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Mulberry leaf polysaccharide extracted by response surface methodolog suppresses the proliferation, invasion and migration of MCF-7 breast cancer cells

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Abstract

Breast cancer with highly heterogeneous characteristics is the main cause of cancer deaths in women all over the world. Chemotherapy-assisted diet therapy has become an effective way to improve the quality of life of cancer patients. Currently numerous dietary components have been found to inhibit the molecular events and signalling pathways associated with various stages of breast cancer development, but the dietary components extraction and its regulation mechanism were long-term and complex. In this study, low-temperature ultrasonic-assisted method was used to extract MLP, and through employing the Design-Expert software, the optimal conditions for MLP extraction were ultrasonic power 179.34 W, ultrasonic time 13.92 min, and ratio (v/w, mL/g) of water to raw material 23.55. Subsequent experiments evaluated the antioxidant activity of mulberry leaf polysaccharide and found that it has obvious ability to scavenge DPPH free radicals. Interestingly, the extracted MLP could inhibit the cell viability, migration and invasion of breast cancer cells in vitro. Therefore, we suggested MLP could been used as an antioxidant supplement that contains these micronutrients for cancer patients or used to develop anti-oxidant functional health food for breast cancer patients.

Keywords: mulberry leaf polysaccharide; response surface methodolog; antioxidant; breast cancer; cell viability.

Practical Application: Research about antioxidant activities and anti-cancer function of mulberry leaf polysaccharide.

1 Introduction

Cancer is considered to be the leading cause of death from diseases worldwide. According to relevant research predictions, there will be 14 million new cancer cases occurred each year by 2035 (Pilleron et al., 2019). The incidence of different types of tumors varies greatly between men and women, and breast cancer is the main cause of cancer death in women (Akram et al., 2017). Recent statistical data studies from China, India and Russia showed that breast cancer was the second leading cause of death in women after lung cancer (Basu et al., 2020). Chemotherapy was one of the main breast cancer treatment strategies. Its effect was to kill tumor cells with chemical/natural compounds, but its effect was systemic and it exposed a wide range of side effects (Liu et al., 2021). Therefore, some researchers have proposed to develop creative eating habits with the same therapeutic properties as the reference drug, but with fewer systemic side effects to prevent and limit the malignant transformation of tumors (Demuth & Czerniak, 2019; Rafiq et al., 2018; Gaspar-Pintiliescu et al; 2020), thereby improving the quality of life of breast cancer patients.

Herbal compounds from natural resources have a long history of application as anti-cancer drug resources (Lou et al., 2018). They were usually low-cost, rich in content, and have almost no side effects in clinical practice. In addition to stimulating anti-inflammatory, anti-tumor or anti-metastatic reactions, the tumor preventive and protective effects of herbal compounds are also related to their cellular defense properties, such as detoxification and antioxidant phenomena (Balthazar et al., 2021; Rafiq et al., 2020; Teodor et al., 2020; Weng et al., 2021). Mulberry (*Morus alba L.*) is one of the sources of natural medicinal materials and has been widely used as a medicinal and edible plant in many Asian countries since ancient times (Yuan & Zhao, 2017; He et al., 2018). With its proven nutritional and health benefits, Mulberry leaves have been considered to be a suitable ingredients for the wider application of functional foods (Jan et al., 2021; Chen et al., 2021). Mulberry leaves had high nutritional value due to its low lipid value and high content of polysaccharide, protein, fiber, vitamins, organic acids, and minerals that were comparable to other berries. These natural biologically active compounds have strong biological activity and have been shown to exhibit excellent pharmacological effects on various diseases, including anti-diabetic, anti-obesity, and antibacterial properties (Hassan et al., 2020), but there are few studies on the prevention and treatment of tumors due to the complexity and long-term nature of the anti-cancer research.

Mulberry leaves polysaccharide (MLP) was the main active ingredient of mulberry leaves. Like other herbal polysaccharides, it has received more and more attention due to its multiple biological activities, but how to improve the extraction rate of mulberry leaf polysaccharide was a key factor that limited its functional development (Liao et al., 2017). For polysaccharides extraction, the conventional hot water extraction technology was still the main and classic method (Passos & Coimbra, 2013),

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but high water temperature could easily lead to polysaccharides hydrolysis, thereby destroying the effective activity of polysaccharide components. Therefore, low-temperature ultrasonic-assisted extraction has received extensive attention and application. The ultrasonic-assisted extraction method has been used to extract the organic compounds from soil, animal and plant tissues due to its lower energy consumption, lower consumption of solvents and higher extraction efficiency (Wen et al., 2018; Chemat et al., 2017; Savic & Savic Gajic, 2020). A number of studies have indicated ultrasonic at different power levels (40-200 W) used for extraction of polysaccharides could signifcantly improve the yield (Zhang et al., 2020), but most of these studies used the heat extraction method.

