Extraction and isolation of the active ingredients of dandelion and its antifungal activity against *Candida albicans*

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Abstract. In this study, six compounds were isolated and purified from dandelion, and only sample I exhibited notable antifungal effect on *Candida albicans* (CA). high-performance liquid chromatography-diode-array detector-electrospray ionization-tandem mass spectrometry analysis showed that sample I comprised 4-coumaric acid, ferulic acid, quercetin pentoside, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, luteolin, and two unknown compounds, at a relative percent composition of 11.45, 3.96, 10.48, 34.24, 3.91, 11.80, 3.65 and 4.21%, respectively. Further antimicrobial experiments showed that the minimum inhibitory concentration of sample I was 32.0 mg/ml, and sample I mainly acts on bacterial growth in the exponential phase of CA growth. Optical density and infrared analyses conclusively suggested that sample I damages the structure of CA cells, particularly the cell wall and cell membrane, resulting in macromolecule leakage of intracellular nucleic acids and cell metabolism disruption. In conclusion, dandelion sample I was reported to increase CA cell membrane permeability by affecting the glycosidic bond in β-(1-3)-D glucan and destroying the cell wall, ultimately leading CA to death.

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Abbreviations: CA, *Candida albicans*; VVC, vulvovaginal candidiasis; DAIS, dandelion active ingredients; LC-DAD-ESI-MS/MS, high performance liquid chromatography-diode-array detector-tandem mass spectrometry; MIC, minimum inhibitory concentration; SEM, scanning electron microscopy; OD, optical density

Key words: dandelion, active ingredients, *Candida albicans*, bacteriostatic mechanism, β-(1-3)-D glucan

Introduction

Vulvovaginal candidiasis (VVC) is a common disease that is unique to women and is mainly caused by *Candida albicans* (CA) (1,2). In recent years, the incidence of VVC has increased significantly, particularly in younger women, due to the intensified influence of environmental pollution, increased pressure of competitiveness, accelerated work pace, lifestyle changes, and many other factors (3). The extensive use of broad-spectrum antibiotics, hormones, and immunosuppressive agents has also contributed to the prevalence of VVC. Moreover, some patients fail to follow advice from doctors and insist on taking drugs or irregular treatments, resulting in repeated relapses of VVC (4,5). CA herbal treatment based on syndrome differentiation in traditional Chinese medicine directly inhibits or eliminates the disease (6-8) and its pathogens by enhancing the body's immunity. It also possesses antipyretic, analgesic, and anti-inflammatory properties, with minimal adverse reactions, thereby addressing the symptoms as well as the root cause of the problem (9). Among the natural products that have been traditionally used to treat CA are black and green teas. These products have demonstrated appreciable inhibitory effects on the growth of CA, possibly due to the arrest of CA biofilm formation upon the impairment of proteasome activity (10). Antimicrobial experiments have shown that propolis extracts derived from bees can also effectively control the proliferation of CA (11). Lavender honey is another product that has been reported to have a certain inhibitory effect on CA and the growth of pathogenic yeasts (12). *In vitro* antimicrobial activity assays reveal that the natural formula traditionally used in Chinese herbal medicine for curing CA targets 23 strains of *Staphylococcus aureus,* among others (13). The andrographolide derivative, Omeprazole, inhibits CA proliferation and biofilm formation in rats by interfering with the transcription and expression of CA adhesion-related genes (14). In addition, *Pulsatilla* decoction, another Chinese herbal medicine, has been shown to effectively prevent the recurrence of CA vaginitis in 49 patients (15). Such valuable effects of Chinese herbal medicine in the treatment of VVC have prompted great interest in this particular field of research.

Dandelion is included in the 2015 edition of 'Chinese Pharmacopoeia' for the Asteraceae (*Taraxacum spp.*) perennial

herb dandelion (*T. mongolicum* Hand-Mazz.), Alkaline dandelion (*T. sinicum Kitag.*) (16). Several studies have demonstrated that dandelion has anti-viral (9), anti-tumor, hypolipidemic (17) and other functions, particularly anti-bacterial and anti-inflammatory effects (18). At present, the use of dandelion in the treatment of human and animal diseases, especially mastitis is extremely extensive. Studies have shown that dandelion active ingredients (DAIS) possess notable bacteriostatic activity against the main pathogens of recessive mastitis in dairy cows, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* (19). Moreover, dandelion water extract has been reported to significantly reduce the expression of tumor necrosis factor-α and intracellular adhesion molecule 1 in the mastitic microvascular endothelium of rats (20). It may also be effective in treating breast edema (21) and triple-negative breast cancer by inducing endoplasmic reticulum (an organelle with protein synthesis and folding as its major function) stress-related cell apoptosis (22). Despite the abundance of studies concerning the therapeutic effect of dandelion extract on mastitis and cancer, its activity against VVC requires further investigation.

