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Effect of gardenia tea on apoptosis of human thyroid cancer cell line SW579 through PI3K-Akt pathway

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Abstract

The purpose of this study was to observe its in vitro inhibitory effect on human thyroid cancer cell line SW579 through PI3K-Akt pathway. In this study, the survival of cells and the inhibitory effect of gardenia tea extract (GTE) on SW579 cancer cells were firstly observed by MTT method. Further qPCR experiments were used to observe mRNA expression in SW579 cancer cells. The active compound composition in the GTE was then also determined by HPLC. The experimental results showed that GTE had almost no effect on normal primary thyroid cells and had no toxic effect, but GTE could significantly inhibit the proliferation of SW579 cancer cells. The detection of mRNA expression in SW579 cancer cells showed that GTE could up-regulate the expressions of Bax, caspase-3, caspase-8 and caspase-9 and down-regulate the expressions of Bcl-2, Bcl-xL, PI3K and Akt in SW579 cancer cells. The chemical composition test showed that GTE mainly contains three compounds, namely rutin, quercetin and phloretin. It can be seen that gardenia tea can regulate the PI3K-Akt pathway of SW579 cancer cells through the effective active substances contained in it, and exert its anti-cancer effect. It is a health-care tea with good anti-cancer effect.

Keywords: gardenia tea; human thyroid cancer cell line SW579; functional food; anticancer; PI3K-Akt pathway.

Practical Application: Gardenia tea, as a traditional health drink, has the effect of improving immunity, but its specific mechanism of action and quantitative efficacy lack effective proof. In this study, the anti-cancer effect of gardenia tea was verified by in vitro experiments, and the mechanism of action was clarified, which is conducive to the better promotion and utilization of gardenia tea.

1 Introduction

Gardenia (scientific name: *Gardenia jasminoides* Ellis) is the fruit of the *Rubiaceae* plant *Gardenia*, which is often used to make beverages in China to make gardenia tea, and is also a commonly used plant in traditional Chinese medicine. Gardenia tea is traditionally known as a drink to prevent symptoms of fever, jaundice, blood heat and vomiting (Chen et al., 2017). Modern research has further confirmed that gardenia tea has a choleretic effect, can promote bile secretion, and can reduce blood bilirubin, which can promote rapid excretion of bilirubin in blood; it has inhibitory effects on hemolytic streptococcus and dermatophytes; it has antipyretic effects, analgesic, sedative, antihypertensive and hemostatic effects (Deng & Sun, 2021).

Thyroid cancer is a malignant tumor, and it has a high chance of returning to normal after surgical treatment. Thyroid cancer is one of the tumors with the best treatment effect of systemic malignant tumors. Therefore, prevention and adjuvant therapy are necessary (Nix et al., 2005). In traditional Chinese medicine, natural plants are often used as a treatment for thyroid cancer, but the effect of cure has not yet been achieved. Combined with Western medical surgery, some natural plant-derived health foods can play a good role in adjuvant therapy. Kiwi fruit, shiitake mushrooms, *Ficus carica* Linn., *Dioscorea polystachya* Turczaninow etc. have all been proven to have preventive or adjuvant therapeutic effects on thyroid cancer (Zhang et al., 2020; Wang et al., 2021).

This study also focused on gardenia tea, a traditional health-care tea, to observe the effect of gardenia tea on thyroid cancer cells cultured in vitro. At the same time, the role of gardenia tea on the PI3K-Akt pathway was observed and verified by molecular biology methods. The purpose of this study is to accumulate theoretical basis for the further application of gardenia tea through research, so as to facilitate the better development and utilization of gardenia tea in health food.

2 Materials and methods

2.1 Gardenia tea extract

The gardenia tea (Fujian Life Element Technology Co., Ltd., Quanzhou, Fujian, China) was freeze-dried and then pulverized, 100 g of the pulverized sample was taken out, 1 L of distilled water was added to the sample powder, and extracted at 90 °C for 1 h. After filtration, the filter residue

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was taken out and 1 L of distilled water was added to extract once. The two extracts were combined and the extract was collected by 732 exchange resin for 3 h. Finally, the extract was evaporated to dryness by rotary evaporation to obtain gardenia tea extract (GTE).