Therefore, the aim of the present work was to determine the effects ofultrasonic power, ultrasonic time and ratio of water to raw material in low-temperature condition on the yield of polysaccharides from mulberry leaves. The anti-oxidant activities of extracted polysaccharides was measured by using the DPPH radical scavenging assay in vitro, and MTT assay was used to detect the effect of MLP on breast cancer cell viability. In addition, the migration and invasion of MLP on breast cancer cells were also researched.

2 Matrials and methods

2.1 Materials and chemicals

The mulberry leaves were collected from the mulberry plantation in Suzhou (Anhui, China), washed with top water, air dried at room temperature and ground into fine powder. 1,1-diphenyl-2-picrylhydrazyl (DPPH), glucose, Vitamin C, absolute ethanol, concentrated sulfuric acid, phenol, and other reagents are all domestically produced analytically pure.

2.2 Cell line and cell culture

Human breast cancer cell lines MCF-7 was maintained in our laboratory. Cell line was cultured in DMEM, MEM, RPMI 1640 (Gibco, USA) supplemented with 10% fetal calf serum (Gibco), and 1% penicillin and streptomycin (Invitrogen, USA) at 37 °C in a humidified atmosphere with 5% CO_2.

2.3 Extraction of polysaccharides

In order to remove most of the monosaccharides, pigments and other small molecules, the powder of mulberry leaves was treated twice with 80% ethanol at room temperature (16 °C) for 24 h. The resulting residue was freeze-dried and used for next extraction step. The dried pretreated sample was extracted in the container with ice-water by the designed ratio of extraction solvent (deionized water) to raw material, ultrasonic power, and ultrasonic time. The resulting solution was filtered, mixed with a triple volume of absolute ethanol and kept overnight. The precipitates were collected by centrifugation at 4000 rpm for 15 min, washed with absolute ethanol and acetone, and dried to obtain the MLP. Mulberry leaf polysaccharides were weighed out 1.0 g and distilled water to make the volume to 100 mL as a sample solution for use. The extraction rate was calculated according to the following formula (Equation 1 (Savic & Savic Gajic, 2020):

$$Extraction\ yield\ (\%) = \frac{W_1}{W_0} \times 100 \quad (1)$$

where W1 and W0 are the weights of MLP and pretreated sample respectively.

2.4 Experimental design of RSM

Effects of extraction parameters including ultrasonic power, extraction time, extraction cycles and ratio of water to raw material on the yields of MLP were conducted by single-factor tests (data not shown). Accordingly, three major factors (ultrasonic power, ultrasonic time and ratio of water to raw material) were chosen and their appropriate ranges were determined based on the preliminary experimental results. In addition, a three-level, three-variable BBD was used to determine the optimal levels of extraction variables, including the ultrasonic power (X_1), ultrasonic time (X_2) and water to raw material ratio (X_3) for the extraction of MLP. For statistical calculation, the variables were coded according to the Equation 2 (Savic & Savic Gajic, 2020):

$$X_i = \frac{\chi_i - \chi_0}{\Delta\chi_i} \quad (2)$$

where Xi is the coded value of independent variable, χi is the actual value of the independent variable, χ0 is the actual value of the independent variable at the central point, and Δχi is the step change of the variable. Table 1 shows the range of independent variables and their levels.

The whole design consisted of 17 experimental runs, including 12 factorial points and 5 axial points. The 5 axial points were used to allow for estimation of a pure error sum of squares. The experiments were carried out in random order, and the experimental data (Table 1) were fitted to the following second-order polynomial mode (Equation 3) (Savic & Savic Gajic, 2020)

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \quad (3)$$

where *Y*, extraction yield of MLP, is the predicted response; β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; X_i and X_j are the independent variables ($i \neq j$).

2.5 Antioxidant activity assay of MLP by DPPH radical scavenging experiment in vitro

The measurement of DPPH free radical scavenging activity was slightly modified using the previously reported method (Liu et al., 2004). Briefly, MLP was dissolved in deionized water to provide a range of concentrations (0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/mL). Then, 50 μL of sample solution, 25 μL of DPPH-ethanol solution (0.4 mM) and 100 μL of deionized water were mixed in a 96-well plate. The mixture was kept in the dark at room temperature for 30 minutes, and the absorbance

Table 1. Box–behnken design matrix and the response values for the yield of MLP.