In this study, DAIS was obtained using D101 and LSA-30 macroporous resins, screened using bacteriostatic experiments, and detected by high-performance liquid chromatography-diode-array detector-electrospray ionization-tandem mass spectrometry (HPLC-DAD-ESI-MS/MS). In particular, minimal inhibitory concentration, bacteriostatic kinetics, cell ultrastructure, cell membrane permeability, and synthesis of cell wall component β-(1-3)-D glucan were assessed in order to elucidate the mechanism of DAIS antifungal activity against CA. The results reported herein constitute the fundamental theoretical background required to conduct further, more detailed research on the development and utilization of dandelion resources.

Materials and methods

Chemicals and materials. Anhydrous ethanol, ethyl ether, petroleum ether, n-butanol, concentrated hydrochloric acid (37%), sodium hydroxide, concentrated sulfuric acid (98%), Tween 80, Tris (hydroxymethyl) aminomethane, dimethyl sulfoxide, glutaraldehyde, sodium dihydrogen phosphate, disodium hydrogen phosphate, KBr, phosphoric acid, and ethyl acetate were of analytical grade and were purchased from Tianjin Fuyu Fine Chemical Co. Ltd. Analytical grade glucose, peptone, yeast extract, and agar powder were provided by AOBOX. Spectrally pure potassium bromide was purchased from Beijing Inluck Science and Technology Development Co. Ltd., whereas chromatographically pure methanol and acetonitrile were purchased from Honeywell (Burdick & Jackson). D101 and LSA-30 macroporous resins were purchased from Sunresin.

Fresh dandelion whole-grass samples were collected and identified as *Taraxacum mongolicum* by Professor Xinsheng Li of the Shaanxi University of Technology. The samples were dried to constant weight at 55˚C, crushed, passed through a 60-mesh screen, then dried again. Active ingredients of dandelion were extracted using the method illustrated in Fig. 1.

Samples of lyophilized CA (ATCC10231) provided by the China Food and Drug Administration were stored in a low-temperature refrigerator (Thermo Fisher Scientific, Inc.) at -80˚C. Prior to use, the samples were rehydrated, passaged, then cultured in Sabouraud agar medium (10 g of peptone, 40 g of glucose, 15 g of agar and 0.1 g of chloramphenicol added to distilled water with final volume 1L). No agar was added to the liquid culture, and other components remained unchanged.

Antibacterial assays. CA (1 µl; OD_{600}=0.4) was inoculated in liquid Sabouraud medium then cultured in a shaker at 37˚C and 200 rpm until the optical density $(OD)_{600nm}$ reached 0.60 (Ultraviolet Spectrophotometer, Shimadzu). Next, 100 µl of the CA suspension were pipetted onto six Sabouraud agar plates and spread evenly using an L-shaped glass rod. Later, 10 µl dandelion extract samples I-VI were each pipetted onto sterile filter paper (6 mm in diameter). Then sterile filter papers were each transferred to Sabouraud agar medium (23,24). The plates were incubated at 37˚C for 36 h, and the antifungal activity of DAIS was determined based on the inhibition zone of the plate. The same active ingredients were used for subsequent experimental studies. All of the experiments were repeated three times for verification.

HPLC-DAD-ESI-MS/MS. An Agilent 6410B HPLC-ESI-MS/MS system (Agilent Technologies, Inc.) was used to analyze DAIS. The ingredients were separated on a DiKMA Diamonsil C18 column (250x4.6 mm, 5 cm) using a mobile phase composed of acetonitrile (eluent A) and 1.0% formic acid (v/v, eluent B). Gradient elution was performed at a flow rate of 1 ml/min, starting with 8% A/92% B at 0 min and passing through 14% A/86% B at 24 min, then 23% A/77% B at 35 min, 24% A/76% B at 44 min, 32% A/68% B at 60 min, 37% A/63% B at 66 min, and finally back to 8% A/92% B at 68 min (maintained for 6 min) (24,25). The detection wavelength of the HPLC system was set to 280 nm and the injection volume to 10 µl. The ESI source was kept at 110˚C, and the desolvation temperature was set to 400˚C. The nebulizer pressure, fragmentor voltage, and capillary voltage were maintained at 35 psi, 135 V, and 3,000 V, respectively. Full spectra were recorded in the negative ionization mode over the m/z range of 100-1,000 Da.

