Cytotoxicity of Propolis Extracts obtained using Dichloromethane and Hexane Solvent on Human Salivary Gland Tumor Cell Line

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Aim: This in vitro study aimed to investigate the effect of propolis extracts from two different solvents on human submandibular salivary gland (HSG) tumor cell line. Materials and Methods: Propolis was extracted by dichloromethane (DCM) and hexane (HEX). Crude extracts were prepared from 6.25 to 200 µg/mL in Dulbecco’s modified eagle medium without serum. Flavonoid and total phenolic contents of crude extracts were measured using a modified colorimetric method. The cytotoxicity was evaluated by 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium (MTT) assay and lactate dehydrogenase (LDH) release assay. The statistics were analyzed by independent sample t-test. Results: Propolis extracts obtained using DCM and HEX exhibited comparable % yield (38.58 and 38.25) and physical characteristics and different amounts of flavonoid (0.439 ± 0.02 and 0.250 ± 0.01 mg catechin/g sample) and total phenolic compounds (3.759 ± 0.03 and 1.618 ± 0.03 mg gallic acid equivalents/g sample). The DCM group at 25, 50, 100, and 200 µg/mL as well as the HEX group at 50, 100, and 200 µg/mL significantly displayed a decrease in % cell viability and an increase in % cytotoxicity, compared with the untreated control group (P < 0.05). The DCM group showed the half-maximal inhibitory concentration (IC50) of MTT (42.93 ± 2.70) and LDH (34.94 ± 0.22). The HEX group showed the IC50 of MTT (61.30 ± 5.39) and LDH (42.32 ± 1.00). Propolis extracts obtained using both DCM and HEX are effective to inhibit HSG viability. Conclusion: Regarding to the cell morphological observation, MTT and LDH assays, propolis extracts obtained using DCM and HEX exhibited the cytotoxic effect on HSG tumor cell line. Based on our knowledge, this research demonstrates the first preliminary result suggesting propolis as a natural product of choice for salivary gland cancer prevention and therapy. Keywords: Cytotoxicity, dichloromethane, hexane, propolis, salivary gland tumor

INTRODUCTION

Head and neck cancer is one of the highly prevalent cancers worldwide and potentially increases due to changes in demographics and lifestyles. Various studies have demonstrated that salivary gland cancers are rare, constituting 3%-11% of all head and neck neoplasms and 0.2% of all malignancies. Nevertheless, it can cause patients’ low quality of life even after the complete salivary gland cancer treatment, resulting from the complications of salivary gland dysfunction.[1] Until now, the medical research has still been challenged to figure out an ideal cancer treatment. Using the natural product as a choice for cancer prevention and hopefully for a combined option with the conventional treatment is promising.

Propolis is a mixture of lipophilic materials collected from many plants and added to beeswax. Propolis from...
different sources demonstrates their main chemical components, which are flavonoids and phenolic acids.\(^{2,3}\) This compound has been reported for several biological activities such as antibacterial and antimitotic, wound-healing, anti-inflammatory, and antioxidant properties.\(^{4}\)

Propolis has also shown the anticancer property in cell culture and animal models.\(^{5,6}\) In addition, propolis exhibited the different effect in several cancer cell types because of a variety of solvents.\(^{7}\) The hexane (HEX) extract inhibited the proliferation of intestinal, breast, liver, lung, and gastric cancers.\(^{8}\) The water extract decreased the cell proliferation and induced the necrosis of colon cancer.\(^{9}\) The methanol extract diminished the growth of human pancreatic cancer cells.\(^{10}\) The ethanol extract inhibited the cell proliferation of prostate cancer cells, colon cancer, and malignant melanoma cells.\(^{11}\) Recently, the comparison of the effects of chloroform, HEX, and ethanol extracts on human colorectal cancer has shown that all extracts were able to inhibit cancer cells; however, the chloroform extract exhibited the most superior result.\(^{12}\) Surprisingly, there is few research investigating the effect of dichloromethane (DCM)- and HEX-extracted propolis on oral cancer cells. For example, the study by Utispan et al. has reported that DCM-extracted propolis displayed the cytotoxicity against primary and metastatic head and neck cancer cell lines.\(^{13}\) Recently, Węgowiec et al. have demonstrated the anticancer effect of Polish extract by ethanol and HEX-ethanol on human tongue cancer cells.\(^{14}\)