Run	Ultrasound power		Ultrasound time		Ration of water to raw material (mL/g)		MLP
	X1	Code X1	X2	Code X2	X3	Code X3	yield (%)
1	150	0	10	-1	30	1	5.16
2	100	-1	10	-1	25	0	5.35
3	150	0	15	0	25	0	6.13
4	100	-1	15	0	30	1	5.55
5	150	0	15	0	25	0	6.10
6	150	0	15	0	25	0	6.28
7	150	0	15	0	25	0	6.17
8	200	1	15	0	30	1	4.40
9	150	0	15	0	25	0	6.23
10	150	0	20	1	20	-1	4.10
11	200	1	15	0	20	-1	5.03
12	100	-1	20	1	25	0	4.05
13	200	1	10	-1	25	0	4.30
14	200	1	20	1	25	0	3.71
15	150	0	10	-1	20	-1	5.48
16	100	-1	15	0	20	-1	5.01
17	150	0	20	1	30	1	4.84

(Abs) at 517 nm was measured by a microplate reader (Bio-RAD, USA). Ascorbic acid was used as positive control. DPPH radical scavenging activity was calculated by the following formula: DPPH radical scavenging activity (%) = $[1 - (Abs_1 - Abs_2)/Abs_0] \times 100$, where Abs_0 is the Abs of the control (deionized water instead of sample), Abs_1 is the Abs of the sample, and Abs_2 is the Abs of the sample under identical conditions as Abs_1 with ethanol instead of DPPH-ethanol solution.

2.6 Antioxidant activity assay of MLP by hydroxyl radical (·OH) scavenging experiment in vitro

Hydroxyl radical (·OH) scavenging activity was measured using the method of Liao et al. (2017). Briefly, MLP was dissolved in deionized water to provide a range of concentrations (0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg/mL). Then, 500 μL of sample solution, 500 μL of o-phenanthroline ethanol solution (0.75 mM), 500 μL of $FeSO_4$ solution (0.75 mM), 500 μL of H_2O_2 solution (0.01%) and 1000 μL were mixed. The mixture was kept in the water bath at 37 °C for 1 h, and the absorbance (Abs) at 512 nm was measured by a microplate reader (Bio-RAD, USA). Ascorbic acid was used as positive control. Hydroxyl radical (·OH) scavenging activity was calculated by the following formula: Hydroxyl radical (·OH) scavenging rate (%) = (Abs1 − Abs0)/(Abs2 −Abs0) × 100%, where Abs0 is the Abs of the control (deionized water instead of sample), Abs1 is the Abs of the sample, and Abs2 is the Abs of the sample under identical conditions as Abs1 with H_2O_2 solution instead of deionized water solution.

2.7 Cell viability detected by MTT assay

To determine cell viability, the colorimetric MTT metabolic activity assay was used. MCF-7 cells (1×10^4 cells/well) were cultured in a 96-well plate at 37 °C, and exposed to vary concentrations of MLP at 24 h, 48 h and 72 h. Cells treated with medium only served as a negative control group. After removing the supernatant of each well and washing twice by PBS, 20 μL of MTT solution (5 mg/mL in PBS) and 100 μL of medium were then introduced. After incubation for another 4 h, the resultant formation crystals were dissolved in dimethyl sulfoxide (100 μL) and the absorbance intensity measured by a microplate reader (Bio-RAD, USA) at 490 nm. All the experiments were performed in triplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

2.8 Cell migration and invasion assays

The cells were harvested 48 h after treated with MLP and were resuspended in medium. The cells were then plated at a density of 2.0×10^6 cells/mL. In total, 0.2 mL cells was added to the upper chamber of transwell chambers (24-well inserts, 8-μm pore size; Millipore, Bedford, MA, USA), and 0.6 mL medium containing 10% fetal bovine serum was added to the lower chamber as a chemoattractant.

For invasion assays, dissolve Matrigel overnight at 4 °C, dilute Matrigel with a pre-cooled serum-free medium at a volume ratio of 1:3, add 40 μL into the pre-cooled transwell chamber, and incubate at 37 °C for 2 hours to solidify Matrigel. Aspirate the excess liquid in the chamber, and add 100 μL and 600 μL serum-free medium to the upper and lower chambers respectively, and equilibrate overnight at 37 °C. On the second day of cell transfection, resuspend 1×10^5 cells with 100 μL serum-free DMEM-F12 and MEM medium, add thm to the upper chamber of the transwell chamber, and add 600 μL complete medium to the lower chamber. After incubating at 37 °C for 24 or 48 hours, remove the cell, wipe the cells in the upper chamber with a cotton swab, and wash with PBS. Fix with 4% paraformaldehyde for 10 minutes, wash once with PBS, stain with crystal violet for 10 minutes, wash once with PBS, and take photos for statistics.

2.9 Statistical analysis

Each independent experiment was repeated at least three times. Statistical analysis between two groups was performed by using Student's t test through SPSS software (SPSS Inc., USA). Variance analysis between multiple groups followed by Tukey's test was used to calculate the statistical significance of the differences. Multiple groups of normalized data were analyzed using one-way ANOVA. Data was shown as mean ± standard deviation. If not specified above, a p-value of less than 0.05 was considered to indicate a statistically significant difference.

