Chemical composition, antioxidant activity and antitumor activity of tumorous stem mustard leaf and stem extracts

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ABSTRACT

The swollen succulent stem of tumorous stem mustard (Brassica juncea var. tumida Tsen et Lee) is the raw material in the processing of Chinese Fuling zhacài. We found that total polyphenols and flavonoids contents of tumorous stem mustard leaf extracts (TSMLE) were significant higher than that of tumorous stem mustard stem extracts (TSMSE). The percentage of isorhamnetin 5-O-hexoside, methyl quercetin O-hexoside, and luteolin O-hexosyl-O-hexosyl-O-hexoside in TSMLE were 170, 230 and 694 times higher than that in TSMSE. TSMLE presented stronger antioxidant capacity against 2,2’-Azino-bis[3-ethylbenzothiazoline]-6-sulfonic acid cationic free radical (ABTS⁺) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals, and it also showed higher reducing power and ferric reducing antioxidant power. Both TSMLE and TSMSE inhibited lung carcinoma cell growth in a dose-dependent manner, while TSMLE was more effective against A549 cells than TSMSE. This is the first report indicates that tumorous stem mustard, especially its leaf, is a potential diet source in preventing of oxidative stress and cancer.

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Composición química, actividad antioxidante y antitumoral de extractos de hojas y tallos de la planta de mostaza de tallo tumoral

RESUMEN

El tallo hinchado y succulento de la mostaza de tallo tumoral (Brassica juncea var. tumida Tsen et Lee) es la materia prima para el procesamiento de la verdura china Fuling zhacài. Al respecto, se constató un contenido total de polifenoles y flavonoides significativamente más alto en los extractos de hojas de mostaza de tallos tumoriales (TSMLE) que en los extractos de tallos de mostaza de tallos tumoriales (TSMSE). El porcentaje de isorhamnetina 5-O-hexosa, metil quercetina O-hexosa y luteolina O-hexosil-O-hexosil-O-hexosa presente en TSMLE fue 170, 230 y 694 veces mayor que en TSMSE. Asimismo, los TSMLE mostraron mayor capacidad antioxidante contra los radicales libres ABTS⁺ + y DPPH, además de un poder reductor y un poder antioxidante reductor del ion férrico más elevado. Tanto los TSMLE como los TSMSE inhibieron el crecimiento celular del carcinoma de pulmón de manera dependiente de la dosis, mientras que los TSMLE fueron más eficaces contra las células A549 que los TSMSE. Este es el primer informe que indica que la mostaza de tallo tumoral, especialmente su hoja, es una fuente dietética con potencial para prevenir el estrés oxidativo y el cáncer.

1. Introduction

Tumorous stem mustard (Brassica juncea var. tumida Tsen et Lee, Figure 1(a)), a native cruciferous vegetable crop in Asia countries, is a very important fresh and processed vegetable in winter and spring (Xie et al., 2014). The swollen succulent stem of tumorous stem mustard is the critical raw material in the processing of Chinese Fuling zhacài, which is as famous as French sour-cucumber and German sweet-sour-cabbage. However, thousands of tons of tumorous stem mustard leaf was discarded in farmland per year since zhacài become a commodity in China from 1899 (Yuan, 2016). This not only causes an environmental problem but also is a great waste of dietary resources. Although previous literature has been reported regarding phenolic compounds of tumorous stem mustard leaf (Xie et al., 2014), a large percentage of compounds present remain unknown and need to be identified before the health-promoting properties of tumorous stem mustard is properly elucidated.

Malignant neoplasms have become one of the leading causes of death, only second to heart diseases (Li et al., 2013). Among them lung cancer is now the most common cause of cancer-related deaths, which accounts for more than one million worldwide annual deaths (Lee, Kang, Jung, Kim, & Kim, 2011). Recently, it is reported that a high intake of vegetables is one of the cornerstones of a healthy diet and has been recommended to the general public to reduce the risk of cancer (Aune et al., 2017). The increasing researches also indicate that vegetal foods and nutrients play an important role in the prevention of cancer development (Key et al., 2004). In this regard, ingestion of leaf and swollen succulent stem of tumorous stem mustard may be a potential strategy for anticancer. Therefore, the main endeavor of the current study was to investigate the potential anti-proliferation activity of tumorous stem mustard leaf...
and stem extracts. To further illuminate mechanism and material basis of the anti-proliferation activity, the antioxidant activity was assessed in vitro, and the phytochemistry profiles were determined by ultra-performance liquid chromatography tandem mass spectrometry.