2.2 Culture of human normal thyroid cells

Normal thyroid tissue was obtained from the para-adenoma tissue during surgery for benign thyroid adenomas, routine thyroid cell primary culture. The isolated thyroid tissue was removed from the capsule and connective tissue, washed with PBS, cut into 1 mm^3 pieces, digested with 2.5 g/L trypsin and 5 g/L collagenase at 37 °C for 90-120 min, and collected every 30 min. The cells were filtered and centrifuged through a 150-mesh stainless steel sieve and then cultured in dulbecco's minimum essential medium (DMEM, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) medium containing 100 mL/L fetal bovine serum. The next day, the medium was changed, and thyroid stimulating hormone (TSH) containing 1 mu/mL was added. The cells were used for experiments after the cells were in a subconfluent state (Zhang et al., 2008).

2.3 MTT assay to determine the survival rate of thyroid cancer cells

Primary normal human thyroid cells and SW579 thyroid cancer cells (Ningbo Mingzhou Biotechnology Co., Ltd., Ningbo, Zhejiang, China) were inoculated in DMEM liquid medium containing 10% inactivated calf serum, and then placed in a CO_2 incubator for culture. After one week of culture, the upper layer of DMEM medium was aspirated and discarded, 0.05% Trypsin-EDTA (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was used to digest the adherent cells, the cells were collected by centrifugation at 3000 rpm/min, and then the cells were added to DMEM medium to prepare a concentration of 1×10^4 cells/mL of cell suspension, and inoculated 200 μL per well in a 96-well culture plate, continued to culture in a CO_2 incubator for 24 h, and discarded the upper liquid medium after the cells adhered to the wall, and then added to each well. 200 μL GTE (20, 40 and 80 mg/mL) of different concentrations were incubated for 48 h.

2.4 Detection of mRNA expression in cells by quantitative polymerase chain reaction (qPCR) method

Trizol reagent (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) was used to extract total RNA from cells, and then the total RNA concentration of each treatment group was adjusted to 1 μg/μL (Nano-300, Hangzhou Allsheng Instruments Co., Ltd., Zhejiang, China). Precisely add 2 μg of RNA extracted from each group to a PCR test tube containing 1 μL each of oligodT18, RNase, dNTP and MLV enzymes, and 10 μL of 5 × Buffer to synthesize cDNA (Thermo Fisher Scientific, Waltham, MA, USA). The cells were synthesized at 4 °C for 3 min, and then the expression level was detected by qRT-PCR (StepOne Plus, Thermo Fisher Scientific). Then, 2 μL of cDNA was synthesized, 1 μL of upstream and downstream primers at a concentration of 10 μmol/L, 10 μL of SYBR Premix Ex Taq II (2×), 0.4 μL of ROX reference Dye (50 ×), and 5.6 μL of Sterilized double-distilled water was mixed, and then placed in a quantitative PCR machine for the reaction (Hu et al., 2022; Long et al., 2022).

2.5 Determination of the main components of GTE by High Performance Liquid Chromatography (HPLC)

The diluted 10 μL GTE solution was pushed into the machine by the autosampler for detection. The chromatographic column was an Accucore C18 column (2.6 μm, 4.6 × 150 mm); the mobile phase A was 0.5% acetic acid water and the mobile phase B was acetonitrile; the flow rate was 0.6 mL/min, the column temperature is 35 °C, and the detection wavelength was 285 nm (UltiMate 3000, Thermo Fisher Scientific).

2.6 Statistical analysis

The mean of the results of three parallel experiments was analyzed by one-way ANOVA method using SAS9.1 statistical software to analyze whether the data in each group had significant differences ($p < 0.05$).