Although the anticancer property of propolis has been reported in several cancer cells, there is still a lack of knowledge about the propolis on salivary gland cancer, particularly mucoepidermoid carcinoma and adenoid cystic carcinoma, which are two types of common salivary gland cancers. Moreover, the current treatments consisting of surgery, radiotherapy, chemotherapy, or the combination still have a limited efficiency and can cause severe complication. Therefore, this study aimed to investigate the effect of propolis extracted by DCM and HEX on human salivary gland tumor cell line. The result from this study would preliminarily broaden a future opportunity to develop propolis as a natural product of choice for salivary gland cancer therapy.

**Materials and Methods**

**Propolis sample preparation**

Propolis was collected from honeybees (*Apis mellifera* in the *Dimocarpus longan* garden, Lamphun, the northern Thailand). It was crudely extracted by two solvents separately, DCM and HEX in 1:5 ratio (w/v). The solution was filtrated in a shaker incubator (Stuart, UK) with a speed of 100 rpm in the dark at the room temperature for 24h. The crude extracts were filtered and evaporated in a rotary evaporator. The extracts were lyophilized and kept in −20°C until use. The % yield was calculated as follows: (Actual weight of extract/total weight of propolis) × 100.

The stock solution (0.05 g/mL) was prepared in 100% dimethyl sulfoxide (DMSO) (Sigma-Aldrich, USA) and filtered through sterile syringe filter with a 0.2 μm diameter (Whatman, UK). Different concentrations of propolis solution were prepared by the two-fold dilution for 200, 100, 50, 25, 12.5, and 6.25 μg/mL in Dulbecco’s modified eagle medium (DMEM) (Gibco, USA) without serum mixed with 0.4% (v/v) DMSO.

**Estimation of flavonoid content**

The flavonoid content of the sample was determined according to the method of Wolfe et al.\(^{15}\) A volume of 0.25 mL of a known dilution of test sample was mixed with 1.25 mL of reverse osmosis (RO) water and 0.075 mL of 5% sodium nitrite solution and incubated at room temperature for 5 min. Then, 0.15 mL of 10% aluminum chloride was added. After 6 min, 0.5 mL of 1M sodium hydroxide was added, and the mixture was diluted with another 0.275 mL of RO water. The absorbance of the reaction mixture was measured at 510 nm with a Shimadzu spectrophotometer and compared with a standard curve of prepared catechin solutions in ethanol. Using the standard curve, the total flavonoid content was expressed as milligrams of catechin equivalents per 1 g sample (mg catechin/g sample).

**Estimation of total phenolic content**

The total phenolic contents of the sample were measured using a modified colorimetric Folin-Ciocalteu reagent according to the method of Wolfe et al.\(^{15}\) A volume of 125 μL of a known dilution of test sample was mixed with 500 μL of RO water and 125 μL of Folin-Ciocalteu reagent and incubated at room temperature for 6 min. Then, 1250 μL of 7% sodium carbonate and 1000 μL of RO water were added, and then the mixture was allowed to stand for 90 min. The absorbance of the reaction mixture was measured at 760 nm with a Shimadzu spectrophotometer and compared with a standard curve of prepared gallic acid solutions in ethanol. Using the standard curve, the total phenolic content was expressed as micrograms of gallic acid equivalents per 1 g sample (mg GAE/g sample).

**Cell culture**

Human submandibular salivary gland (HSG) cell line was cultured at 37°C, 5% CO\(_2\) in growth medium containing DMEM with 4.5 g/L glucose, L-glutamine, and sodium pyruvate, 10% fetal bovine serum, 100 units/
mL penicillin with 100 μg/mL streptomycin (Gibco, USA). In a 96-well culture plate, 10,000 cells/cm² were plated (Thermo scientific, China) in a growth medium at 37°C, 5% CO₂ for 24 h. The next day, cultured cells were treated in different concentrations of propolis extracted by various solvents for 24h. The experimental groups were propolis-treated cells, whereas the control group was untreated cells. The photographs of untreated and treated cells were taken before further analysis.