2. Materials and methods

2.1. Materials and reagents

The entire plant of fresh tumorous stem mustard (*Brassica juncea var. tumida* Tsun et Lee) was obtained from a local agricultural market. Human lung carcinoma cell (A549) was obtained from Cell Bank of Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and dihydroethidium (DHE) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2ʹ-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and trichloroacetic acid were provided from AppliChem (Darmstadt, Germany). Tea polyphenols (phenolics >95%) was obtained from Shanghai yuanye biotechnology co. LTD (Shanghai, China). Chromatographic grade acetone and methanol were purchased from Kemiu Chemical Reagent Co., Ltd ( Tianjin, China). Deionized water was obtained from Hangzhou Wahaha Group Co., Ltd (Hangzhou, China). All the cell culture reagents were purchased from Sinopharm (Beijing, China). All other chemical reagents were analytical reagents.

2.2. Preparation of tumorous stem mustard extracts

The extraction was performed as described previously (Han, Li, Huang, & Yang, 2016). At first, the rootless tumorous stem mustard leaf and swollen succulent stem were separately homogenated. The homogenates were extracted with two-fold (w/v) 80% methanol in 1000 mL conical flask at 70°C for 60 min for three times. After vacuum filtration, filter liquors from each extraction were combined and cooled to room temperature. The methanol extract was concentrated at 45°C with a vacuum rotary evaporator (RE-52AA, Shanghai arong biochemical instrument factory, Shanghai, China). After that, the concentrated solution was further extracted by ethyl acetate in a 1:2 ratio (v/v) for three times to isolate and enrich lipophilic phytochemicals. The extracted solution of ethyl acetate was concentrated 45°C with a vacuum rotary evaporator. The residual aqueous solution (about 50 mL) containing phytochemicals was freeze-dried (RLPHR 1–2 LD, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz Germany). The extracts yielded green and white powder, defined as TSMLE (tumorous stem mustard leaf extracts) and TSMSE (tumorous stem mustard stem extracts) from tumorous stem mustard leaf and swollen succulent stem, respectively.

2.3. Spectrophotometric determination of total polyphenols and total flavonoids

The total polyphenols in the extracts were analyzed using Folin-Ciocalteu’s colorimetric assay as described by Nie, Ren, Lu, Sun, and Yang (2015). The powder of TSMLE and TSMSE were dissolved in 80% methanol (w/v = 2:1). Fifty microliters of the measured solution and 10 μL Folin-Ciocalteu reagent were mixed in 96-well plates. After six minutes, 7% Na2CO3 (100 μL) and deionized water (80 μL) were added and mixed thoroughly. After 90 min of incubation in darkness at room temperature, the absorbance at 760 nm was measured (PT-3502C, Beijing putian new bridge technology co. LTD, Beijing, China). The results were expressed as microgramme of gallic acid equivalent per milligramm of leaf or stem extracts (μg GAE/mg, y = 70.421x – 0.0351, R² = 0.9946). All detections were performed in five repetitions.

The total flavonoids contents of extracts were measured by the method reported by González-Barrio, Periago, Luna-Recio, Javier, and Navarro-González (2018), with some modification. Aliquots of 10 μL measured solution were mixed with 5% NaNO2 (10 μL). After 6 min, 10 μL of 10% AlNO3 was added and the mixture was left for another 6 min before the addition of 4% NaOH (100 μL), and 60% ethanol (120 μL) was added, followed by a thorough mixing and further standing for 15 min. The absorbance of the mixture was determined at 510 nm using a microplate reader (PT-3502C, Beijing putian new bridge technology co. LTD, Beijing, China). The results were expressed as microgramme of rutin equivalent per milligramm of leaf or stem extracts (μg RE/mg, y = 3169.5x – 0.23, R² = 0.9960). All detections were carried out in five repetitions.