Determination of the minimum inhibitory concentration (MIC). A single activated CA colony was inoculated in liquid sabourand medium, incubated at 37˚C and shaken at 200 rpm for 12 h, then adjusted to a CA concentration of 10^7 CFU/ml. A 400 mg/ml DAIS mother liquor solution was also prepared. Both solutions were added to test tubes containing liquid Sabouraud medium. The final DAIS concentrations in the tubes were adjusted to 4.0, 8.0, 16.0, 32.0, 48.0, 64.0, or 128 mg/ml, whereas the amount of added CA suspension was set to 5% (v/v) in all of the tubes. A test tube containing an equivalent volume of pure water was used as control. Fluconazole was added to test tubes containing liquid Sabouraud medium and 5% CA suspension at final concentrations of 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, and 1.40 mg/ml, as positive control group. Next, the mixtures were incubated at 37˚C and shaken at 200 rpm for 24 h. The MIC was taken to be the lowest sample group concentration beyond which changes in OD_{600} values compared with the next sample group were ≤5% (26,27).

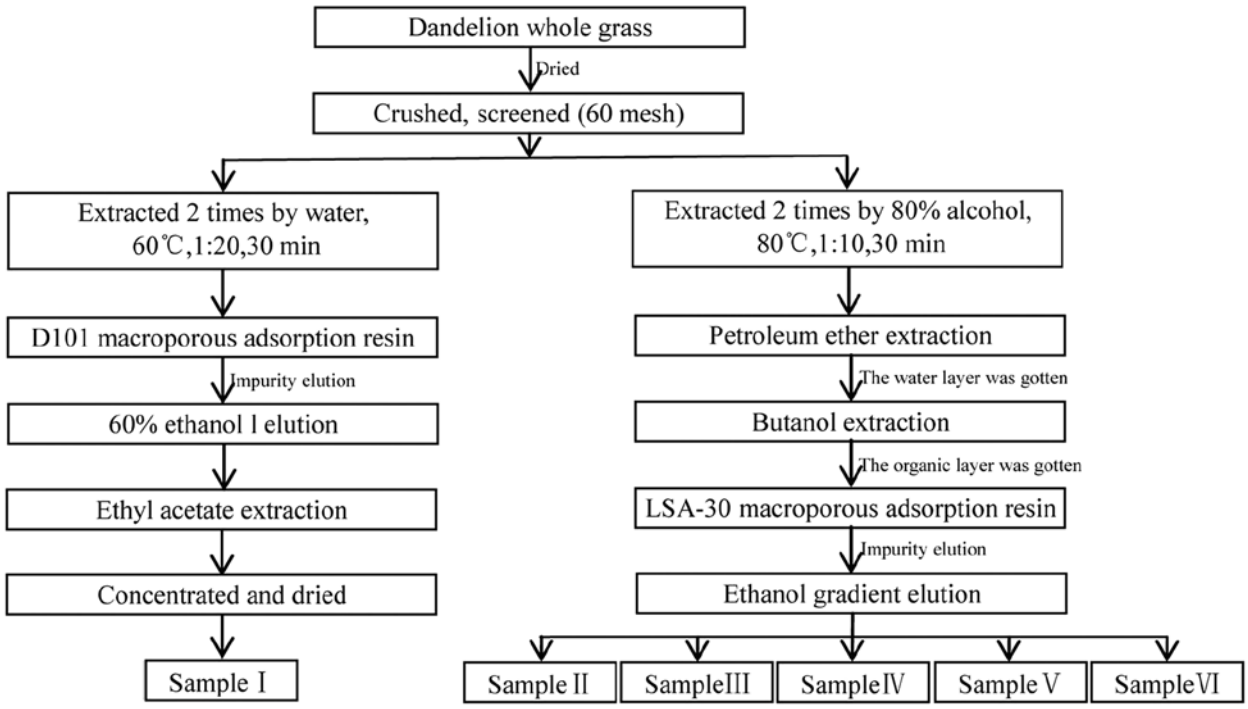

Figure 1. Schematic of the dandelion active ingredients extraction protocol.