3 Results

3.1 Effects of GTE on in vitro survival of primary thyroid cells and SW579 thyroid cancer cells

It could be seen from Figure 1 that GTE had no effect on the proliferation of primary thyroid cells at a concentration of

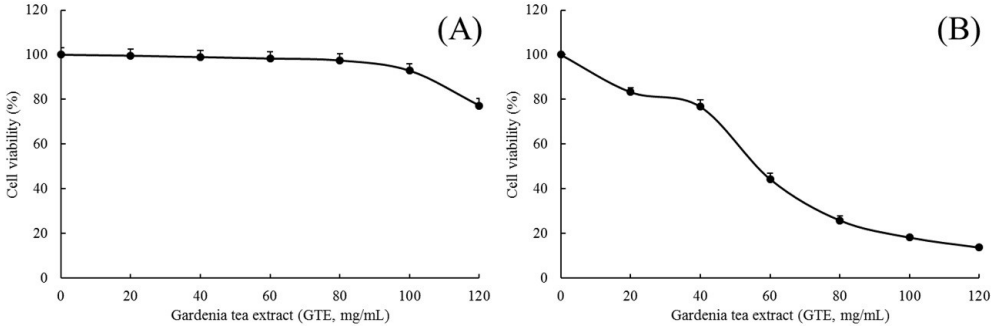

Figure 1. Survival of primary thyroid cells (A) and SW579 thyroid cancer cells (B) after treated with different concentration of gardenia tea extract (GTE).

0-80 mg/mL, the primary thyroid cells proliferate normally, and the survival rate was close to 100%. Under the same concentration of GTE, the *in vitro* proliferation rate of SW579 thyroid cancer cells was negatively correlated with GTE concentration. The higher the concentration of GTE effect, the lower the viability of SW579 cells. Therefore, 20, 40 and 80 mg/mL concentrations of GTE were chosen for subsequent experiments.

3.2 Effects of GTE on in vitro survival of primary thyroid cells and SW579 thyroid cancer cells

According to the results of MTT experiment (Table 1), the inhibition rates of 20, 40 and 80 mg/mL GTE on SW579 thyroid cancer cells reached 20.2%, 55.8% and 79.6%, respectively. GTE had a significant proliferation inhibitory effect on SW579 cancer cells cultured *in vitro*.

3.3 Effects of GTE on Bax, Bcl-2 and Bcl-xL mRNA expressions in cancer cells

According to the results (Figure 2), GTE at 20, 40 and 80 mg/mL could up-regulate Bax mRNA expression and down-regulate Bcl-2 and Bcl-xL expression in SW579 cancer cells to varying degrees. With the increase of GTE concentration, its up-regulation of Bax and down-regulation of Bcl-2 and Bcl-xL expression were enhanced.

3.4 Effects of GTE on caspase-3, -8 and -9 mRNA expressions in cancer cells

According to the results (Figure 3), SW579 cancer cells showed the strongest expression of caspase-3, -8 and -9 at 80 mg/mL of GTE treatment. And 40 mg/mL GTE could also make caspase-3, -8 and -9 expression stronger than 20 mg/mL GTE.

Table 1. Inhibitory effect of Gardenia Tea Extract (GTE) on the proliferation of SW579 thyroid cancer cells.

Group	Control	Gardenia tea extract (GTE, mg/mL)		
		20	40	80
OD_{490}	0.483 ± 0.009^{a}	0.403 ± 0.012^{b}	0.371 ± 0.009^{c}	0.125 ± 0.009^{d}
Inhibitory effect (%)	/	16.69 ± 1.83^{c}	23.23 ± 3.00^{b}	74.06 ± 1.90^{a}

a-d: different lowercase letters indicate significant differences between the corresponding groups ($p < 0.05$).