2.4. Identification of phytochemicals by UPLC-MS/MS analysis

The measured solution was diluted (v/v = 1:2) with 80% methanol, and then each sample was centrifuged and filtered with a 0.22 μm PVDF membrane for ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) analysis. A 30A liquid chromatography system (Shimadzu, Tokyo, Japan), equipped with a binary pump and coupled to a 4500 QTRAP mass spectrometer detector (AB Sciex Pte. Ltd., CA, USA), was used. UPLC liquid chromatography was performed using a Waters ACQUITY UPLC HSS T3 C18 column (1.8 μm, 2.1 mm × 100 mm), which was maintained at 40°C. Elution with solvent A (water with 0.04% acetic acid, v/v) and solvent B (acetoneitrile with 0.04% acetic acid, v/v) in a linear gradient manner was conducted at a flow rate of 0.4 mL/min as: 5%-

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**Figure 1.** Plant of tumorous stem mustard (*Brassica juncea var. tumida* Tsun et Lee) (a); and total polyphenols and total flavonoids contents in extracts from tumorous stem mustard leaf and stem, respectively (b).

| TSMLE | TSMSE |
|-------|-------|
| Total polyphenols | 273 | 160.42, R² = 0.9946 |
| Total flavonoids | | |
95% B (0–11 min), 95%-95% B (11–12 min), 95%-5% B (12–15 min). The injection volume was 5 μL, and source conditions were the following: electrospray ionization (550°C), capillary voltage was 5.5 kV and curtain gas was 25 psi.

To facilitate phytochemicals identification, our collaborator (Metware, Wuhan, China) established the Metware database (MWDB). On the basis of accurate mass and obtained migration time of these phytochemicals, peak correction and peak integration method was edited using MultiQuant (AB Sciex Pte. Ltd., CA, USA). Furthermore, these phytochemicals were also verified based on two main online databases: HMDB and Metlin.

2.5. Determination of antioxidant capacity

2.5.1. DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated according to the method reported by Bannour et al. (2017) with slight modifications. Briefly, 150 μL of measured solutions at various concentrations (0, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/mL) were mixed with 150 μL DPPH working solution (1 mmol/L) in a 96-well plate and allowed to react at room temperature for 30 min in dark before the absorbance was recorded at 517 nm. The scavenging activity against DPPH (%) = \[1-(A_j-A_0)/A_0\] × 100%, where A0 was the absorbance without the sample, Aj was the absorbance with the sample, and A0 was the absorbance of ground color.

2.5.2. ABTS radical scavenging activity

ABTS assay was carried out by using a modified method reported earlier (Peng, Li, Li, Deng, & Zhang, 2017). Briefly, the ABTS+ solution was prepared by adding 100 mL of ABTS solution (7 mmol/L) to 100 mL of K2S2O8 (2.45 mmol/L) and the mixture was kept in the dark at room temperature for 24 h before analysis. The ABTS+ working solution was diluted with 80% ethanol to the absorbance 0.70 ± 0.05 at 734 nm. Ten microliters of samples were reacted with 200 μL of ABTS+ working solution in the dark at room temperature for 5 min in a 96-well plate and the absorbance was measured at 734 nm. The scavenging activity against ABTS+ (%) = \[1-(A_1-A_0)/A_0\] × 100%, where A0 was the absorbance without the sample, A1 was the absorbance with the sample, and A0 was the absorbance of ground color.

2.5.3. Ferric reducing antioxidant power (FRAP assay)

FRAP assay followed the method in the previous report with some modifications (Bannour et al., 2017). Fifty microliters of samples were reacted with 100 μL of ferric-TPTZ reagent (prepared by mixing 0.3 mol/L acetate buffer, pH = 3.6, 10 mmol/L TPTZ, 20 mmol/L FeCl3 at ratio of 10:1:1 (v/v/v)) and 200 μL of distilled water, and the mixed-solution was warmed to 37°C before use. The absorbance reading was taken after 30 min at 593 nm. The increased absorbance of the reaction mixture suggested its enhanced reducing power (Nie et al., 2015).

2.5.4. Measurement of total reducing power

The total reducing power (TRP) of the two extracts was determined as reported by Ren, Zhao, Nie, Yang, and Yang (2014). Two hundred microliters of extracts were combined with 250 μL of 0.2 mol/L phosphate buffer saline (pH = 6.6) and 250 μL of 1% K2Fe(CN)6 solution (w/v). The mixture was incubated at 50°C for 20 min, and then 250 μL of 10% TCA solution was added, and the mixture was centrifuged at 1000 rpm/min for 5 min. The supernatant liquor (100 μL) was mixed with 80 μL deionized water and 0.1% FeCl3 solution (w/v), and the absorbance at 700 nm was detected by a microplate reader (PT-3502C, Beijing putian new bridge technology co. LTD., Beijing, China). A high absorbance of the reaction mixture indicated strong reducing power (Nie et al., 2015).

