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Effects of *Platostoma palustre* ethanolic extracts and commercial herbal tea on the cell viability of colorectal cancer cells

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Abstract

Platostoma palustre jelly is a traditional food. *Platostoma palustre* has been used as folk medicine and is effective against heat-shock, hypertension and diabetes. Therefore, the aim of this study was to determine the effects of ethanolic extracts and commercial herbal tea of *Platostoma palustre* in inhibiting colorectal cancer cell viability. The ethanolic extracts of *Platostoma palustre* by using 90% ethanol for extraction. In this study, 2-fold serial dilution of 100 mg/mL *Platostoma palustre* extracts were applied. On other hand, the same dilution fold was also performed for 100% commercial herbal tea with *Platostoma palustre*. Additionally, CT-26 and HT-29 colorectal cancer cell lines were also used in this study. After co-culturing for 24 hours, the cell viability of CT-26 and HT-29 colorectal cancer cell lines were performed by using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. According to these data, the 1.56-100 mg/mL *Platostoma palustre* extracts possessed the significant inhibition effects of CT-26 colorectal cancer cell viability. The 3.13-100% commercial herbal tea with *Platostoma palustre* possessed the significant inhibition effects of CT-26 colorectal cancer cell viability. The 6.25-100 mg/mL *Platostoma palustre* extracts possessed the significant inhibition effects of HT-29 colorectal cancer cell viability. The 25-100% commercial herbal tea with *Platostoma palustre* possessed the significant inhibition effects of HT-29 colorectal cancer cell viability. However, the 0.39-3.13 mg/mL *Platostoma palustre* extracts possessed the significant promoting effects of HT-29 colorectal cancer cell viability. The 0.39-12.5% commercial herbal tea with *Platostoma palustre* also possessed the significant promoting effects of HT-29 colorectal cancer cell viability. Comparison of CT-26 and HT-29 cell lines was on the cell viability after *Platostoma palustre* ethanolic extracts and commercial herbal tea treatments, CT-26 cell line was better sensitive than HT-29 cell line on the inhibition of cell viability after treatment of *Platostoma palustre* ethanolic extracts and the commercial herbal tea. Taken these results together, *Platostoma palustre* ethanolic extracts and commercial herbal tea may have a potential for inhibiting the growth of colorectal cancer cells.

Keywords: Cell viability; Colorectal cancer; Commercial herbal tea; Ethanolic extraction; *In vitro; Platostoma palustre* extracts

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1. Introduction

Cancer is a major public health problem worldwide. The top 10 cancer types for estimated deaths in Taiwan in 2020 were in order as lung cancer, liver cancer, colorectal cancer, breast cancer (female), prostate cancer, oral cavity cancer, pancreatic cancer, stomach cancer, esophageal cancer, and ovarian cancer. Therefore, the research R&D of novel anti-tumor drugs and other therapeutic strategies are urgently needed [1-3].

Platostoma palustre is an annual plant that is mainly distributed in tropical and subtropical regions, including Taiwan, Indonesia, Vietnam, southern China, and Burma [4-6]. The related food with *Platostoma palustre* as tea, herbal jelly, and sweet soup with herbal jelly are popular during the summer. Additionally, the heated herbal jelly with *Platostoma palustre* is admired by many Taiwanese in winter. *Platostoma palustre* has been used as folk medicine. *Platostoma palustre* has been verified that possessed many functional compounds such as polysaccharides (gum) with a unique aroma and texture, sterol compounds, stigmasterol, α-sitosterol, tripterpene compounds, oleanolic acid, volatile compounds (caryophyllene oxide, α-caryophyllene, eugenol, benzene acetaldehyde, and 2,3-butanedione etc), essential oil (n-hexadecanoic acid, linoleic acid, and linolenic acid), volatile oil (chavibetol, n-hexadecanoic acid, and α-cadinol), and ursolic acid [4-8]. Therefore, theses functional compounds of *Platostoma palustre* have indicated that many biological effects is effective against and attenuating the metabolic syndrome, heat-shock, hypertension, diabetes, liver disease, muscle and/or joint pains, hyperglycemia, inflammation, oxidant activity, free radical scavenging effects, acute and chronic hepatitis, and caner growth [9-12].