3 Results

3.1 Optimization of extraction parameters

Predicted model and statistical analysis

The experimental design along with the extraction rate were shown in Table 1, and the data were analyzed by Design-Expert software. As a result, a second-order polynomial equation describing the correlation between the MLP yield and the test variables was obtained as follows (Equation 4) (Savic & Savic Gajic, 2020):

$$Y = +6.18 - 0.32X_1 - 0.45X_2 + 0.041X_3 + 0.18X_1X_2 - 0.29X_1X_3 + 0.27X_2X_3 - 0.86X_1^2 - 0.97X_2^2 - 0.32X_3^2 \quad (4)$$

where Y represents the extraction yield of MLP, X_1, X_2 and X_3 represent ultrasonic power, ultrasonic time and ratio of water to raw material, respectively.

The ANOVA, lack-of-fit and the adequacy of the model are indicated in Table 2. The model F-value of 132.23 implied that the model was significant. The determination coefficient (R^2) and the adjusted determination coefficient (adj-R^2) were 0.9942 and 0.9866, respectively, which indicated that the experimental and predicted values of MLP production are in good agreement with the goodness of fit of the regression equation. The P-value is used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller of the P-value represented the more significant of the corresponding coefficient. Table 2 showed that the linear coefficients (X_1 and X_2), quadratic term coefficients (X_1^2, X_2^2 and X_3^2) and cross product coefficients (X_1X_2, X_1X_3, and X_2X_3) were significant on extraction yield of MLP due to P-value < 0.05. The results also showed that among the independent variables, the extraction ultrasonic power was the most important parameter that affected the output of MLP, followed by the ultrasonic time and the ratio of water to raw materials.

Response surface plot and contour plot

The 3D response surface and 2D contour plots were provided as the graphical representations of the regression equation. They showed the type of interactions between two tested variables and the relationship between responses of each variable and the levels of experiment. In the present study, the response surface and contour plots (Figure 1) were obtained by using Design-Expert. Figure 1A and 1B showed the effects of ultrasonic power (X_1), ultrasonic time (X_2) and their interaction on extraction yield when the ratio of water to raw material (X_3) was fixed at 0 level. The X_1 and X_2 demonstrated quadratic effects on the extraction yield. The yield of MLP increased to the maximum with increase of ultrasonic power, and then decreased slightly with the further increase of extraction ultrasonic power. The ultrasonic time showed a similar effect on the yield of MLP. Generally, higher ultrasonic power and longer ultrasonic time can promote the dissolution of polysaccharides from plant tissues. However, too high ultrasonic power and too long ultrasonic time may result in degradation of polysaccharides and hence decrease the polysaccharides yield. Similarly, Figure 1C and 1D show the quadratic effects of X_2 and X_3 on the extraction yield when X_1 was fixed at level 0, and Figure 1E and 1F show the effects of X_1 and X_3 and their reciprocal interaction on the extraction yield when X_2 was fixed at level 0.

Table 2. ANOVA for response surface quadratic model of MLP extraction.

Source	Sum of squares	df	Mean square	F-value	P-value
Model	11.38	9	1.26	132.23	< 0.0001 [a]
X1	0.79	1	0.79	82.99	< 0.0001 [a]
X2	1.61	1	1.61	168.43	< 0.0001 [a]
X3	0.014	1	0.014	1.42	0.2717 [b]
X1X2	0.13	1	0.13	13.18	0.0084 [a]
X1X3	0.34	1	0.34	35.78	0.0006 [a]
X2X3	0.28	1	0.28	29.37	0.0010 [a]
X12	3.14	1	3.14	328.23	< 0.0001 [a]
X22	3.93	1	3.93	410.78	< 0.0001 [a]
X32	0.43	1	0.43	45.36	0.0003 [a]
Residual	0.067	7	9.565E-003		
Lack of Fit	0.045	3	0.015	2.82	0.1709 [b]
Pure Error	0.021	4	5.370E-003		
Cor Total	11.45	16			

R^2 = 0.9942, adjusted R^2 = 0.9866. [a] 5% significance level. [b] Not significant relative to the pure error.