2.6. Cell proliferation assessment

The anti-proliferation of lung cancer cell assay was carried out by our previous method (Li, He, Tian, Shi, & Yang, 2016). A549 cells (2 × 105 cells/well) were seeded in 96-well plates and treated with different doses of TSMLE or TSMSE at a concentration of 0, 30, 60, 120, 240, and 480 μg/mL at 37°C for 48 h. Moreover, the A549 cells (2 × 105 cells/well) were also treated with TSMLE at a concentration of 0, 30, 60, 120, 240 and 480 μg/mL at 37°C for 12, 24, and 48 h. After the exposure time, 10 μL of MTT solution (5 mg/mL) in phosphate-buffered saline was added to each well at a final concentration of 0.5 mg/mL and then the plate was further incubated for 4 h. The formed crystal formazan was dissolved in 150 μL of a solution containing 10% SDS, 0.01 mol/L hydrochloric acid and 5% isobutyl alcohol. Subsequently, the absorbance was measured at 570 nm, and the percentage of cell survival was expressed as follows: Cell viability = (absorbance values in treated cells/absorbance of control cells) × 100%.

2.7. Reactive oxygen species (ROS) assay in lung carcinoma cells

Intracellular production of ROS, namely, hydrogen peroxide and superoxide anion were measured by the method of Li et al. (2013) with minor modifications. A549 cells (1 × 105 cells/well) were seeded in six-well plates in triplicate and then exposed to TSMLE at two concentration of 60 μg/mL and 120 μg/mL at 37 °C for 12 h. After incubation, cells were detached with trypsin-EDTA and washed twice with phosphate-buffered saline. Then, the cells were exposed to 10 μmol/L DHE probes at 37°C for 30 min. The morphological changes of cells and ROS levels were visualized by fluorescence microscopy (Leica DML LED; Leica, Solms, Germany) (Li et al., 2016).

2.8. Statistical analysis

Results are expressed as the mean ± standard deviations (SD). Significant differences (p-value <0.05) were assessed through the analysis of ANOVA with Sidak’s multiple comparisons test using the GraphPad Prism 7.0 (GraphPad Software, Inc.).

3. Results and discussion

3.1. Total polyphenols and total flavonoids contents

We found that the extraction yield of TSMLE and TSMSE from leaf and stem of tumorous stem mustard, which was extracted with 80% methanol, was 0.15% and 0.1%, respectively. It is well known that polyphenols are important phytochemicals present in all vegetal tissues, and it plays a key role in preventing diseases such as cancers, cardiovascular diseases, and chronic inflammatory diseases (Díaz-García, Obón, Castellar, Collado, & Alacid, 2013). In this work, the total polyphenols contents of TSMLE and TSMSE analyzed using the Folin-Ciocalteu method were 28.92 μg of gallic acid equivalent per milligram of leaf extracts and 24.15 μg of gallic acid equivalent per milligram of stem extracts, respectively (Figure 1(b)). Generally, the antioxidant
activity of flavonoids was stronger than that of phenolic acids. Thus, the flavonoids levels of the two extracts were assessed in the current study. As shown in Figure 1(b), the contents of total flavonoids in TSMLE and TSMSE were 18.44 microgramme of rutin equivalent per milligram of leaf extracts and 6.95 µg of rutin equivalent per milligram of stem extracts, respectively. These quantitative assays indicated that TSMLE contained a significantly higher number of total polyphenols and total flavonoids than TSMSE, suggested that the TSMLE has higher antioxidant activity and anti-tumor activity than TSMSE.