The objective of this study was to evaluate the effects of *Platostoma palustre* extracts and commercial herbal tea on the cell viability of colorectal cancer cells. Therefore, we hypothesize that *Platostoma palustre* extracts via 90% ethanolic extraction and commercial herbal tea with *Platostoma palustre* can be effective in inhibiting CT-26 and HT-29 colorectal cancer cell viability *in vitro*. In addition, we also want to compare the tumoral inhibition abilities between CT-26 and HT-29 colorectal cancer cell lines after treating with *Platostoma palustre* extracts via 90% ethanolic extraction and commercial herbal tea with *Platostoma palustre*.

2. Material and methods

2.1. Cell Lines and Culture Condition

CT-26 cells (ATCC® CRL-2638™) and HT-29 (ATCC® HTB-38™) were purchased from ATCC (Manassas, VA 20110). McCoy's 5a medium, RPMI-1640 medium, fetal bovine serum (FBS), and antibiotics (penicillin and streptomycin) were purchased from Sigma-Aldrich. CT-26 cells were cultured in RPMI-1640 medium and HT-29 cells were cultured in McCoy's 5a medium. Both McCoy's 5a medium and RPMI-1640 medium was supplemented with 10% FBS and 1% penicillin and streptomycin. The cells were incubated at 37°C with 5% CO_2. Cells were sub-cultured to replace flesh media per 2-3 days when they became confluent.

2.2. Source of Dried *Platostoma palustre* and Commercial Herbal Tea

The dried *Platostoma palustre* were collected from the traditional markets in Miaoli, Taiwan. The commercial herbal tea with *Platostoma palustre* were also purchased (Guanxi, Hsinchu, Taiwan).

2.3. Chemical and Reagents

Cell viability assay kit [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; MTT] was purchased from abcam®.

2.4. 90% Ethanolic Extraction

10-15 g samples of *Platostoma palustre* powder were soaked for 24 h at room temperature with 200 mL of 90% ethanol, followed by filtration through Whatman #1 filter paper. The filtrates were then evaporated in vacuo to dryness and weighed to determine the yields.

2.5. Cell Viability Assay

CT-26 and HT-29 cells (5×10^4/mL) were initially incubated for 24 h in a 96 well plate, respectively. The *Platostoma palustre* extracts and commercial herbal tea with *Platostoma palustre* were respectively diluted with culture media. Two-fold serial dilution of 100 mg/mL *Platostoma palustre* extracts were applied (0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100 mg/mL). On other hand, the commercial herbal tea with *Platostoma palustre* was also performed 2-fold

serial dilution (0, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, and 100%). After 24 h-experimental point, cell viability was detected by MTT cell viability assay kit. The reduced purple dye intensity of color was estimated by reading at optical density 570 nm in a spectrophotometer.

2.6. Statistical Analysis

The data were expressed as mean ± SD. All comparisons were made by one-way ANOVA and all significant differences are reported at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

3. Results

3.1. Suppression of Colorectal Cancer Cell Viability after Treatments of *Platostoma palustre* Ethanolic Extracts and Commercial Herbal Tea with *Platostoma palustre*