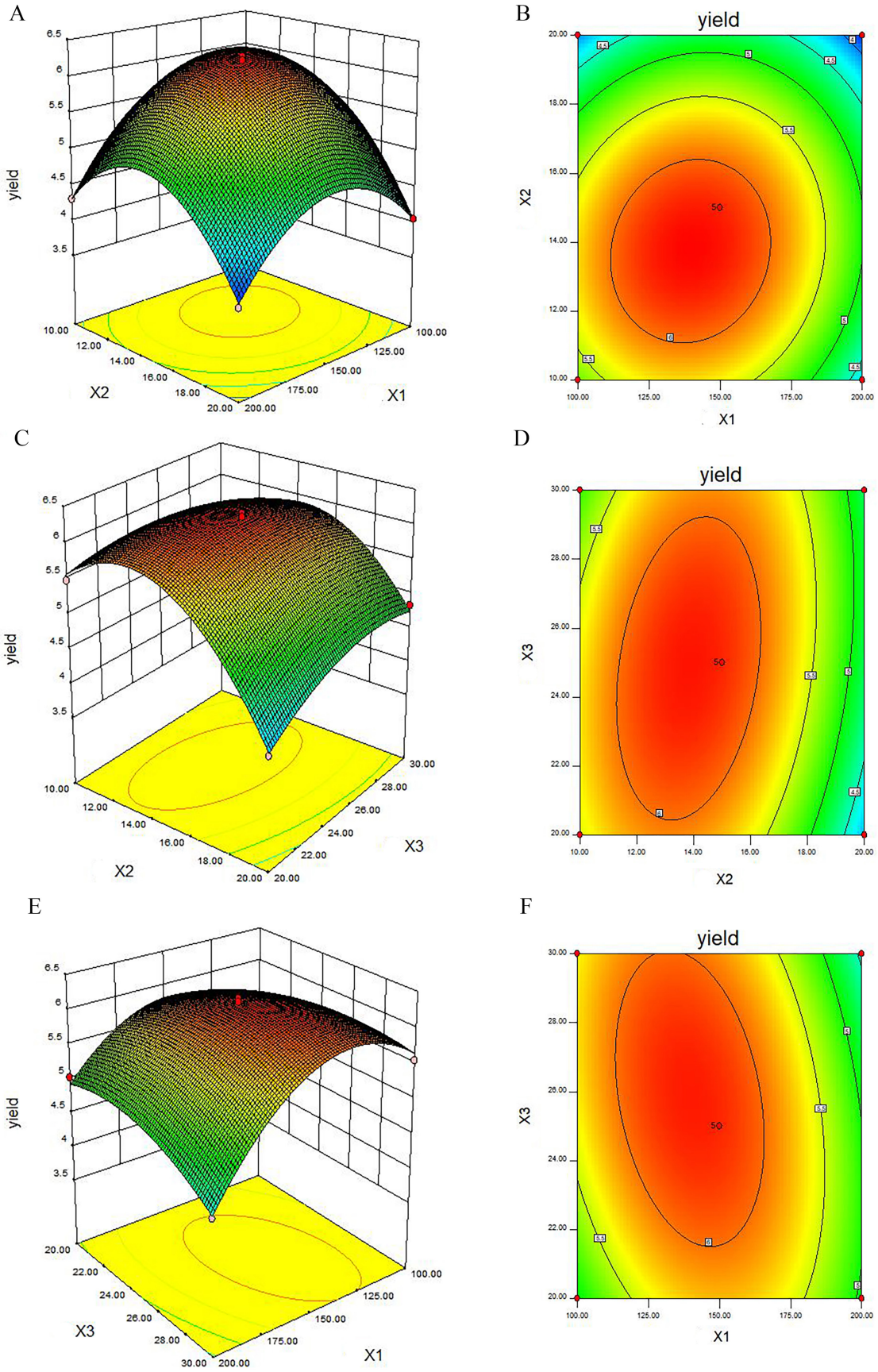

Figure 1. Response surface plots (A, C and E) and contour plots (B, D and F) showing the effects of variables (X_1, ultrasonic power; X_2, ultrasonic time; X_3, ratio of water to material) and their mutual effects on the extraction yield of MLP.

Optimization of extracting parameters and validation of the model

Through employing the Design-Expert software, the optimal conditions for MLP extraction were ultrasonic power 179.34 W, ultrasonic time 13.92 min, and ratio (v/w, mL/g) of water to raw material is 23.55. Under the optimal conditions, the maximum predicted yield of MLP was 5.76%. For the convenience of operation, the optimal parameters were determined as follows: ultrasonic power 179 W, ultrasonic time 14 min, and ratio (v/w, mL/g) of water to raw material 24. In order to ensure that the predicted results were not biased toward the actual value, experimental rechecking were carried out by using the deduced optimal conditions. A average value of 5.72 ± 0.5% (n = 3) was obtained from actual experiments, which proved the validation of the RSM model and indicated that the model was sufficient for the extraction of MLP.

3.2 Antioxidant activity assay of MLP by DPPH radical scavenging experiment

DPPH free radical was a stable free radical with a characteristic absorption at 517 nm, which would be significantly reduced when exposed to proton-donating substances. Therefore, it has been widely used to evaluate the antioxidant activity of natural antioxidants. In the present study, the scavenging ability of MLP on DPPH free radicals was examined and the results are shown in Figure 2. The scavenging rates of MLP and Vc increased with the increase of sample concentration. At a concentration of 4.0 mg/mL, the DPPH free radical scavenging activities of MLP and Vc were 66.34% and 91.31%, respectively.