Bacteriostatic kinetic analysis. The CA culture and DAIS mother solution, prepared as aforementioned, were added to liquid medium in three test tubes at DAIS concentrations of 0, 1 and 3X MIC, and a CA concentration of 5% (v/v); 0X MIC served as the control group. The mixtures were then incubated at 37˚C and 200 rpm followed by obtaining the OD_{600nm} every 3 h for a total of 36 h (Ultraviolet Spectrophotometer, Shimazu). The CA survival and antifungal rates were calculated according to equations (1) and (2) (28-30):

$$\text{Survival rate}(\%) = \frac{OD_{600nm}\ \text{Sample group}}{OD_{600nm}\ \text{Control group}} \quad (1)$$

$$\text{Antifungal rate}\% = 1 - \text{Survival rate}(\%) \quad (2)$$

Morphological observation by scanning electron microscopy (SEM). Mixtures of 0, 0.5, 1.0, and 2.0X MIC DAIS and 5% (v/v) CA were incubated at 37˚C and shaken at 200 rpm for 5 h. 1 ml aliquots of each sample were centrifuged at 7,104 x g for 6 min (Eppendorf) at 4˚C then washed three times with PBS (0.01 M, pH 5.8). Subsequently, the centrifuged aliquots were fixed overnight at room temperature with 2.5% glutaraldehyde (prepared using 25% glutaraldehyde, deionized water, and PBS at a ratio of 1:4:5 in a dark, enclosed environment to prevent evaporation), dehydrated with a graded alcohol solution, and lyophilized for 12 h using a vacuum freeze dryer (Four-Ring). Finally, the samples were coated with gold using an ion coater (31-33) prior to SEM analysis at different magnifications (x2,000, x5,000 and x10,000 times (JEOL, Ltd.).

Cell membrane permeability analysis. CA cultures prepared as aforementioned were centrifuged at 3,996 x g for 10 min at 4˚C then washed three times with PBS (0.01 M, pH 5.8). The cultured cells were subsequently suspended in 0.01% Tween 80/PBS (0.01 M, pH 5.8) and their concentration was adjusted to 10^9 CFU/ml. DAIS mother solution was added to four test tubes containing suspended CA cells to attain final DAIS concentrations of 0, 0.5, 1.0 and 2.0X MIC. The 0X MIC group served as the control group. The mixtures groups were then incubated at 37˚C and 200 rpm. Subsequently, 200 μl aliquots of each sample were collected after 0, 1, 2, 4, and 6 h of incubation at room temperature. The OD_{260nm} values of supernatants were measured after centrifugation at 3,996 x g for 10 min (34) at 4˚C.

Effects on the synthesis of cell wall β-(1-3)-D-glucan. The 5% (v/v) CA suspensions were added to and 0.5X MIC DAIS in liquid Sabouraud medium. The 0 X MIC group was considered as the control group. After incubation at 37˚C and 200 rpm for 48 h, the CA cells were inactivated at 121˚C for 20 min then collected by centrifugation at 3,996 x g for 10 min at 4˚C. The collected cells were washed three times with 0.01% Tween 80/PBS (0.01 M, pH 5.8) and once with sterile water, followed by lyophilization and weighing.

Cell wall β-(1-3)-D-glucan was extracted according to the method reported in the literature (35), and the extraction rate was determined according to the dextran/cell dry weight. The extracted β-(1-3)-D-glucan was analyzed using infrared spectrometry (Nicolet; Thermo Fisher Scientific, Inc.) in the range of 4,000-500 cm^{-1}.

Statistical analysis. Data analysis was performed using SPSS 22.0 (IBM, Corp.) and Origin 8.0 (OriginLab) software. ChemDraw v. 17 (CambridgeSoft) software was used to illustrate chemical structures. Experimental data were analyzed using one-way analysis of variance followed by a Tukey's post hoc test to determine the significant differences between the groups. $P<0.05$ and $P<0.01$ were considered to indicate statistically significant and highly significant differences, respectively. The results were expressed as the mean ± standard deviation.

