Evaluation and Comparison of Antiproliferative Properties of 48 Spices

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Abstract: Spice is widely consumed as flavorings in the world, and shows many bioactive activities. In this study, the antiproliferative activities of 48 spices on three kinds of cancer cells were systematically evaluated. The spices were extracted with 50% aqueous ethanol, and the antiproliferative effects of these extracts on human breast cancer cells MCF-7, human lung cancer cells A549 and human hepatoma cell HepG2 were investigated by the MTT assay. Furthermore, the dose-effect relationships of eight spice extracts having the highest antiproliferative activities on three kinds of cancer cells were studied. The results found that the antiproliferative activities varied greatly in different kinds of spices and different cancer cells. In addition, several spices had strong inhibition on the proliferation of all three kinds of cancer cells, such as garlic, rocambole, pod pepper, little green pepper, leek and leek flower stem. Moreover, most of eight spice samples could dose-dependently inhibit growth of three cancer cells. This paper would be helpful for the understanding of the anticancer activities of these spices, and several spices with strong antiproliferative activities could be of great potential to be developed into anticancer agents or functional foods.

Keywords: spice; anticancer; breast cancer; lung cancer; liver cancer.
1. Introduction

Cancer is among the most leading causes of death worldwide, and is a serious public health problem (Wu et al., 2022; Xu et al., 2020). According to WHO, there were 19.29 million of people newly diagnosed with cancer and 9.96 million of deaths caused by cancer in 2020, which could be induced by a variety of factors, such as malnutrition, excessive alcohol consumption, smoking, environmental pollution and genetic inheritance (Wang et al., 2022). The most commonly diagnosed types of cancers in the world include lung cancer, liver cancer, stomach cancer, colorectal cancer and breast cancer, which could vary greatly in incidence by country, gender and age (Tao et al., 2020; Wu et al., 2022). The threatening morbidity and mortality of cancer over the last few decades aroused the great interest in searching for the effective strategies for the prevention and treatment of cancers. At present, the most common and efficient therapies for cancer include chemotherapy, radiotherapy, immunotherapy and surgery (Xiong et al., 2022). However, these therapeutic strategies have shown limited efficacy and associated adverse responses, such as fatigue, anorexia, anxiety, depression as well as liver and kidney damage (Yang et al., 2022). More and more attention has paid to the intervention using some natural products, like fruits, vegetables, tea and spices, to prevent and treat with cancer because of their low toxicity and promising effects (Zheng et al., 2016).

Spices have been well-known for their flavor, taste and color for thousands of years, and widely consumed as seasonings worldwide (Zheng et al., 2016). Besides, more and more attention has focused on the medicinal value of spices. Extensive research in recent years has shown that spices have promising potential in the prevention and treatment of various chronic diseases, such as diabetes mellitus, cardiovascular diseases, obesity, and some cancers (Deekshith et al., 2021; Gupta et al., 2022; Okaiyeto et al., 2021; Qiblawi et al., 2020). Although they are consumed in small amounts, certain spices contain high levels of phytochemicals, especially polyphenols, which have contributed to their various bioactivities (Aggarwal et al., 2008; Srinivasan, 2014). For instance, turmeric, clove, ginger, cinnamon and black cumin are reported to be able to inhibit the development of cancers (Jaksevicius et al., 2017; Jayakumar et al., 2012; Kammath et al., 2021). Additionally, the mechanisms by which these spices mediate anticancer activities are also extensively studied, and inhibiting the proliferation of cancer cells is showed to play a vital role in the effects (Zheng et al., 2016). However, the anti-proliferative activities of different spices could vary greatly. In this study, the antiproliferative activities of 48 spices on tree kinds of cancer cells were evaluated by the MTT assay, and eight spice extracts having the highest antiproliferative activities were chosen to further assess their dose-effect relationships. This paper could provide a better understanding about the anticancer activities of these spices, and could be helpful for some spices with strong antiproliferative activities to be developed into anticancer agents or functional foods.
2. Materials and Methods

2.1. Chemicals and Materials

Dubelcco’s modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from invitrogen corporation (Grand Island, USA). Penicillin, streptomycin, dimethyl sulfoxide (DMSO), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the experiments were analytical grade, and deionized water was used throughout the experiment. Forty-eight spice samples were bought from Guangzhou, China. The wet spice samples were purchased from local supermarkets, and the dry ones were provided by local herbal medicine stores.