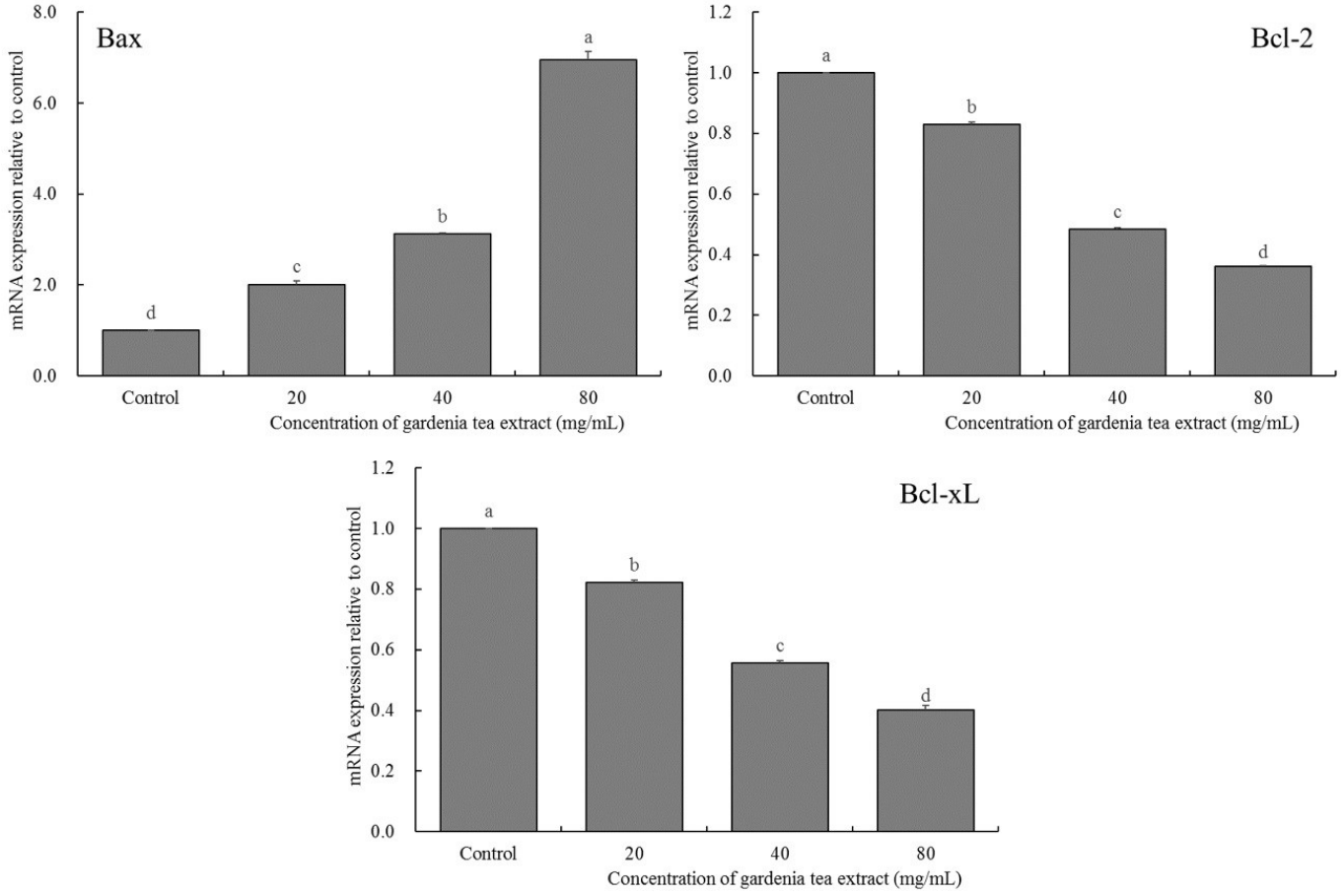

Figure 2. Effects of gardenia tea extract (GTE) on Bax, Bcl-2 and Bcl-xL mRNA expressions in cancer cells. a-d: different lowercase letters indicate significant differences between the corresponding groups ($p < 0.05$).