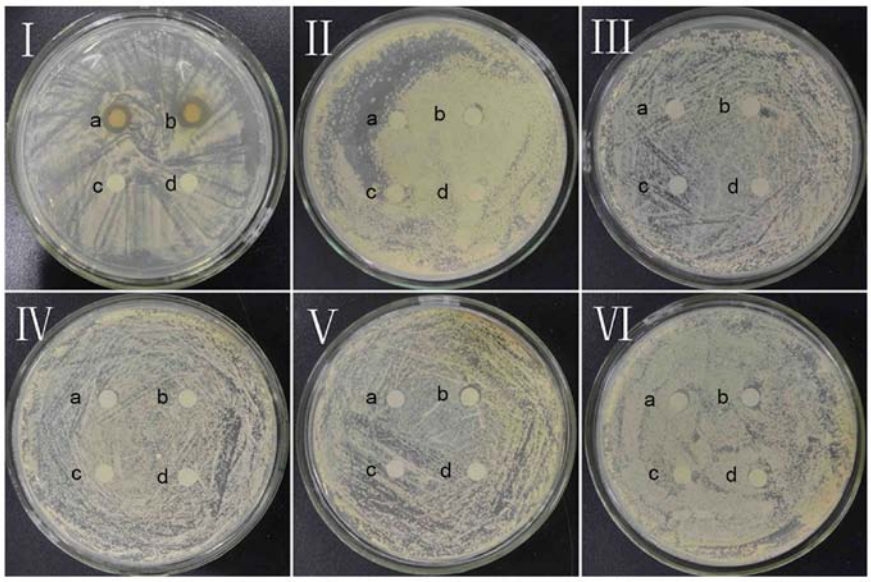

Figure 2. Antifungal effect of dandelion active ingredients in samples I, II, III, IV, V, and VI against *Candida albicans*. a and b, experimental groups; c and d, control groups in I, II, III, IV, V, VI.

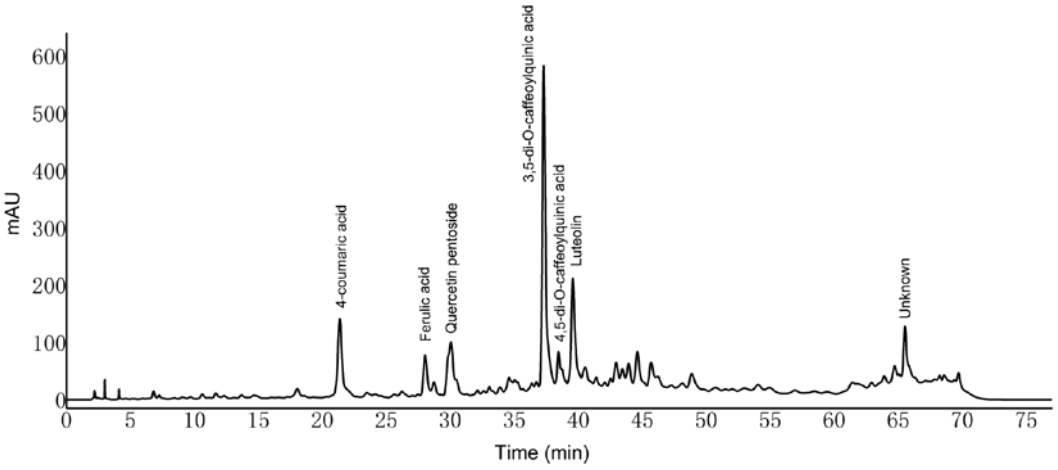

Figure 3. High-performance liquid chromatography-diode-array detector profiles of dandelion sample I at 280 nm. Peak 1, 4-coumaric acid; 2, ferulic acid; 3, quercetin pentoside; 4, 3,5-di-*O*-caffeoylquinic acid; 5, 4,5-di-*O*-caffeoylquinic acid; 6, luteolin; 7, unknown; 8, unknown.

Results

Study of the antimicrobial activity of DAIS. In vitro and *in vivo* assessments have confirm the antifungal activity of dandelion, a plant that has been commonly used in China to treat gynecopathy (20), such as mammitis and vaginitis. Dandelion contains various phytochemicals, including flavonoids, phenolic acids, alkaloids, and terpenes (25). In the present study, 6 samples (I, II, III, IV, V, VI) of DAIS were extracted and purified using hot-water (Fig. 1). The antifungal activity of the dandelion extracts was determined by assessing their effect on the growth of CA using the K-B paper disk method. As presented in Fig. 2, dandelion sample I is the only sample to exhibit an inhibition zone, indicating that all of the other samples (II, III, IV, V and VI) lack notable antifungal activity with respect to CA. The mean diameter of the CA inhibition zone in sample I (concentration=53.2 mg/ml) was found to be 10.38±0.15 mm, signifying marked antifungal effects against CA.