3.2. Phytochemicals profile of tumorous stem mustard leaf and stem extracts

Besides polyphenols, terpenoids, alkaloids, and some vitamins also have strong antioxidant activity and anti-tumor activity (Dillard & German, 2000). Taking into consideration the point mentioned above, the targeted-metabolomics analysis was performed to reveal the phytochemicals profile of TSMLE and TSMSE (supplementary Figure S1A, B). The identity of each compound was obtained on the base of the MS² fragmentation data and retention time by matching them with the characterization data available in our MWDB database and others. The identification of phytochemical compounds by UPLC-MS/MS allowed the characterization of 32 different classes of phytochemicals in the tumorous stem mustard leaf and stem extracts (Figure 2(a)). As can be seen in Figure 2(a), amino acids, organic acids, flavonol, nucleotide, and its derivates, hydroxycinnamoyl derivatives, flavanone and flavone accounted for a large proportion in extracts. Of note, the percentages of powerful anti-oxidants and antineoplastics including organic acids, flavonol, coumarins, anthocyanins, and catechin derivatives in TSMLE were higher than that in TSMSE (Figure 2(a)), suggested that TSMLE has stronger bioactivity than TSMSE.
Tumorous stem mustard was a native cruciferous vegetable crop in Asia countries (Xie et al., 2014). It may have a high level of glucosinolates like other cruciferous vegetables (Brown & Morra, 1995). We found that the proportion of sinigrin hydrate (Figure 2(b)), a potent anticancer glucosino-late (Awasthi & Saraswathi, 2015), was higher in TSMLE than TSMSE. Although these organic acids can stabilize polyphenols to maintain antioxidant and anticancer activities (Dillard & German, 2000), our results showed that the levels of three main organic acids (l(-)-malic acid, methylmalonic acid, and succinic acid) in TSMLE were slightly higher than that in TSMSE. Thus, these results strongly suggest that the TSMLE possess stronger antioxidant and anticancer activities than TSMSE.

Thus, these results strongly suggest that the TSMLE possess stronger antioxidant and anticancer activities than TSMSE. Although l(-)-malic acid, methylmalonic acid, and succinic acid have no direct antioxidant and anticancer activities, these organic acids can stabilize polyphenols to maintain antioxidant and anticancer activities (Dillard & German, 2000). Our results showed that the levels of three main organic acids (l(-)-malic acid, methylmalonic acid, and succinic acid) in TSMSE were slightly higher than that in TSMSE. Apart from these identified main antioxidants and anticancer compounds, we also identified 11 other compounds including 7 amino acids, one nucleotide and 3 other compounds in TSMSE.
the TSMLE and TSMSE (Figure 2(b,c)). Furthermore, we also found that these extracts of tumorous stem mustard contained vitamin C, alkaloids, flavone C-glycosides, isoflavone, terpenoids, and other important antioxidants and/or anticancer compounds (supplementary Table S1). It is worth noting that, TSMLE had higher contents of potential antioxidants including caffeic acid O-glucoside, 4-hydroxy-3,5-diisopropylbenzaldehyde, gallocatechin-gallocatechin, 7 anthocyanins, 4 coumarins, 2 flavanone, 5 flavone, 7 flavone C-glycosides, 9 flavonol, 3 organic acids, and 10 quinate derivatives, relative to TSMSE. Totally, the present results lend support to previous findings (Xie et al., 2014) and expand the horizon of phytochemicals profile research findings of tumorous stem mustard to a new high. In addition, the results of phytochemical analysis also suggested that tumorous stem mustard, especially its leaf, was a potential diet source with strong antioxidant and anticancer activities.

3.3. Antioxidant activity of TSMLE and TSMSE

Polyphenols and some other phytochemicals act at different levels as antioxidants due to their ability to transfer hydrogen atoms and/or electrons (Bannour et al., 2017). The antioxidant capacity of plant extracts is affected by various factors and largely depends on both the composition of the extract and the analytical test system (González-Barrio et al., 2018). Regrettably, there is not a standardized method for antioxidant capacity evaluation; thus, it is necessary to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action (González-Barrio et al., 2018). In our work, we used four methods, ABTS', DPPH, TRP and FRAP based on the radical scavenging activity or ferric reducing power. As can be seen in Figure 3(a), TSMLE and TSMSE were found to have the ability to scavenge ABTS' at a tested concentration range of 0–2 mg/mL. The ABTS'-scavenging effects for TSMLE at all tested concentrations were slightly higher than that of TSMSE. The assay for scavenging DPPH· showed that the antioxidant effect of the polyphenol-enriched extract of tumorous stem mustard leaf was more potently than that of tumorous stem mustard stem, where the DPPH·-scavenging ability of TSMLE was from 25% to 50% at concentrations ranging from 0.4 mg/mL to 2 mg/mL, where the effect of TSMSE was only from 13% to 32% following the treatment (Figure 3(b)). The reducing capability was determined by monitoring the transformation of Fe³⁺ to Fe²⁺ in the presence of the extracts (Nie et al., 2015). As shown in Figure 3(c), the reducing power of TSMLE at 2 mg/mL was as high as 0.12 (absorbance), which was almost 50% higher than that of the same concentration of TSMSE. The FRAP analysis was also performed with these extracts, since the ferric reducing ability is frequently used as an indicator of electron-donating activity, which is an important mechanism of antioxidant activity (Khaled-Khodja, Boulekbache-Makhlof, & Madani, 2014). TSMLE showed obviously higher total antioxidant activity than TSMSE (Figure 3(d)). These results indicated that 80%-CH₃OH-extracts from tumorous stem mustard leaf and stem possess antioxidant property, and the antioxidant effect of the extract from stem mustard leaf was observably superior to that from the stem. This difference of antioxidant activity might be caused by the fact that the contents of antioxidants in stem mustard leaf were higher than that of its stem.