3.5 Effects of GTE on PI3K and Akt mRNA expressions in cancer cells

According to the results (Figure 4), GTE could reduce the mRNA expression intensity of PI3K and Akt after the treatment of SW579 cancer cells, and the concentration of 80 mg/mL could reduce the above expression intensity the most.

3.6 Composition of GTE

The experimental results showed that GTE mainly contained three compounds, namely rutin, quercetin and phloretin (Figure 5). And phloretin was the highest content and the most core functional ingredient.

4 Discussion

Gene hyperactivation of PI3K/AKT signaling has been recognized as one of the most common driving mechanisms in many cancers. Among more than 12 solid tumor types, PIK3CA and PTEN were the most frequently somatic point-mutated genes, only after the tumor suppressor gene TP53. Cancers with higher rates of PIK3CA mutational activation include breast, endometrial, bladder, colorectal, and head and neck squamous cell carcinomas (Bosse et al., 2013). Protein kinase B (AKT) serine/threonine kinases are the most well-studied effectors downstream of PI3Ks, regulating cell growth, metabolism, survival, and proliferation (Contreras-Paredes et al., 2009). Excessive activation of the PI3K pathway has been shown to induce tumorigenesis. One study analyzed 315 genes in 60,991 solid tumors and found 18 PI3K-related gene alterations in 44% of tumors, of which PI3Kα mutations were the most frequent. PI3Kα inhibitors have also been confirmed to inhibit related downstream signaling pathways in breast cancer and improve tumor immune regulation and microenvironment to achieve tumor suppression (Bilanges et al., 2019).

The PI3K-Akt pathway has intrinsic effects on the proliferation of cancer cells (Wang et al., 2022), inhibits PI3Kδ signaling through BCR and other pathways, promotes FL cell death, and restores FL cell dependence on Bcl-2 anti-apoptotic protein (McCubrey et al., 2006). Bcl-2 family proteins are apoptosis-regulating proteins and play a key role in apoptosis (Volkmann et al., 2014). Bcl-2 and Bcl-xL are typical apoptosis inhibitors, which can prevent mitochondria from releasing cytochrome C to promote cell proliferation (Ola et al., 2011; Liu et al., 2022b). On the contrary, Bax is a typical apoptosis-promoting factor, and the balance of Bax and Bcl-2 and Bcl-xL in the body is related to the success rate of cancer treatment (Czabotar et al., 2014).