**MTT assay**

3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium (MTT) assay was performed with cells in a 96-well plate. Cells were washed with 100 μL of phosphate buffer saline (PBS) after the media removal. Cells were added to 200 μL of 0.05% MTT (Invitrogen, USA) in serum-free DMEM at 37°C, 5% CO₂ for 2h in dark. Then, cells were washed twice with 100 μL of PBS and the formazan crystals in cells were dissolled by 100 μL of DMSO. Next, the optical density (OD) at 540 nm was read by the microplate reader (BioTek, USA). The % cell viability was calculated as follows: [(OD treated−OD blank)/(OD untreated−OD blank)] × 100.

**LDH assay**

Lactate dehydrogenase (LDH) assay was conducted with cells in a 96-well plate by CytoTox 96 nonradioactive cytotoxicity assay (Promega, USA). The supernatant of cells was collected. From each sample, 50 μL of supernatant was mixed with 50 μL of assay buffer and incubated at the room temperature for 30 min in dark. Then, the reaction was stopped by adding 50 μL of stop solution before reading the OD at 490 nm. The % cytotoxicity was calculated as follows: (OD cell of LDH release−OD no cell of LDH release/OD maximum LDH release−OD no cell of LDH release) × 100.

**Statistical analysis**

The half-maximal inhibitory concentration (IC50) was calculated by using CurveExpert 1.4. The statistic was analyzed by SPSS version 18 with independent sample t-test to compare the differences between groups (propolis extract group versus untreated control group) at the 95% confidential interval.

**Results**

Propolis that was extracted by various solvents demonstrated the different % yield of dry weights and physical appearances [Figure 1]. The percent yields of DCM and HEX were 38.58% and 38.25%, respectively. The crude extracts from both solvents also exhibited sticky materials in yellow color. Propolis extracts obtained using DCM and HEX showed the flavonoid compound by 0.439 ± 0.02 and 0.250 ± 0.01 mg catechin/g sample, as well as the total phenolic compound by 3.759 ± 0.03 and 1.618 ± 0.03 mg GAE/g sample, respectively [Table 1]. Untreated cells (0 μg/mL or control group) were polyhedral, indicating the regular epithelial cell morphology [Figure 2A]. Treated cells with 6.25 and 12 μg/mL of both solvent groups did not show the morphological alteration (data not shown). Meanwhile, cells in the DCM group (25 μg/mL) [Figure 2B], but not in the HEX groups (25 μg/mL) [Figure 2F], were shrunken. Compared with the untreated cells [Figure 2A], cells treated with 50 μg/mL of DCM and

| Type of Solvent | % Yield | Physical Characteristics |
|-----------------|---------|--------------------------|
| Dichloromethane (DCM) | 38.58 | Sticky with dark yellow |
| Hexane (HEX) | 38.25 | Sticky with light yellow |

Figure 1: The % yield and physical characteristics of propolis obtained using from dichloromethane and hexane. The % yield is the ratio of the actual dry weight of the extract to the total weight of propolis, expressed as a percentage.
HEX extracts were round and shrunken [Figure 2C, G]. Noticeably, cells treated with propolis extracts (100 and 200 µg/mL) in both solvents showed the cell morphological changes [Figure 2D, E, H, I].

The HSG viability varies depending on the propolis concentrations and the types of solvent [Figure 3], which was shown by the MTT assay. Compared with the untreated cells, there is no statistical difference in the % cell viability of cells treated with 6.25 and 12.5 µg/mL of propolis in DCM and HEX. Interestingly, cells in the DCM group first showed a decrease in % cell viability at the 25 µg/mL with a significantly statistical difference. The reduction in % cell viability at the 50 µg/mL of DCM and HEX groups was observed. Apparently, the cell viability in the DCM and HEX groups gradually insignificantly declined with the dose-dependent manner. The IC50 of each extract is shown: DCM (42.93 ± 2.70) and HEX (61.30 ± 5.39) [Table 2].

The HSG toxicity varies depending on the propolis concentrations and the types of solvent [Figure 4]. As expected, the result of the LDH assay demonstrated the opposite trend compared with that of MTT assay. Compared with the untreated cells, there is no difference in % cytotoxicity among cells treated with low concentrations (6.25 and 12.5 µg/mL) of crude extracts.