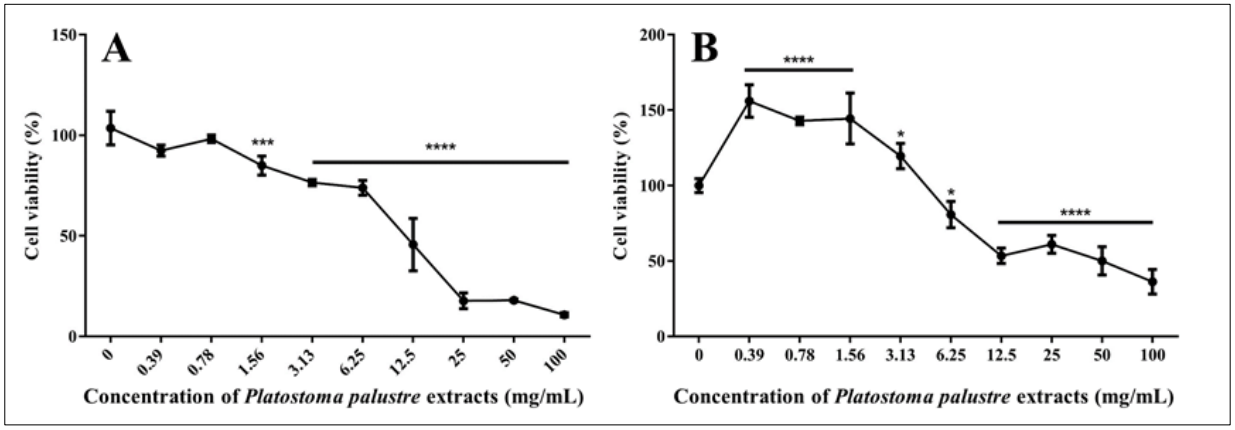

Figure 1 Inhibition cell viability abilities of colorectal cancer cell lines (CT-26 and HT-29) after *Platostoma palustre* extract treatment (24 hours). (A) CT-26 colorectal cancer cell line. (B) HT-29 colorectal cancer cell line. All data are expressed as mean ± SD for three replicates. All significant differences compared to each other after *Platostoma palustre* extract treatment were reported at $^{*}p < 0.05$, $^{***}p < 0.001$, and $^{****}p < 0.0001$

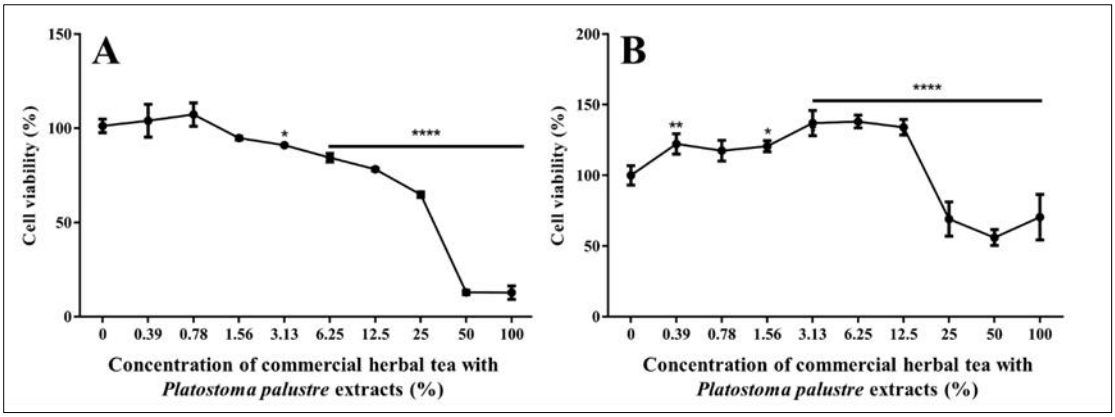

Figure 2 Inhibition cell viability abilities of colorectal cancer cell lines (CT-26 and HT-29) after the commercial herbal tea with *Platostoma palustre* treatment (24 hours). (A) CT-26 colorectal cancer cell line. (B) HT-29 colorectal cancer cell line. All data are expressed as mean ± SD for three replicates. All significant differences compared to each other after the commercial herbal tea with *Platostoma palustre* treatment were reported at $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$

The ethanolic extracts of *Platostoma palustre* by using 90% ethanol for extraction. In this study, 2-fold serial dilution of 100 mg/mL *Platostoma palustre* extracts were applied. On other hand, the commercial herbal tea with *Platostoma palustre* was also performed 2-fold serial dilution. Additionally, CT-26 and HT-29 colorectal cancer cell lines were also used in this study. After co-culturing for 24 hours, the cell viability of CT-26 and HT-29 colorectal cancer cell lines were

performed by using MTT assay. According to these data, the 1.56-100 mg/mL *Platostoma palustre* ethanolic extracts possessed the significant inhibition effects of CT-26 colorectal cancer cell viability (Fig. 1A). The 3.13-100% commercial herbal tea with *Platostoma palustre* possessed the significant inhibition effects of CT-26 colorectal cancer cell viability (Fig. 1B). The 6.25-100 mg/mL *Platostoma palustre* extracts possessed the significant inhibition effects of HT-29 colorectal cancer cell viability (Fig. 2A). The 25-100% commercial herbal tea with *Platostoma palustre* possessed the significant inhibition effects of HT-29 colorectal cancer cell viability (Fig. 2B). However, 0.39-3.13 mg/mL *Platostoma palustre* extracts possessed the significant promoting effects of HT-29 colorectal cancer cell viability (Fig. 2A). The 0.39-12.5% commercial herbal tea with *Platostoma palustre* also possessed the significant promoting effects of HT-29 colorectal cancer cell viability (Fig. 2B).