3.3 Antioxidant activity assay of MLP by hydroxyl free radical (·OH) scavenging experiment

Hydroxyl free radicals are the most reactive oxygen radicals in the cells of organisms, and are generated by the catalytic action of metal ions in superoxide anion and hydrogen peroxide. Antioxidant substances in the substance to be evaluated can compete to capture hydroxyl radicals in the system. Therefore, this method is also an important part of evaluating the antioxidant activity of natural plant polysaccharides. In the present study, the hydroxyl free radical scavenging ability of MLP was examined and the results are shown in Figure 3. The scavenging rates of MLP and Vc increased with the increase of sample concentration. At a concentration of 1.0 mg/mL, the hydroxyl free radical scavenging activities of MLP and Vc were 83.46% and 92.91%, respectively.

3.4 MLP inhibited cell proliferation of MCF-7 cells

To investigate whether the antioxidant activity of MLP could modulate cell viability in breast cancer, MCF-7 cells were treated with different concentration MLP by different incubation time (Figure 4). MTT assays experiments showed that the MLP could obviously decrease the cell proliferation of MCF-7, and this inhibitory effect has the concentration and time dependent characteristics. Compared with the control group, the cell inhibition rate of the treatment group treated with 100 μg/mL MLP for 48 hours decreased most significantly, and it was statistically significant.

3.5 MLP inhibited cell migration and invasion of MCF-7 cells

To investigate whether the antioxidant activity MLP could regulate cell migration and invasion in breast cancer cells, transwell experiments were conducted. As shown in Figure 5, the transwell chamber was serially observed for 48 h following the cells treated with MLP at 100 μg/mL concentration on the plate. As shown in Figure 5A, the speed of migration was faster in MCF-7 cells, compared with that in the MCF-7 treated with MLP groups. Moreover, the speed of

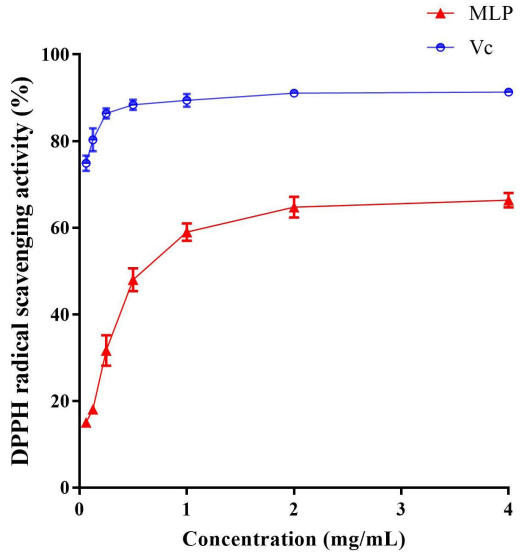

Figure 2. Scavenging effects on DPPH radical activity of crude MLP *in vitro*. Vc represented the vitamin C as control, MLP represented mulberry leaves polysaccharide. The data are shown as means ± SD.

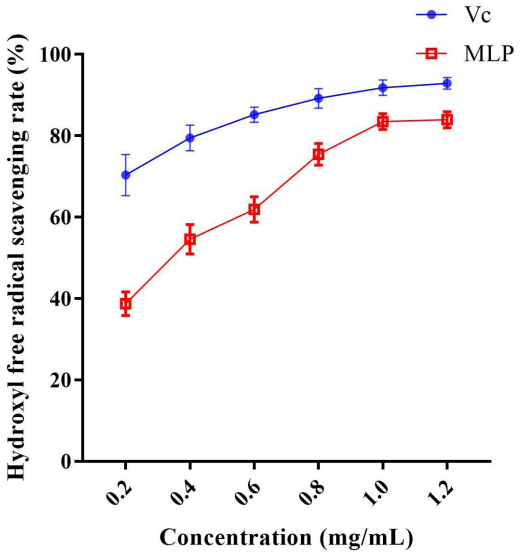

Figure 3. Scavenging effects on hydroxyl free radical activity of crude MLP *in vitro*. Vc represented the vitamin C as control, MLP represented mulberry leaves polysaccharide. The data are shown as means ± SD.

invasion was faster in MCF-7 cells, compared with that in the MCF-7 treated with MLP groups (Figure 5B). This indicated that the MLP could obviously decreased cell migration and invasion in vitro.