Table 1: Flavonoid and total phenolic compound contents of propolis extracts from two different solvents

| Type of solvent | Flavonoid compound** | Total phenolic compound*** |
|----------------|----------------------|---------------------------|
| Dichloromethane| 0.439 ± 0.02         | 3.759 ± 0.03              |
| Hexane         | 0.250 ± 0.01         | 1.618 ± 0.03              |

**mg catechin/g sample  
***mg GAE/g sample. The measurements (n = 3) were done in triplicate

Table 2: Values of half-maximum inhibiting concentration (IC50) of propolis extracted by different solvents on HSG cells evaluated by MTT and LDH assays were shown

| Type of solvent | Inhibiting concentration (IC50) (µg/mL) |
|----------------|----------------------------------------|
|                | MTT         | LDH         |
| Dichloromethane| 42.93 ± 2.70 | 34.94 ± 0.22 |
| Hexane         | 61.30 ± 5.39 | 42.32 ± 1.00 |

MTT = 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium, LDH = lactate dehydrogenase

Figure 2: Morphological changes of human salivary gland were observed under inverted microscope. (A) Untreated group as a control, (B)–(E) dichloromethane (DCM) group, (F)–(I) hexane (HEX) group. Treated groups were cultured in different concentrations of propolis; scale bar = 50 µm

Figure 3: MTT assay demonstrates the % cell viability of propolis extracts on human salivary gland. Values from three independent experiments (n = 3) are shown as the percentage of mean ± standard deviation. *P < 0.05 compared to the untreated group

Figure 4: Toxicity assay: (A) Untreated group as a control, (B)–(E) dichloromethane (DCM) group, (F)–(I) hexane (HEX) group. Treated groups were cultured in different concentrations of propolis; scale bar = 50 µm
However, % cytotoxicity of cells in the DCM group exhibited a dose-dependent increase from 25 to 200 µg/mL. Cells in the HEX group at 50, 100, and 200 µg/mL presented approximately 60%–80% of cytotoxicity. The IC50 of each propolis extract is shown: DCM (34.94 ± 0.22) and HEX (42.32 ± 1.00) [Table 2].

**DISCUSSION**

This study is aimed to evaluate the cytotoxicity effect of propolis extracts by two different solvents, DCM and HEX, on HSG cell line using cell morphological changes, MTT and LDH assays. The HSG cell line, labeled as a submandibular ductal cell line, is commonly used as an in vitro model to study for salivary gland research. However, this cell line has recently been considered as HeLa derived by short tandem repeat (STR)-DNA profiling.[16] Nevertheless, the in vivo transplantation in mice showed these cells were able to generate a mass resembling salivary gland-like tumor.[17]

Compared with various concentrations of propolis extracts, 50, 100, and 200 µg/mL of DCM- and HEX-extracted propolis clearly demonstrated the cytotoxic effect on this cell line. However, the DCM group exhibited the superior outcome than the HEX group. The HSG demonstrated their morphological alteration, which was prominently observed in the high concentrations of propolis, 100 and 200 µg/mL. Nevertheless, HSG initially changed their morphology after treated with 25 µg/mL of propolis in the DCM group. Regarding IC50 of MTT and LDH assays, propolis extracts by both DCM and HEX were toxic to HSG cells, compared with the untreated control cells. Interestingly, we observed that the sensitivity of LDH assay was higher than that of MTT assay. Additionally, the DMSO toxicity to the cells might affect the interpretation of propolis’ anticancer property. Our experiment did not show any differences in % cell viability (data not shown) between untreated cells cultured in complete medium and complete medium plus 0.4% DMSO, indicating the nontoxicity of 0.4% DMSO. This suggests the anticancer effect due to the propolis composition.

The MTT assay demonstrated that each extract exhibited distinct viability of HSGs. The propolis extracts by DCM and HEX inhibited the HSG proliferation in different degrees. This may be due to different types and/or amounts of active compounds potentially extracted by each solvent. A previous study indicated that the anticancer chemical compositions of propolis were better extracted in the solvents with less polarity such as DCM, and with no polarity such as HEX, compared with water or ethanol, which are solvents with the high polarity.[18,19] Our results are similar to the study by Teerasripreecha et al. that crude extracts obtained using DCM and HEX were able to inhibit the proliferation of several types of cancer cells.[20] However, our study first reported the effects of different solvent-extracted propolis on HSG cancer cell line.