Qualitative and quantitative analyses of dandelion sample I by LC-DAD-ESI-MS. Eight compounds in dandelion sample I were separated within 78 min and identified at 280 nm using HPLC (Fig. 3). The compounds were further identified by tandem mass spectrometry based on their $[M-H]^-$ values and corresponding ion fragments (Table I). Peak with retention times of 21.35 min in Fig. 3 was identified as 4-coumaric acid based on the $[M-H]^-$ m/z value of 163. The ion fragment at m/z=119 corresponds to the loss of a CO_2 moiety. With an $[M-H]^-$ m/z value of 193 and ion fragment at m/z=134, a peak with a retention time of 28.3 min was identified as ferulic acid (36). Similarly, a peak with a retention time of 30.05 min was identified as quercetin pentoside based on the molecular ion peak at m/z=433 and the ion fragment at m/z=301 (formed upon the removal of pentose) (25). Peaks with retention times of 37.29 and 38.45 min both yielded an $[M-H]^-$ m/z value of 515, and ion fragments at m/z=353 and 173. However, the

Table I. Analysis of dandelion sample I by high performance liquid chromatography-electrospray ionization-tandem mass spectrometry.

Author, year	Peak no.	Identity	Retention time (min)	$[M-H]^-$ (Online parent ion)	Fragment ions (Online, MRM mode, daughter ion)	$[M-H]^-$ (Reported parent ion)	Fragment ions (Reported daughter ion)	Relative percentage (%)	(Refs.)
Kenny *et al*, 2014	1	4-coumaric acid	21.35	163	119[M-H-CO_2]	163	119	11.45	(36)
Kenny *et al*, 2014	2	Ferulic acid	28.03	193	134	193	134	3.96	(36)
Chen *et al*, 2012	3	Quercetin pentoside	30.05	433	301[M-H-pentose]	433	301	10.48	(25)
Dias *et al*, 2014	4	3,5-D*i*-*O*-caffeoylquinic acid	37.29	515	353[M-H-caffeoyl], 191[M-H-2 caffeoyl], 173[M-H-caffeoyl-quinic], 163	515	353, 191, 179, 135, 173, 163	34.24	(37)
Dias *et al*, 2014	5	4,5-Di-O-caffeoylquinic acid	38.45	515	353[M-H-caffeoyl], 173[M-H-caffeoyl-quinic], 179	515	353, 173, 179, 191, 135	3.91	(37)
Kenny *et al*, 2014	6	Luteolin	39.56	285	133	285	133	11.80	(36)
Agregán *et al*, 2017	7	Unknown	44.61	327	-	327	283	3.65	(38)
	8	Unknown	65.22	297	-	-[a]	-[a]	4.21	

[a]No reported data available.

Table II. Minimum inhibitory concentration of sample I and fluconazole against CA.

	Negative control group	Experimental group						Positive control group
Concentration (mg/ml)	0	4.00	8.00	16.00	32.00	64.00	128.00	0.60
The growth of CA		-	-	-	+[a]	+	+	+

[a]+, change in the optical density at 600 nm was ≤5% compared with the next sample group. Fluconazole was used as the positive control. CA, *Candida albicans*.

former showed a unique fragment at m/z=191, whereas the distinguished fragment in the latter was detected at m/z=179. Based on these values, these two peaks were identified as 3,5-di-*O*-caffeoylquinic acid and 4,5-di-*O*-caffeoylquinic acid, respectively (37). The detected fragments at 353, 191, and 173 correspond to the loss of caffeoyl, two caffeoyl, and caffeoyl + quinic groups, respectively (37). The peak with a retention time of 39.56 min was identified as luteolin due to the detection of an ion fragment at m/z=133 (38). Finally, the peaks with retention times of 44.61 and 65.22 min were unidentified. Comparing with reports of Kenny *et al* (36), 4-coumaric acid, ferulic acid, Quercetin and luteolin had the same $[M-H]^-$ values and corresponding ion fragments. 3,5-Di-O-caffeoylquinic acid and 4,5-di-O-caffeoylquinic acid had same $[M-H]^-$ values and similar corresponding ion fragments compared with Dias *et al* (37); quercetin pentoside had the same $[M-H]^-$ value and corresponding ion fragment compare with Chen *et al* (25). Thus, the composition of sample I included 4-coumaric acid, ferulic acid, quercetin pentoside, 3,5-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, luteolin and two unknown compounds. The relative percentages of peaks 1, 2, 3, 4, 5, 6, 7, and 8 were found to be 11.45, 3.96, 10.48, 34.24, 3.91, 11.80, 3.65 and 4.21%, respectively. Therefore, 3,5-di-*O*-caffeoylquinic acid was recognized as the main ingredient in dandelion extract sample I.