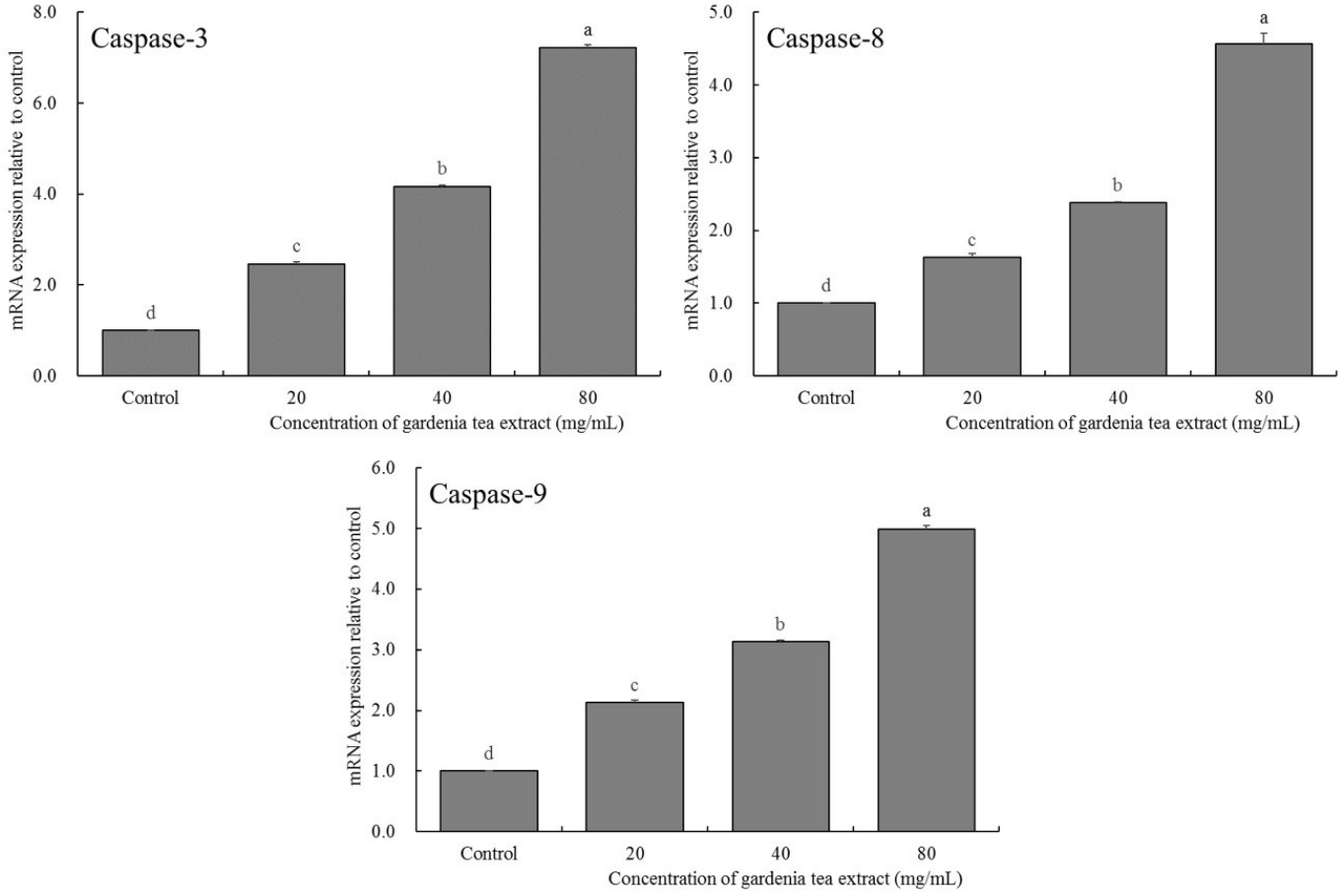

Figure 3. Effects of gardenia tea extract (GTE) on caspase-3, -8 and -9 mRNA expressions in cancer cells. a-d: different lowercase letters indicate significant differences between the corresponding groups ($p < 0.05$).