To better understand the effect of various solvent-extracted propolis inducing the cell death, LDH assay was exploited on HSG cells. The amount of LDH in the culture is directly proportional to the number of damaged cells.[21] DCM- and HEX-extracted showed an increased LDH activity with dose-dependent manner. Noticeably, the DCM-extracted propolis at 25 µg/mL exhibited the superiority to enhance the HSG damage, compared with the HEX-extracted propolis at the same concentration. However, the cell death mechanism of propolis-treated salivary gland tumor cells would be further addressed.

Previous studies have reported variable chemical compositions of propolis depending on geographical sources of propolis. These may influence the difference in active components and biological activity of propolis.[22] Propolis from Europe, North America, and Asia had high flavonoid and phenolic contents.[23] In contrast, Brazilian propolis showed high terpenoid and coumaric acid.[24] Nevertheless, various sources of propolis have still mostly shown the anticancer effect. For example, Chinese propolis showed the significant anticancer effect on breast cancer cells.[25] Portuguese propolis had the cytotoxicity on lung cancer cells.[26] Unexpectedly, there is no research on the effect of propolis extracts on salivary gland tumor cells. Therefore, our study first demonstrated that Thai propolis extracts inhibited HSG proliferation and also induced cell death, suggesting its anticancer property on salivary gland tumor cells.
Propolis extracts by various solvents displayed a variety of active components.[27] The common solvents used for extraction are water, methanol, ethanol, chloroform, DCM, ether, and acetone.[23] A study by Teerasripreecha et al. has revealed that the crude DCM and HEX sequential extracts of propolis exhibited antiproliferative activities across several cancer cell lines.[20] Consequently, we chose DCM and HEX to directly dissolve the propolis and examine their anticancer effect on salivary gland cancer cell line. In our study, DCM-extracted propolis had the percentage of yield comparable to that of HEX-extracted propolis. However, propolis extracts by DCM showed higher flavonoid and total phenolic compounds than those by HEX. Our results are similar to Sambou et al. that the yield of propolis and total phenolic and flavonoid contents of DCM were higher than those of HEX.[28] This may explain why propolis in the DCM group exhibited lower IC50 than that in the HEX group.

Several pharmacological compounds in propolis and their anticancer mechanisms have been reported.[4,7,10,29,30] Flavonoid (e.g., quercetin, chrys, naringenin) and total phenolic compounds (e.g. caffeic acid phenethyl ester [CAPE] and p-coumaric acid) from propolis have been shown to diminish the in vitro cytotoxicity in human tumor cells and tumors in animals.[11] There are accumulating reports that many flavonoids exert anticancer activity, and the molecular mechanisms responsible for this effect have been studying.[32] CAPE suppressed human oral cancer cell proliferation by modulating tumor suppressor genes and downregulating the oncogenes and survival via inhibiting Akt signaling.[33] CAPE also exhibited potent therapeutic activity by decreasing cyclooxygenase-2 (COX-2) expression and showed immunomodulation, resulting in a suppression of prostaglandin-2 synthesis in human oral epidermal carcinoma KB cells.[34] In addition, CAPE induced apoptosis by altering the pro-and antiapoptotic protein expression as well as kinase C modulation and inhibited tyrosine kinase signaling.[35] Cardanol and cardol, which are phenolic compounds, exhibited as cytotoxic constituents in Thai propolis.[20] Consequently, we conducted the phytochemical testing to demonstrate the presence of both flavonoid and total phenolic components. The result suggests that the ability of propolis to inhibit salivary gland cancer cell line may come from the differences between two extracts, giving us a hint why DCM extract seems better. Nevertheless, the active ingredient of propolis affecting the salivary gland tumor cells needs to be further explored.

**Conclusion**

Based on the cell morphological observation, MTT and LDH assays, propolis extracts obtained using DCM and HEX exhibited the cytotoxic effect on HSG tumor cell line by affecting HSG viability and cell death in different manners depending on its concentrations.

**Future scope/clinical significance**

The solvent type and concentration may influence the type and different amounts of active components in propolis. The further study is essential to identify the biological effect of active components in extracts by different solvents in salivary gland tumor cells. This would be applied for a future research and therapeutic strategy in salivary gland cancer. For instance, animal models may be used to demonstrate the effect of propolis on the prevention and treatment of salivary gland lesions. As the prevention purpose, the animals should be medicated with propolis before creating the lesion in the salivary gland. As the therapeutic purpose, the animal would be medicated after the lesion generated. Our current research is the first preliminary study providing the knowledge to use propolis as a natural product of choice for salivary gland cancer prevention and therapy.