2.2. Sample Extraction

The extraction of spice samples was carried out according to the literature (Fu et al., 2010). Briefly, the wet spice samples were washed with distilled water and given an airing at room temperature. Then, the wet and dry samples were homogenized with a food processor. The wet samples were stored at −20 °C, and dry samples were kept at room temperature in a dry place for further analysis. The 0.10 g of dry sample or 1.00 g of wet sample were weighed and added into the 15 mL centrifuge tube. The 50% aqueous ethanol was added to the sample to 10 mL. The mixture was placed in a shaking water bath (37 °C) for 24 h for extraction, and then centrifuged at 3,500 rpm for 15 min. The supernatant was collected and evaporated to dryness by rotary vaporization at 50 °C. Subsequently, the extract was dissolved in deionized water to obtain the 200 mg/mL stock solution with a minimum amount of DMSO. Finally, the solution was filtrated with a 0.22 μm sterilized filter and stored at 4 °C for further experiment.

The initial weight of the wet samples before drying and their final weight after drying were carefully recorded. The water contents of wet samples were among 59.2% - 95.0%. The results of the wet samples were converted into dry weight (DW) to compare with dry samples. All the experiments were performed three times, and the results were presented as mean ± standard deviation (SD).

2.3. Cell Lines and Cell culture

Human breast cancer cell line MCF-7, human lung cancer cell line A549 and human hepatoma HepG2 cell line were provided by KeyGen Biology Technology Company (Nanjing, China). The three kinds of cell lines were grown in DMEM, which was supplemented with 10% FBS and 1% penicillin-streptomycin. All the cell lines were maintained in an incubator with the temperature of 37 °C and the CO₂ concentration of 5% for further evaluation.

2.4. Evaluation of Antiproliferative Effects of Spice on Cancer Cell Lines
The MTT colorimetric assay was performed to determine the effects of the spice samples on the viability of different cancer cell lines according to the literature with minor modification (Li et al., 2013). Briefly, the stock solutions of the spices were further diluted with DMEM to obtain the concentrations of 20 mg/mL. The cancer cells were seeded in 100 μL DMEM complete medium in 96-well plates with different density according to the difference of cells growth rate (MCF-7 cells: 1 × 10⁴ cells/well, A549 cells: 6 × 10³ cells/well, and HepG2 cells: 8 × 10³ cells/well), and incubated for 24 h for attachment. Then the culture medium was discarded and cancer cells were treated with 200 μL medium containing 20 mg/mL of the extracts for 48 h in a humid atmosphere (37 °C, 5% CO₂). Control wells received the growth medium without the extracts to treat cancer cells, and blank wells contained no cells and treated with 200 μL of growth medium, which were then incubated for 48 h. Subsequently, the culture medium was removed, and PBS was used for washing for twice. The MTT solution (5 mg/mL) was diluted with PBS to produce a final concentration of 0.5 mg/mL of MTT, and then 200 μL of diluted MTT solution was added to the wells and incubated for another 4 h. After that, the medium was removed and formazan crystals were dissolved in 150 μL of DMSO. Then, the 96-well plate was placed on an orbital shaker for 10 min of shaking. The absorbance was recorded at 490 nm using a Bio-assay reader (BioRad, USA). Antiproliferative activity of the extracts was measured as percent compared with control wells, and all samples were conducted in triplicate.

2.5. Determination of the Dose-dependent Manner of Spice

The eight spice extracts with strong antiproliferative activities were chosen for further evaluation of dose-effect relationship by the MTT method as described above. The cancer cells were seeded in 100 μL DMEM complete medium in 96-well plates with different density and incubated for 24 h. Subsequently, the culture medium was removed and the cells was treated with 200 μL of various concentrations (1, 5, 10, 15, 20, 40 mg/mL) of the extracts, which was obtained by the dilution with medium and incubated for 48 h. The medium containing sample extracts was discarded, and the wells were washed with PBS twice. The 200 μL of 0.5 mg/mL MTT solution was added to the wells and incubated for another 4 h, and then the medium was removed and formazan was dissolved in 150 μL of DMSO. Control wells and blank wells were also set and treated as above. The 96-well plate was placed on an orbital shaker for 10 min of shaking. The absorbance was recorded at 490 nm, and the inhibition ratio was calculated. All samples were conducted in triplicate.