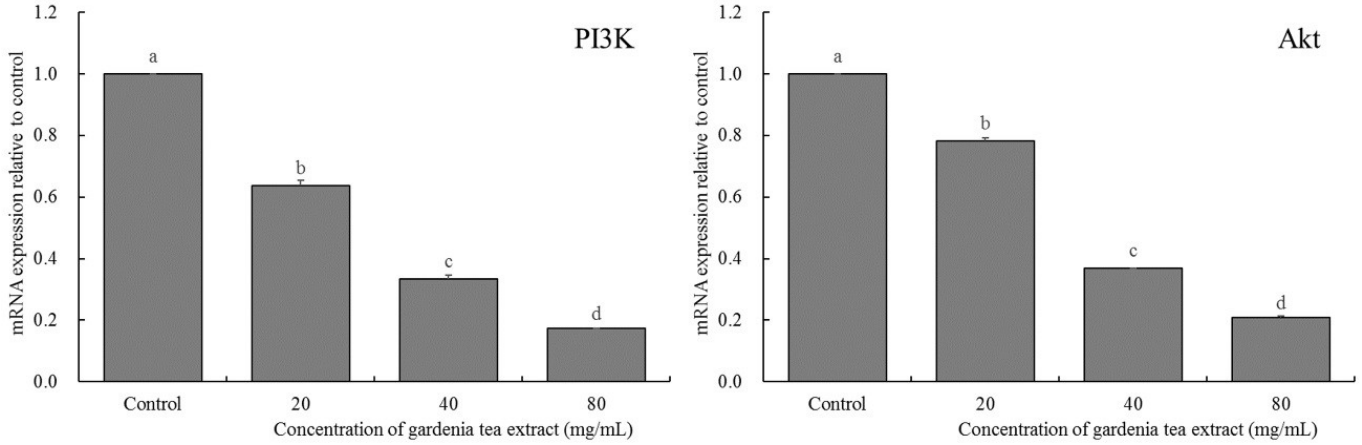

Figure 4. Effects of gardenia tea extract (GTE) on PI3K and Akt mRNA expressions in cancer cells. a-d: different lowercase letters indicate significant differences between the corresponding groups ($p < 0.05$).