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**Conflicts of interest**

There are no conflicts of interest to be declared.

**Authors’ contributions**

Jirattikarn Kaewmuangmoon: Concept, design, investigation, data analysis; Kanokwan Charoonpatrapong: Writing-review and editing, validation; Kajohnkiart Janebodin: Data analysis, writing original draft, review and editing, validation.

**Ethical policy and institutional review board statement**

Not applicable.

**Patient declaration of consent**

Not applicable.

**Data availability statement**

Not applicable.
REFERENCES

1. Lin HH, Limesand KH, Ann DK. Current state of knowledge on salivary gland cancers. Crit Rev Oncog 2018;23:139-51.
2. Bankova V. Chemical diversity of propolis and the problem of standardization. J Ethnopharmacol 2005;100:114-7.
3. de Groot AC. Propolis: A review of properties, applications, chemical composition, contact allergy, and other adverse effects. Dermatitis 2013;24:263-82.
4. Anjum SI, Ullah A, Khan KA, Attaullah M, Khan H, Ali H, et al. Composition and functional properties of propolis (bee glue): A review. Saudi J Biol Sci 2019;26:1695-703.
5. Orsolić N, Basić I. Antitumor, hematostimulative and radioprotective action of water-soluble derivative of propolis (WSPDP). Biomed Pharmacother 2005;59:561-70.
6. Orsolić N, Sver I, Terzić S, Tadić Z, Basić I. Inhibitory effect of water-soluble derivative of propolis and its polyphenolic compounds on tumor growth and metastasizing ability: A possible mode of antitumor action. Nutr Cancer 2003;47:156-63.
7. Król W, Bankova V, Sforcin JM, Szlizszka E, Czuba Z, Kuropanicki AK. Propolis: Properties, application, and its potential. Evid Based Complement Alternat Med 2013;2013:807578.
8. Umthong S, Puthong S, Chanchao C. Trigona laeviceps propolis from Thailand: Antimicrobial, antiproliferative and cytotoxic activities. Am J Chin Med 2009;37:855-65.
9. Sulaiman GM, Ad’hiah AH, Al-Sammarrae KW, Bagnati R, Frapolli R, Bello E, et al. Assessing the anti-tumour properties of Iraqi propolis in vitro and in vivo. Food Chem Toxicol 2012;50:1632-41.
10. Premtranachai P, Chanchao C. Review of the anticancer activities of bee products. Asian Pac J Trop Biomed 2014;4:337-44.
11. Kubina R, Kabała-Dzik A, Dziedzic A, Bielec B, Wojtyczka RD, Kuropatnicki AK. Propolis: Properties, application, and its potential. Evid Based Complement Alternat Med 2013;2013:807578.
12. Valença I, Morais-Santos F, Miranda-Gonçalves V, Ferreira AM, Almeida-Aguiar C, Baltazar F. Portuguese propolis disturbs glycolytic metabolism of human colorectal cancer in vitro. BMC Complement Altern Med 2013;13:184.
13. Ulispan K, Chithkul B, Koontongkaew S. Cytotoxic activity of propolis extracts from the stingless bee Trigona sirindhornae against primary and metastatic head and neck cancer cell lines. Asian Pac J Cancer Prev 2013;14:1051-5.
14. Węgowiec J, Wieczynska A, Wiekiewicz W, Kulbacka J, Saezko J, Pachura N, et al. Polish propolis-chemical composition and biological effects in tongue cancer cells and macrophages. Molecules 2020;25:2426.
15. Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. J Agric Food Chem 2003;51:609-14.
16. Lin LC, Elkashty O, Ramamoorthy M, Trinh N, Liu Y, Sunavala-Dossabhoy G, et al. Cross-contamination of the human salivary gland HSG cell line with HeLa cells: A STR analysis study. Oral Dis 2018;24:1477-83.
17. Janebodin K, Reyes M. Neural crest-derived dental pulp stem cells function as ectomesenchyme to support salivary gland tissue formation. Dentistry 2012;S13:001.
