclusion

Nanoparticles of *Saussurea lappa* exhibit a dose-dependent cytotoxic effect on HeLa cancer cells, significantly reducing cell viability at higher concentrations (≥400 µg/mL), suggesting strong anticancer potential. In contrast, *Saussurea lappa*nanoparticles inhibit SHED proliferation in a concentration- and time-dependent manner, with lower concentrations exerting early inhibitory effects and higher concentrations showing cumulative suppression over 72 hours. These findings indicate that while *Saussurea lappa* nanoparticles may serve as a promising anticancer agent, their impact on normal stem cell proliferation warrants careful dose optimization for potential therapeutic applications.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Madhuri K, Elango K, Ponnusankar S. Saussurea lappa (Kuth root): review of its traditional uses, phytochemistry and pharmacology. Orient Pharm Exp Med. 2012;12(1):1–9.
2. Ali SI, Venkatesalu V. Botany, traditional uses, phytochemistry and pharmacological properties of Saussurea costus – An endangered plant from Himalaya- A review. Phytochem Lett. 2022;47:140–155.
3. Alnahdi HS, Danial EN, Elhalwagy MEA El, Ayaz NO. Phytochemical Studies, Antioxidant Properties and Antimicrobial Activities of Herbal Medicinal Plants Costus and Cidir Used in Saudi Arabia. Int J Pharmacol. 2017;13(5):481–487.
4. Rahman MdA, Hong J-S, Huh S-O. Antiproliferative properties of *Saussurea lappa* Clarke root extract in SH-SY5Y neuroblastoma cells via intrinsic apoptotic pathway. Anim Cells Syst (Seoul). 2015;19(2):119–126.
5. Ali S, Ahmed D. Comparing DES-mediated ultrasound- and heat-assisted extraction of bioactive metabolites from Saussurea lappa and optimization by RSM and validation studies. GAC. 2023;7:100080.
6. Urvashi, Kaur R. Saussurea Lappa C.B. Clarke: Kushta/Kut. In: Immunity Boosting Medicinal Plants of the Western Himalayas. Singapore: Springer Nature Singapore; 2023. p. 433–462.
7. Pavan Kumar Ch, Devi A, Ashok Yadav P, Rao Vadaparthi R, Shankaraiah G, Sowjanya P. "Click" reaction mediated synthesis of costunolide and dehydrocostuslactone derivatives and evaluation of their cytotoxic activity. J Asian Nat Prod Res. 2016;18(11):1063–1078.
8. Barrios FJ. Chemistry of Sesquiterpene Lactones. In: Sesquiterpene Lactones [Internet]. Cham: Springer International Publishing; 2018. p. 93–117.
9. Peasari J reddy, Motamarry S sri, Varma KS, Anitha P, Potti RB. Chromatographic analysis of phytochemicals in Costus igneus and computational studies of flavonoids. Inform Med Unlocked. 2018;13:34–40.
10. Park K-S, Kim H, Kim MK, Kim K, Chong Y. Synthesis and biological evaluation of flavonol-glucose conjugates for cosmeceutical development. J Korean Soc Appl Biol Chem [Internet]. 2015 Jun 11;58(3):317–323.
11. Kumar R, Patel SK, Verma A, Joshi GS, Bisen HK, Asrani RK. Saussurea costus (falc.) lipsch: A promising ally against cancer. PRENAP. 2024;5:100123.
12. Pandey MM, Rastogi S, Rawat AKS. Saussurea costus: Botanical, chemical and pharmacological review of an ayurvedic medicinal plant. J Ethnopharmacol. 2007;110(3):379–90.
13. Elshaer SE, Hamad GM, Sobhy SE, Darwish AMG, Baghdadi HH, H. Abo Nahas H. Supplementation of Saussurea costus root alleviates sodium nitrite-induced hepatorenal toxicity by modulating metabolic profile, inflammation, and apoptosis. Front Pharmacol. 2024;15: 1378249.
14. Kumar A, Kumar S, Maurya AK, Agnihotri VK. NMR Based Metabolic Profiling of *Saussurea lappa* Roots and Aerial Parts from Western Himalaya. Anal Chem Lett. 2020;10(4):428–441.
15. Mohd Nor NH, Mansor NI, Mohd Kashim MIA, Mokhtar MH, Mohd Hatta FA. From Teeth to Therapy: A Review of Therapeutic Potential within the Secretome of Stem Cells from Human Exfoliated Deciduous Teeth. Int J Mol Sci. 2023;24(14):11763.
16. Chunarkar-Patil P, Kaleem M, Mishra R, Ray S, Ahmad A, Verma D. Anticancer Drug Discovery Based on Natural Products: From Computational Approaches to Clinical Studies. Biomedicines. 2024;12(1):201-210.
17. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT Assay: Utility, Limitations, Pitfalls, and Interpretation in Bulk and Single-Cell Analysis. Int J Mol Sci. 2021;22(23):12827.
18. Mansour HH, Rifaat Ismael NES, Hafez HF. Ameliorative effect of septilin, an ayurvedic preparation against γ-irradiationinduced oxidative stress and tissue injury in rats. Indian J Biochem Biophys. 2014;51(2). 135-141.
19. Lim JS, Lee SH, Lee SR, Lim H-J, Roh Y-S, Won E. Inhibitory Effects of Aucklandia lappa Decne. Extract on Inflammatory and Oxidative Responses in LPS-Treated Macrophages. Molecules. 2020;25(6):1336.
20. Choi YK, Cho S-G, Woo S-M, Yun YJ, Jo J, Kim W. *Saussurea lappa* Clarke-Derived Costunolide Prevents TNF α -Induced Breast Cancer Cell Migration and Invasion by Inhibiting NF- κ B Activity. eCAM. 2013;2013:1–10.
21. Kim H-R, Kim J-M, Kim M-S, Hwang J-K, Park Y-J, Yang S-H. Saussurea lappa extract suppresses TPA-induced cell invasion via inhibition of NF-κB-dependent MMP-9 expression in MCF-7 breast cancer cells. BMC Complement Altern Med. 2014;14(1):140-170.
22. Dong G, Shim A-R, Hyeon JS, Lee HJ, Ryu J-H. Inhibition of Wnt/β-Catenin Pathway by Dehydrocostus Lactone and Costunolide in Colon Cancer Cells. Phytother Res. 2015;29(5):680–6.
23. Chio HC, Jeong NH. Effect of saussurea lappa root extract on proliferation and hair growth-related signal pathway in human hair follicle dermal papilla cells. Appli Chem for Eng. 2021;32(6). 647-652.