MIC analysis. The results summarized in Table II revealed that the CA growth inhibition capacity of dandelion sample I increases with increasing concentration of the extract. The MIC was taken to be the lowest sample group concentration beyond which changes in OD_{600} values compared with the next sample group are less than or equal to 5%. Therefore, the MIC of sample I was determined as 32.0 mg/ml. The MIC of fluconazole was 0.60 mg/ml which was notably lower than the MIC of sample I because sample I was a mixture and its purity low. The low purity of samples I led to low activity.

Kinetic analysis. Microbial growth curves include four phases corresponding to a delay period, an exponential growth period, a stable period, and a decay period. The CA mechanism of inhibition of dandelion sample I was investigated by analyzing the exponential phase. The kinetic equation of the CA exponential growth period is expressed as (39):

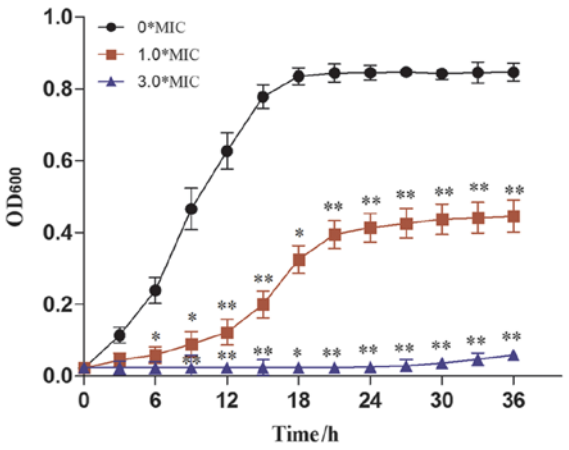

Figure 4. Inhibitory curves of *Candida albicans* containing sample I at different apparent concentrations. Each value represents the mean ± standard deviation (n=3). Tukey's post hoc test was applied to the experimental results $^*P<0.05$ and $^{**}P<0.01$ vs. 0X MIC group. 0X MIC, no addition of sample I; 1.0X, MIC, sample I at 1.0 times the minimum inhibitory concentration; 3.0X MIC, sample I at 3.0 times the minimum inhibitory concentration. MIC, minimum inhibitory concentration; OD, optical density.

$$r = \frac{dN}{dt} = \mu N$$

N is the number of CA at time t, and μ is the growth rate of CA.

Integration on both sides yields the following equation:

$$\int_{N_2}^{N_1} dN/N = \int_{t_2}^{t_1} \mu dt$$

At constant specific growth rate of microorganisms, the integrated form of kinetic equation given by equation (3), which can be rearranged to produce equation (4), where $\Delta t = t_2 - t_1$.

$$\ln[\frac{N_2}{N_1}] = \mu \Delta t \quad (3)$$

$$\mu = \frac{\ln[\frac{N_2}{N_1}]}{\Delta t} \quad (4)$$

Antimicrobial kinetics refers to the kinetics of antifungal activity as determined by plotting the inhibitory growth curve of CA. As presented in Fig. 4, the OD_{600nm} of CA was significantly

Table III. Inhibitory rate of sample I in *Candida albicans*.

Sample	Concentration MIC (mg/ml)	OD_{600nm} (3 h)	OD_{600nm} (18 h)	Growth rate/μ	Antibacterial rate/ % (18 h)
Sample I	0 (0)	0.1143±0.0221	0.8352±0.0244	0.04806	0.00
	1.0 (32.00)	0.0451±0.0192	0.3252±0.0231[a]	0.01867	61.06
	3.0 (96.00)	0.0228±0.0132	0.0239±0.0142[a]	0.0007	97.13

Each absorbance value represents the mean ± standard deviation (n=3). The growth rate and inhibitory rate were determined according to formulas (1), (2) and (3). Tukey's post hoc test was applied to the experimental results [a]P<0.05 vs. 0X MIC group. MIC, minimum inhibitory concentration; OD, optical density.

reduced upon the addition of dandelion sample I at a concentration of 1.0X MIC. It was further decreased when the concentration of the dandelion extract was augmented to 3.0X MIC. This indicated that the antifungal activity of sample I increases with increasing DAIS concentration. In terms of values, an inhibitory rate of 97.13% was recorded using a DAIS concentration of 3.0X MIC, with a CA growth rate of only 0.00007 (Table III). The obtained results indicate that dandelion sample I inhibits CA proliferation mainly in the exponential phase, resulting in greatly reduced CA growth rates. Such antifungal activity depends on concentration and exposure time (31).