18. Zuorro A, Iannone A, Lavecchia R. Water-organic solvent extraction of phenolic antioxidants from Brewers‘ spent grain. Processes 2019;7:126.
19. Pukklay P, Chuesaard T. Total phenolic, flavonoid contents and antioxidant activity of propolis extracts from Nan province. PSRU J Sci Technol 2021;6:13-27.
20. Teerassripreecha D, Phuwapraisiran P, Puthong S, Kimura K, Okuyama M, Mori H, et al. In vitro antiproliferative/cytotoxic activity on cancer cell lines of a cardanol and a cardol enriched from Thai Apis mellifera propolis. BMC Complement Altern Med 2012;12:27.
21. Specian AF, Serpelon MJ, Tuttis K, Ribeiro DL, Ciliaó HL, Varanda EA, et al. LDH, proliferation curves and cell cycle analysis are the most suitable assays to identify and characterize new phytotherapeutic compounds. Cytotechnology 2016;68:2729-44.
22. Ahangari Z, Naseri M, Vatandoost F. Propolis: Chemical composition and its applications in endodontics. Iran Endod J 2018;13:285-92.
23. Wagh VD. Propolis: A wonder bees product and its pharmacological potentials. Adv Pharmacol Sci 2013;2013:308249.
24. Kumazawa S, Yoneda M, Shibata I, Kanaeda J, Hamasaka T, Nakayama T. Direct evidence for the plant origin of Brazilian propolis by the observation of honeybee behavior and phytochemical analysis. Chem Pharm Bull (Tokyo) 2003;51:740-2.
25. Xuan H, Li Z, Yan H, Sang Q, Wang K, He Q, et al. Antitumor activity of Chinese propolis in human breast cancer MCF-7 and MDA-MB-231 cells. Evid Based Complement Alternat Med 2014;2014:280120.
26. Demir S, Alyazicioglu Y, Turan I, Misir S, Mentese A, Yaman SO, et al. Antiproliferative and proapoptotic activity of Turkish propolis on human lung cancer cell line. Nutr Cancer 2016;68:165-72.
27. Suran J, Cepanci E, Masek T, Radić B, Radić S, Tlak Gajger I, et al. Propolis extract and its bioactive compounds—From traditional to modern extraction technologies. Molecules 2021;26:2930.
28. Sambou M, Jean-François J, Ndongou Moutombi FJ, Doiron JA, Hebert MPA, Joy AP, et al. Extraction, antioxidant capacity, 5-lipoxygenase inhibition, and phytochemical composition of propolis from Eastern Canada. Molecules 2020;25:2397.
29. Abbasi AJ, Mohammad M, Bayat M, Gema SM, Ghadirian H, Seifi H, et al. Applications of propolis in dentistry: A review. Ethiop J Health Sci 2018;28:505-12.
30. Khuaphiwat S, Tragoolpua Y. Antioxidant and anti-cancer cell proliferation activities of Chinese propolis extracts against primary and metastatic head and neck cancer cell lines. Asian Pac J Cancer Prev 2013;14:6991-5.
31. Vagish Kumar LS. Propolis in dentistry and oral cancer management. N Am J Med Sci 2014;6:250-9.
32. Kopustinskiene DM, Jakstas V, Savickas A, Bernatoniene J. Flavonoids as anticancer agents. Nutrients 2020;12:1-25.
33. Kuo YY, Lin HP, Hsu C, Su LC, Yang J, Hsiao PH, et al. Caffeic acid phenethyl ester suppresses proliferation and survival of TW2.6 human oral cancer cells via inhibition of Akt signaling. Int J Mol Sci 2013;14:8801-17.
34. Chiu H, Yang C, Chi H, Han Y, Shen Y, Venkatarkrishnan K, et al. Cyclooxygenase-2 expression in oral precancerous and cancerous conditions and its inhibition by caffeic acid phenethyl ester-enriched propolis in human oral epithelial carcinoma KB cells. Arch Bio Chem 2017;69:83-91.
35. Yu HJ, Shin JA, Yang HW, Won DH, Ahn CH, Kwon HJ, et al. Apoptosis induced by caffeic acid phenethyl ester in human oral cancer cell lines: Involvement of PUMA and Bax activation. Arch Oral Biol 2017;84:94-9.