2.6. Statistical analysis
All the experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out by SPSS 13.0 and Excel 2003. The difference was considered significant at \( p < 0.05 \).

3. Results and Discussion

3.1. Anti-proliferative Activities of 48 Spices

Lung, breast and liver cancers are among the most common types of cancers (Wu et al., 2022). Cultured cancer cells played an important role in the rapid screening and evaluation of anticancer potential of samples, and the exploration of related mechanism (Li et al., 2013). Therefore, in present study, the antiproliferative effects of 48 spices on three kinds of cancer cells (including human lung cancer cell A549, breast cancer cell MCF-7 and liver cancer cell HepG2) were systematically evaluated and compared by MTT assay. The MTT assay is a simple, practical and widely employed method to evaluate the antiproliferative capacity of samples based on that the number of viable cells is directly proportional to the amount of insoluble purple formazan, which is converted from MTT by the action of a mitochondrial enzyme in living cells (Sugahara et al., 2022; Xu et al., 2021).

The inhibition rate of 48 spice extracts towards three kinds of cancer cells are showed in Figures 1-3. It was clear that there was an obvious difference in the sensitivity of three kinds of cancer cells to the samples. For human breast cancer cell MCF-7, the inhibition rate varied from 7.18 ± 0.82% to 97.73 ± 0.67% with a 14-fold difference. The 10 spice extracts with the strongest inhibition on the growth of MCF-7 cells were liquorice, rocambole, little green pepper, pod pepper, garlic, leek white, leek flower stem, leek, oregano and Villous amomum fruit, while fennel showed the lowest anti proliferative capacity.

For human lung cancer cell line A549, the inhibition rate varied from 5.44 ± 7.44% to 98.96 ± 1.36% with an 18-fold difference. The 10 spice extracts with the strongest inhibition on the growth of A549 cells were Radix Aucklandiae, pod pepper, little green pepper, leek white, rocambole, liquorice, garlic, leek flower stem, leek and middle red pepper, while long pepper had the lowest antiproliferative capacity.

For the human hepatoma HepG2 cell line, the inhibition rate varied from 2.41 ± 11.27% to 99.03 ± 0.11% with a 41-fold difference. The 10 spice extracts with the strongest inhibition on the growth of HepG2 cell were leek white, leek flower stem, leek, pod pepper, garlic, little green pepper, rocambole, celery, Radix Aucklandiae and liquorice, while long pepper had the lowest antiproliferative capacity.

Generally speaking, different kinds of spices had different effects on inhibiting the growth of different cancer cells, and different kinds of cancer cells displayed different sensitivity to the same spice. For example, in this study, celery showed the 40.28 ± 7.13% of inhibition rate on MCF-7 cells, while the 98.06 ± 0.98% and 98.56 ± 0.23% of inhibition rates on A549 and HepG2 cells. The results were in consistent with the previous studies (Al Aboody, 2021; Gomaa et al., 2020; Rana et al., 2022). In addition,
some spices could exert very strong inhibitory effect on the growth of all three kinds of cancer cells, such as garlic, rocambole, pod pepper, little green pepper, leek and leek flower stem, while some spices, such as cumin and red bell pepper, showed very little effect on the three cancer cells. It was similar to a previous study, in which ginger showed strong cytotoxic activity on four human tumor cell lines (HCT116, HepG2, MCF7, and PC3), while the cytotoxicity of black cumin was considerably low on the four cell lines (El-Dawy et al., 2019). It hinted that different spices should be used for the prevention and treatment of different cancers. In addition, some spices could be an important dietary source to prevent cancers and have great promising to be developed into anticancer agents.