4. Discussion

The incidence and mortality of cancer are rapidly growing worldwide. The reasons are complex and both aging and growth of the population are related with the prevalence and distribution of the main risk factors for cancer. World Health Organization (WHO) in 2015 reported that cancer is the first or second leading cause of death in 91 of 172 countries. WHO lists cancer was one of top 10 threats to people health. Nearly, 10 million people die of cancer each year worldwide. Additionally, some reports presented that the global cancer cases will be increase to 60% in 2040. There may be nearly 29.4 million new cases of cancer each year [1-3]. In Taiwan in 2020, the top 5 cancers among Taiwanese are lung cancer, liver cancer, colorectal cancer, female breast cancer, and prostate cancer. Cancer is also a threat to the lives of people in Taiwan. Therefore, the research R&D of novel anti-tumor drugs and other therapeutic strategies are urgently needed.

Previously, the effect of different ethanolic concentrations on antioxidant properties and cytoprotective activities of *Platostoma palustre* has been verified. The antioxidant activities of *Mesona procumbens* ethanolic extracts which displayed variable antioxidant levels. The 60% *Mesona procumbens* ethanolic extracts exhibited higher antioxidant activities that possessed a protective capability for the biological membrane system to prevent and treat oxidative stress-related disorders [13-16]. In this study, 90% *Platostoma palustre* ethanolic extracts were used. The bio-functional indexes and concentrations of 90% *Platostoma palustre* ethanolic extracts were chlorogenic acid (0.30 mg/mg 90% *Platostoma palustre* ethanolic extracts), caffeic acid (0.70 mg/mg 90% *Platostoma palustre* ethanolic extracts), rutin (0.80 mg/mg 90% *Platostoma palustre* ethanolic extracts), astragaloside IV (7.50 mg/mg 90% *Platostoma palustre* ethanolic extracts), and rosmarinic acid (15.90 mg/mg 90% *Platostoma palustre* ethanolic extracts). Among of these bio-functional indexes of 90% *Platostoma palustre* ethanolic extracts, the contents of astragaloside IV and rosmarinic acid were higher than others. Many literatures have been demonstrated that astragaloside IV and rosmarinic acid possessed anti-tumor abilities. Therefore, we want to understand the effects of *Platostoma palustre* extracts and commercial herbal tea on the cell viability of colorectal cancer cells. *In vitro* study showed that comparison of CT-26 and HT-29 cell lines was on the cell viability after *Platostoma palustre* ethanolic extracts and commercial herbal tea treatment, CT-26 cell line was better sensitive than HT-29 cell line on the inhibition of cell viability after treatment of *Platostoma palustre* ethanolic extracts and commercial herbal tea.

5. Conclusion

Platostoma palustre has been used as a folk medicine and is effective against heat-shock, hypertension and diabetes. Therefore, the aim of this research was to determine the effects of ethanolic extracts and commercial herbal tea of *Platostoma palustre* in inhibiting colorectal cancer cell viability. Comparison of CT-26 and HT-29 cell lines was on the cell viability after *Platostoma palustre* ethanolic extracts and commercial herbal tea treatment, CT-26 cell line was better sensitive than HT-29 cell line on the inhibition of cell viability after treatment of *Platostoma palustre* ethanolic extracts and commercial herbal tea. Taken these results together, *Platostoma palustre* ethanolic extracts and commercial herbal tea may have a potential for inhibiting the growth of colorectal cancer cells. We hope that *Platostoma palustre* ethanolic extracts will be more deeply researched in the R&D of new anti-colorectal cancer drug in the future.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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