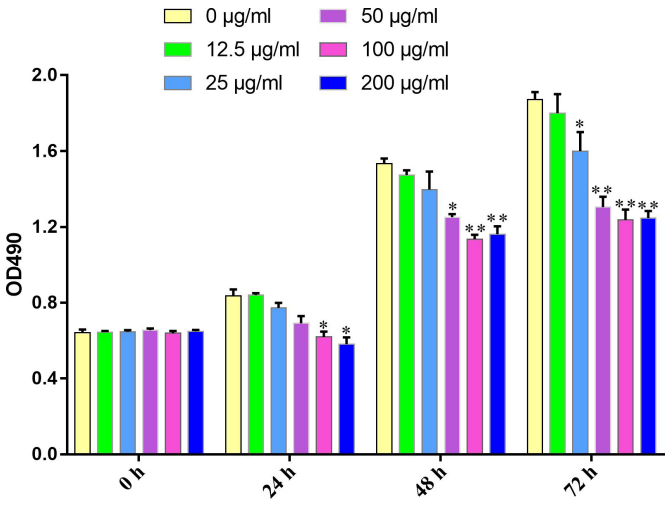

Figure 4. Different concentration MLP inhibited the cell viability of breast cancer cells MCF-7 at 24 h, 48 h or 72 h. The data are shown as means ± SD, and * represented $p < 0.05$, ** represented $p < 0.01$, SPSS.

4 Discussion

Plants are strongly regarded as an important natural resources of phytochemicals with a wide range of effective biological activities, which could be used as drugs for the treatment of various diseases, such as inflammation, cardiovascular diseases, neurodegenerative diseases, cancer, etc. (Fadeyi et al., 2013; Ukwade et al., 2020). Approximately 70-80% of the world population directly or indirectly depended on plant materials as their main source of treatment, and more than 60% of anti-tumor supplements come from many medicinal plants or plant parts. Plant samples and other food extractions have aroused great interest in biomedical research for human benefit (Gao & Watanabe, 2011; Rafiq et al., 2020). Mulberry, a multipurpose agro-forestry plant that belongs to the family of Moraceae, was widely distributed in tropical, subtropical and temperate regions (Agarwal & Kanwar, 2007). It is commonly used as a silkworm (*Bombyx mori L.*) diet, alternative medicine in China and Japan and a kind of tea due to its low toxicity and good therapeutic properties (Chung et al., 2013; Wang et al., 2014). According to reports, mulberry leaves contained a variety of biologically active compounds, including 1-deoxynojirimycin, mulberries, chlorogenic acid, rutin, flavonol glycosides and anthocyanins, etc., which could exert anti-obesity, anti-diabetic, antioxidant and other biological functions are related. Mulberry leaf polysaccharide (MLP) was another main active ingredient that could promote