Figure 1. The inhibition rate (%) of 48 spices on MCF-7 cells. 1, Chinese prickly ash; 2, black pepper; 3, white pepper; 4, nutmeg; 5, white nutmeg; 6, tangerine peel; 7, cumin; 8, star anise; 9, Villous amomum fruit; 10, Tsaoko amomum fruit; 11, bay leaf; 12, liquorice; 13, mint leaf; 14, purple perilla; 15, Angelica dahurica; 16, kenchor; 17, turmeric; 18, long pepper; 19, cinnamon; 20, fennel; 21, Radix Aucklandiae; 22, oregano; 23, Rhizoma Kaempferia; 24, dried pepper; 25, clove; 26, galangal; 27, haw; 28, pod pepper; 29, little green pepper; 30, middle green pepper; 31, middle red pepper; 32, red bell pepper; 33, green bell pepper; 34, yellow bell pepper; 35, onion; 36, chive; 37, Zingiber officinale; 38, rocamboles; 39, garlic; 40, garlic sprout; 41, garlic sprout stem; 42, leek white; 43, leek; 44, leek flower stem; 45, coriander; 46, celery; 47, Apium graveolens leaf; 48, Apium graveolens stick.
Figure 2. The inhibition rate (%) of 48 spices on A549 cells. 1, Chinese prickly ash; 2, black pepper; 3, white pepper; 4, nutmeg; 5, white nutmeg; 6, tangerine peel; 7, cumin; 8, star anise; 9, Villous amomum fruit; 10, Tsaoko amomum fruit; 11, bay leaf; 12, liquorice; 13, mint leaf; 14, purple perilla; 15, Angelica dahurica; 16, kenchor; 17, turmeric; 18, long pepper; 19, cinnamon; 20, fennel; 21, Radix Aucklandiae; 22, oregano; 23, Rhizoma Kaempferiae; 24, dried pepper; 25, clove; 26, galangal; 27, haw; 28, pod pepper; 29, little green pepper; 30, middle green pepper; 31, middle red pepper; 32, red bell pepper; 33, green bell pepper; 34, yellow bell pepper; 35, onion; 36, chive; 37, Zingiber officinale; 38, rocambole; 39, garlic; 40, garlic sprout; 41, garlic sprout stem; 42, leek white; 43, leek; 44, leek flower stem; 45, coriander; 46, celery; 47, Apium graveolens leaf; 48, Apium graveolens stick.
Figure 3. The inhibition rate (%) of 48 spices on HepG2 cells. 1, Chinese prickly ash; 2, black pepper; 3, white pepper; 4, nutmeg; 5, white nutmeg; 6, tangerine peel; 7, cumin; 8, star anise; 9, Villous amomum fruit; 10, Tsaoko amomum fruit; 11, bay leaf; 12, liquorice; 13, mint leaf; 14, purple perilla; 15, Angelica dahurica; 16, kenchor; 17, turmeric; 18, long pepper; 19, cinnamon; 20, fennel; 21, Radix Aucklandiae; 22, oregano; 23, Rhizoma Kaempferiae; 24, dried pepper; 25, clove; 26, galangal; 27, haw; 28, pod pepper; 29, little green pepper; 30, middle green pepper; 31, middle red pepper; 32, red bell pepper; 33, green bell pepper; 34, yellow bell pepper; 35, onion; 36, chive; 37, Zingiber officinale; 38, rocambole; 39, garlic; 40, garlic sprout; 41, garlic sprout stem; 42, leek white; 43, leek; 44, leek flower stem; 45, coriander; 46, celery; 47, Apium graveolens leaf; 48, Apium graveolens stick.

3.2. The Dose-dependent Manner of Spices

According to the above result, 8 spices with the strongest antiproliferative activities on different cancer cells were chosen for further dose-effect relationship experiment, that is, garlic, rocambole, pod pepper, little green pepper, leek, leek flower stem, leek white and liquorice for MCF-7 cells, garlic, rocambole, pod pepper, little green pepper, leek, leek flower stem, Radix Aucklandiae and liquorice for A549 cells, and garlic, rocambole, pod pepper, little green pepper, leek, leek flower stem, leek white and celery for HepG2 cells. Six different concentrations (1, 5, 10, 15, 20, 40 mg/mL) of the spice extracts were used in this study, and the results of dose-effect relationship of 8 top spices with high antiproliferative activities on three kinds of cancer cells are showed in Figures 4-6.

For human breast cancer cell MCF-7 (Figure 4), most of the 8 selected spices showed a strong inhibitory effect on cell proliferation with the inhibition rate > 80% at the concentrations of 1 mg/mL, including leek white, garlic, liquorice, little green pepper and rocambole. Although the inhibition rate of leek (around 70%) and leek flower stem (around 80%) were relatively low at 1 mg/mL of concentrations, they showed a sharp increase and reached the maximum (100%) at the concentrations of 5 mg/mL. The rocambole and pod pepper experienced a relatively slow increase in the inhibition of MCF-7 cell growth with the increase of sample concentrations, which reached the maximum at 10 mg/mL concentrations. Notably, leek white, garlic, and liquorice almost maintained the 100% of inhibition rate on the MCF-7 cell proliferation among different concentrations, which hinted that they could be a promising source for the prevention and treatment of human breast cancer.