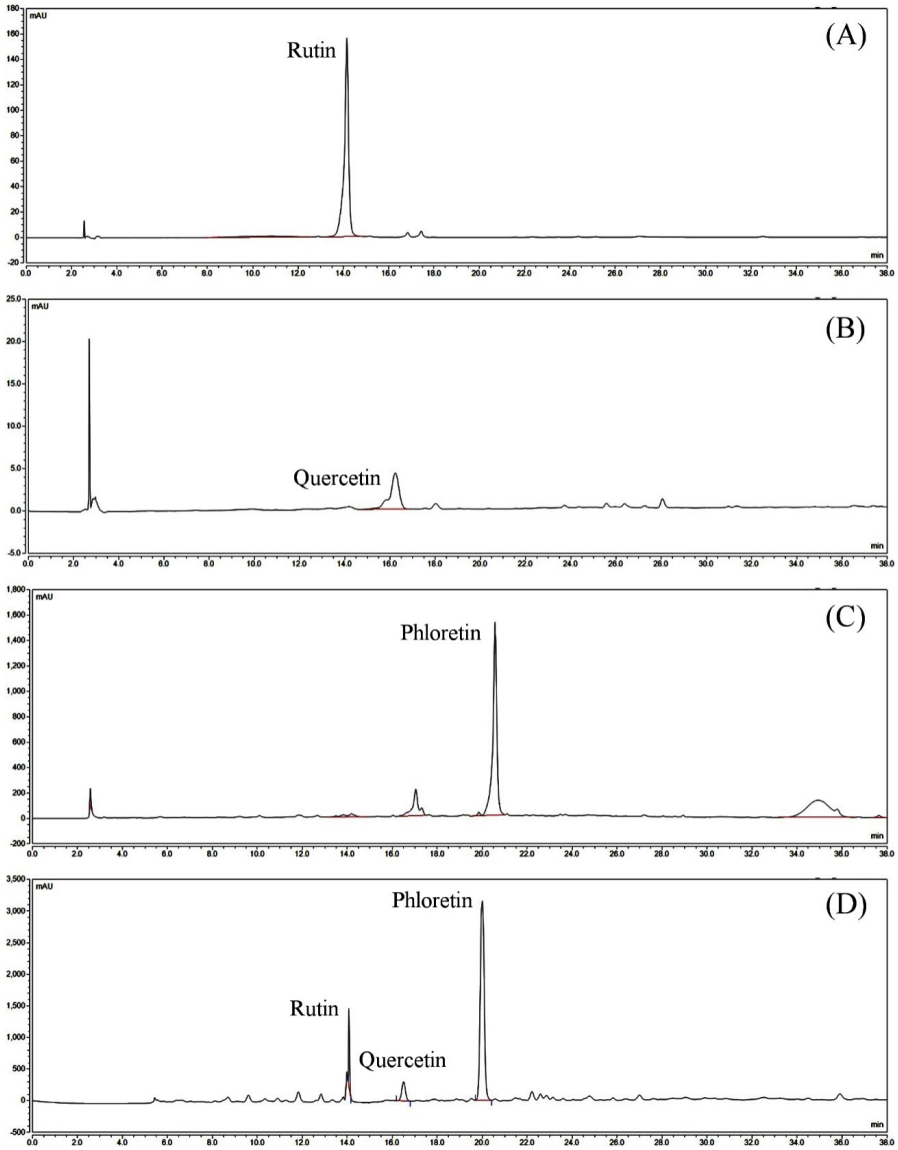

Figure 5. Compound composition of gardenia tea extract (GTE), (A) chromatogram of rutin standard, (B) chromatogram of quercetin standard, (C) chromatogram of phloretin standard, (D) chromatogram of GTE.

Akt in the PI3K-Akt pathway mediates cell survival by directly inhibiting the Bcl-2-associated death promoter and pro-apoptotic proteins such as caspase-9. Activated eNOS by Akt produces nitric oxide, leading to increased vascular permeability and cell migration (Yu et al., 2014). Caspase-3, -8 and -9 are all proteases involved in apoptosis (Sipahli et al., 2022). Caspase-8 and caspase-9 are upstream caspases in the apoptosis pathway and can activate downstream caspase-3 (Suo et al., 2016). Caspase-8 can activate Bax to induce mitochondrial release of cytochrome C, further activate caspase-9 and caspase-3, and promote cancer cell apoptosis (Finucane et al., 1999). In this study, GTE also had a significant regulatory effect on the PI3K-Akt signaling pathway. GTE has no obvious toxic effect on normal thyroid cells, but has a significant down-regulation effect on the expression of PI3K and Akt in thyroid cancer cells. At the same time, GTE can up-regulate the expressions of Bax, caspase-3, caspase-8 and caspase-9 related to the PI3K-Akt signaling pathway, and down-regulate the expressions of Bcl-2 and Bcl-xL.

The PI3K-Akt signaling pathway is involved in a variety of life activities and is directly related to the proliferation of cancer cells (Liu et al., 2022a). Rutin is an active compound with antioxidant effects that inhibit the proliferation of cancer cells. A study also showed that it can inhibit liver cancer by regulating the PI3K-Akt signaling pathway (Jin et al., 2020). Quercetin is also a compound that can modulate the PI3K-Akt signaling pathway, thereby exerting anti-cancer effects, including effects on liver, gastric and oral cancers (Jia et al., 2015; Xiao et al., 2021). The effect of phloretin on the PI3K-Akt signaling pathway has been confirmed, and phloretin can regulate this pathway to induce apoptosis of prostate cancer cells and also inhibit the proliferation of breast cancer cells (Kang et al., 2017; Roy et al., 2022). GTE mainly contains three compounds: rutin, quercetin and phloretin. The combined effect of these active substances constitutes the effect of GTE on regulating the PI3K-Akt pathway and inhibiting thyroid cancer.

Gardenia tea can effectively inhibit the growth of thyroid hepatoma cells *in vitro*, and has no obvious toxic effect on normal thyroid cells, which proves that gardenia tea has a specific inhibitory effect on thyroid cancer. At the same time, further gene expression experiments confirmed that gardenia tea can up-regulate the mRNA expressions of Bax, caspase-3, caspase-8, and caspase-9 in thyroid hepatocellular carcinoma cells and down-regulate the expressions of Bcl-2, Bcl-xL, PI3K, and Akt. It can promote the proliferation of thyroid hepatoma cells. It can be seen that gardenia tea is a health food with anti-cancer activity, and can specifically regulate the PI3K-Akt signaling pathway.

Abbreviations

MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide. qPCR: quantitative polymerase chain reaction. HPLC: high performance liquid chromatography. Bax: Bcl-2-associated X. Bcl-2: B-cell lymphoma-2. Bcl-xL: B-cell lymphoma-extra large. PI3K: phosphatidylinositol 3-kinase. Akt: protein kinase B.

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