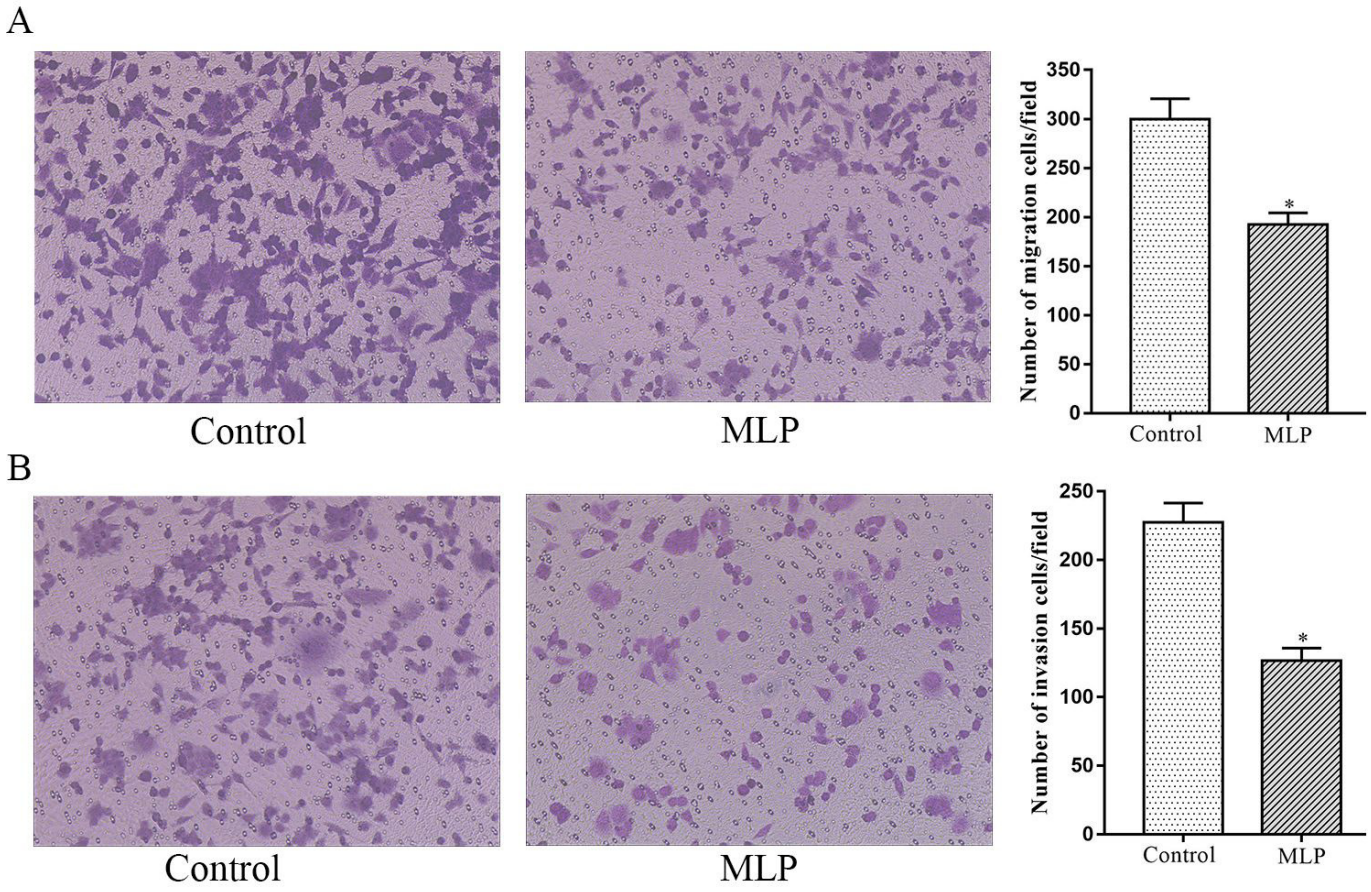

Figure 5. MLP inhibited the cell migration and invasion of breast cancer cells MCF-7. A. MLP inhibited the cell migration of breast cancer cells MCF-7, B. MLP inhibited the cell invasion of breast cancer cells MCF-7. The data are shown as means ± SD, and * represented $p < 0.05$, SPSS.

insulin expression in alloxan-induced diabetic mice and regulate liver glucose metabolism (Li et al., 2011). To some extent, MLP could also act as an antioxidant and antibacterial agent (Zhang et al., 2016). However, most studies on the activity of mulberry leaf polysaccharides focused on anti-diabetic studies, and the activities of mulberry leaf polysaccharides extracted by different methods were significantly different, which may be closely related to the difference in extraction methods.

At present, the extraction of mulberry leaf polysaccharides was mainly the classic thermal extraction method, but high-temperature water extraction was likely to cause the hydrolysis of mulberry leaf polysaccharides, so our research used low-temperature ultrasonic to extract mulberry leaf polysaccharides. Through response surface methodolog, the optimal conditions for MLP extraction were ultrasonic power 179.34 W, ultrasonic time 13.92 min, and ratio (v/w, mL/g) of water to raw material 23.55, and the yield of mulberry leaf polysaccharides was similar to the results of previous studies (Samavati & Yarmand, 2013). Subsequent experiments evaluated the antioxidant activity of mulberry leaf polysaccharide and found that it had obvious ability to scavenge DPPH free radicals. It could be seen that the mulberry leaf polysaccharide extracted by low-temperature ultrasonic had good antioxidant activity, which can provide a reference for the research and development of related drugs. Several epidemiological observations have shown an inverse relation between consumption of plant-based foods, rich in phytochemicals, and incidence of cancer (Chikara et al., 2018; Dini, 2021; George et al., 2021). Phytochemicals, secondary plant metabolites, via their antioxidant property played a key role in cancer chemoprevention by suppressing oxidative stress-induced DNA damage. In additon, they modulated several oxidative stress-mediated signaling pathways through their antioxidant effects, and ultimately protected cells from undergoing molecular changes that trigger carcinogenesis (Chikara et al., 2018). Interestingly, the extracted MLP could inhibit the cell viability, migration and invasion of breast cancer cells in vitro, which implied that mulberry leaf polysaccharide can be used as an antioxidant for cancer patients or used to develop anti-oxidant functional health food for breast cancer patients.

In summary, in this study, we found low-temperature ultrasonic can effectively extract mulberry leaf polysaccharides from mulberry leaves. Through response surface design methods, we have obtained the optimal extraction conditions (ultrasonic power 179.34 W, ultrasonic time 13.92 min, and ratio (v/w, mL/g) of water to raw material 23.55). Experiments In vitro have showed that mulberry leaf polysaccharides had good antioxidant activity, which could inhibit cell viability of MCF-7. Meanwhile, transwell experiments also showed that MLP could obviously inhibited the cell migration and invasion of breast cancer cells. All these results indicated that MLP could be considered as an antioxidant component suitable for wider application of functional foods, which may be an effective diet therapy method to improve the quality of life of breast cancer patients. In the near future, more efforts should be made to fully understand the role of mulberry leaf polysaccharides in anti-tumor, so as to provide better nutritional strategies for preventing and treating tumors and maintaining a healthy life.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

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Author contributions

FF designed and performed experiments, wrote the manuscript. PH and XKT gave suggestion on study design. All authors read and approved the final manuscript.

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