For human lung cancer cell line A549 (Figure 5), contrary to the results from MCF-7 cells, most of the 8 spices showed a low inhibitory activity on cell growth with the inhibition rate < 80% at the 1 mg/mL of sample concentration, except for garlic and leek white. Rocambole, pod pepper and liquorice showed the lowest inhibition on cell proliferation with the inhibition rate < 40%, while they showed rapid increase with the concentrations increased. The inhibition rate of liquorice quickly reached the maximum (100%) at the concentration of 5 mg/mL, and the inhibition rates of rocambole and pod pepper firstly increased to > 80% at the concentration of 5 mg/mL, and reached the maximum at the
concentration of 10 mg/mL. Basically, all the 8 spices showed the 100% inhibition on the A549 cell growth among 10 - 40 mg/mL of concentrations.

For the human hepatoma HepG2 cell line (Figure 6), similar to the results from human lung cancer cell line A549, the majority of 8 spices showed week inhibition on the cell growth at the low concentration of 1 mg/mL, while garlic, little green pepper and leek white displayed strong antiproliferative activities with the inhibition rate > 80%. Leek showed the lowest inhibition rate (< 20%) at concentration of 1 mg/mL, while sharply jumped to > 80% of inhibition rate at the concentration of 5 mg/mL, and reached the maximum at 10 mg/mL concentration. In addition, the inhibition rates of pod pepper, leek flower stem and rocambole showed the similar increase experience as leek, increasing among 1-10 mg/mL of concentrations and reached the maximum at 10 mg/mL of concentration. Basically, most of the 8 spices showed the 100% inhibition on the HepG2 cell growth among 10 - 40 mg/mL of concentrations.

In this study, 6 spices, including garlic, rocambole, pod pepper, little green pepper, leek and leek flower stem, showed strong inhibition on the growth of three different kinds of cancer cells. Some previous studies evaluated the antiproliferative activities of one or several spices, while the results were inconsistent in different studies (Gorinstein et al., 2009; Matsuura et al., 2006; Toigo et al.; Zamri et al., 2019). The reason might be that the origin, variety and cultivated environment of spices were different in different studies. In addition, most of the tested spice samples exhibited a dose-dependently inhibitory effect on the cancer cell viability and proliferation, which was similar to previous studies (Asdaq et al., 2021; Berrington et al., 2012). Notably, some spices could exert a 100% inhibition on the growth of cancer cell even at a low concentration, such as 1 mg/mL of leek white on MCF-7 cells. Moreover, the majority of selected spices could reach the maximum inhibition rate (100%) at the concentrations of around 10 mg/mL. These results suggested that some spices with strong antiproliferative activities at a low concentration would be of great potential to be developed into anticancer agents or functional foods.
Figure 4. The dose-dependent curve of antiproliferative effects of the 8 top spice samples on the human breast cancer cell MCF-7. (a) rocambole and garlic; (b) pod pepper and little green pepper; (c) leek and leek flower stem; (d) leek white and liquorice.

Figure 5. The dose-dependent curve of antiproliferative effects of the 8 top spice samples on the human lung cancer cell line A549. (a) rocambole and garlic; (b) pod pepper and little green pepper; (c) leek and leek flower stem; (d) Radix Aucklandiae and liquorice.
4. Conclusions

In this study, the antiproliferative effects of 48 spices on three kinds of cancer cells were evaluated and compared, and the dose-effect relationships were also studied. The results found that the antiproliferative activities of 48 spices varied greatly. Some spices displayed strong antiproliferative effects on one specific cancer cell, but little effect on the other cancer cells. In addition, several spices could considerably inhibit the proliferation of all three kinds of cancer cells, such as garlic, rocambole, pod pepper, little green pepper, leek and leek flower stem, while some spices showed limited effects on suppressing the growth of these cancer cells, such as cumin and red bell pepper. Besides, the dose-effect relationships were observed for several spices. In the future, the specific components in spices with high antiproliferative activities should be explored for their anticancer effects. This paper would be helpful for the understanding of the anticancer activities of these spices, and several spices with strong antiproliferative activities could be of great potential to be developed into anticancer agents or functional foods.
Potential Conflicts of Interest

The authors declare no conflict of interest.

References