3.4. Antitumor activity of TSMLE and TSMSE

It is well known that the antioxidant activity of phytochemicals was closely related to the anti-tumor activity of phytochemicals.
Thus, our data of antioxidant assessing suggested that there was an obvious difference in antitumor activity between TSMLE and TSMSE. This is the first time to compare the anti-tumor effects of TSMLE and TSMSE in human lung carcinoma A549 cells. As shown in Figure 4(a), the viability of A549 cells was significantly inhibited by TSMLE and TSMSE in a dose-dependent manner. The inhibitory rate of A549 cells treated with TSMLE at 30, 60 and 120 µg/mL for 24 h was 39%, 45%, and 45%, which was significant higher ($p < 0.0001$) than that of TSMSE (12%, 13% and 15%), respectively (Figure 4(a)). This might be the fact that the antioxidant activity of TSMLE was significantly higher than the antioxidant activity of TSMSE. In addition, the anti-tumor effect of TSMLE was also carried out in a time-dependent manner (Figure 4(b)). ROS has been implicated as a second messenger in multiple signaling pathways and can also play an important role in apoptosis by regulating the activity of certain enzymes involved in the cell death pathway (Zhang et al., 2012). The abnormal increase of ROS level in mitochondria can already push tumor cells to the brink of their toxic threshold, which is implicated in the induction of apoptosis.
by several phytochemicals (Li et al., 2013). To investigate if the mitochondrial dysfunction in TSMLE-treated A549 cells was promoted by ROS production, we analyzed intracellular ROS level in terms of fluorescence by DHE. As depicted in Figure 4(c), as compared with the untreated control cells, the level of ROS in 60 μg/mL TSMLE-treated A549 cells for 24 h was distinctly increased. Furthermore, a further treatment of A549 cells with 120 μg/mL of TSMLE led to a dose-dependent elevation of intracellular ROS level (Figure 4c). This image indicated that the apoptotic effect of TSMLE on A549 cells was concerned with an increased accumulation of intracellular ROS and ROS production might lead to apoptotic cell death via the mitochondrial pathway (Li et al., 2016). This anti-tumor activity may be the fact that the extracts form tumorous stem mustard contained polyphenols, sinigrin hydrate and other anti-tumor compounds (supplementary Table S1). Although polyphenols exhibited strong antioxidant capacity in vitro, these antioxidant properties may not be entirely responsible for their chemical prevention effects (Li et al., 2016). Yamashita et al. (1998) reported that polyphenols as antioxidants are also considered to exhibit pro-oxidant properties, which may account for their anticancer properties since chromatin-bound endogenous transitional metal ions were regulated by polyphenols to produce ROS during apoptosis processing of cancer cells (Sarwar et al., 2015). These results indicated that the extracts from tumorous stem mustard leaf and stem induced growth inhibition and apoptosis through enhancing intracellular oxidative stress of lung cancer A549 cells.

4. Conclusions

This is the first study with unequivocal evidence that tumorous stem mustard leaf contains more polyphenols and other antioxidant and anti-tumor compounds, and its extracts have higher antioxidant and anti-tumor activities in human A549 cells than the edible stem. Considering the more than 100 history of uses of edible stems from tumorous stem mustard as food, its leaf might be an excellent source of dietary nutrient in the prevention and/ or treatment of lung cancer. These findings will promote the comprehensive utilization of tumorous stem mustard as food.

Disclosure statement

No potential conflict of interest was reported by the authors.

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