Morphological observation by SEM. Presently, SEM has become necessary for the study of cell morphology. The SEM micrographs of CA cells in the control groups were shown in Fig. 5A-C. CA cells exhibited an almost ellipsoid shape, with complete structure, smooth surface, and notable refraction. The morphology of CA cells was notably unchanged upon treatment with 0.5X MIC of sample I for 5 h; however, the surface became rough, and the refraction was weakened (Fig. 5D-F). After treatment with 1.0X MIC of sample I for 5 h, the CA cell structure remained intact, but most cells were no longer ellipsoidal in shape. In fact, the surfaces of many cells were found to be wrinkled, and some of them appeared to be depressed (Fig. 5G-I). When the concentration of DAIS was further increased to 2.0X MIC, all CA cells showed non-ellipsoidal shape with relatively rough surfaces. The cells also began to shrink, and their morphology was irregular. Some cells showed marked compression, and damaged cell membranes of CA appeared (Fig. 5J-L). These results suggest that dandelion sample I is capable of destroying the membrane of CA cells, changing their normal morphology, shrinking their size, wrinkling their surface, and giving them an asymmetrical structure (40). The reason behind such changes may be attributed to significant leakage in cell content.

Cell membrane permeability of CA. Under normal circumstances, intracellular macromolecules cannot pass through the cell membrane. However, when cells are adversely affected by unfavorable growth conditions or antibacterial agents, their membranes become damaged, thereby allowing UV-absorbing macromolecules, such as DNA and RNA, to leak into the culture medium, leading to an increase in cell absorbance at 260 nm (28). Thus, the integrity of the cell membranes can be estimated based on cellular absorbance at 260 nm.

The UV absorbance values of the culture medium at a wavelength of 260 nm were relatively low in the control group (0X MIC) compared with the sample groups (0.5, 1.0, and 2.0X MIC sample I-treated CA) (Fig. 6). After incubation for 1 h, the OD_{260nm} values of the 0, 0.5, 1.0 and 2.0 X MIC supernatants were found to be 0.0926±0.0252, 0.1027±0.0183, 0.1286±0.0214, and 0.1579±0.0152, respectively. These values increased to 0.2982±0.0333, 0.3594±0.0165, 0.3942±0.0231, and 0.4756±0.0216, respectively, after 6 h of incubation. With increases in treatment time, the absorbance of the culture medium at 260 nm was gradually increased. These results indicated that dandelion sample I could be capable of destroying the cell membranes of CA, resulting in extensive nucleic acid leakage and ultimately, the inhibition of growth (41).

Effects of DAIS on CA cell wall β-(1-3)-D glucan synthesis. β-(1-3)-D glucan is the main component of the CA cell wall (42), and structural abnormalities in this component may lead to cell wall rupture. Therefore, the integrity of the CA cell wall was investigated by assessing the cellular content of β-(1-3)-D-glucan using infrared spectroscopy. The results indicated β-(1-3)-D-glucan contents of 22.13 and 20.86% for the 0 and 0.5X MIC groups, respectively (data not shown). The difference between the two groups is insignificant, which implies that sample I has no significant effect on the concentration of β-(1-3)-D-glucan in the CA wall.

The infrared spectrum of β-(1-3)-D-glucan comprises a peak between 3,000 and 2,800 cm^{-1} corresponding to the 'C-H' stretching vibration (Fig. 7). A wide peak between 800 and 400 cm^{-1} corresponds to the '-CH_2-' moieties. The peaks at 2,920, 1,370, 1,260, and 1,250 cm^{-1} characterize the vibrations of polysaccharides, whereas the peak at 1650 cm^{-1} peak is related to the 'C=O' vibration. Finally, the absorption peak at 890 cm^{-1} is indicative of the presence of a β-configuration polysaccharide glycosidic bond.

Compared with the control group, the 'C-H', 'C=O', and '-CH_2-' absorption peaks were all increased in the 0.5X MIC group. Meanwhile, the absorption peak of the β-configuration polysaccharide glycosidic bond is decreased. However, no significant change was noted in the other absorption peaks. These results indicate that dandelion sample I altered the structure of β-(1-3)-D glucan in CA cell walls, possibly via the partial breaking of the glycosidic bond to form more 'C=O'

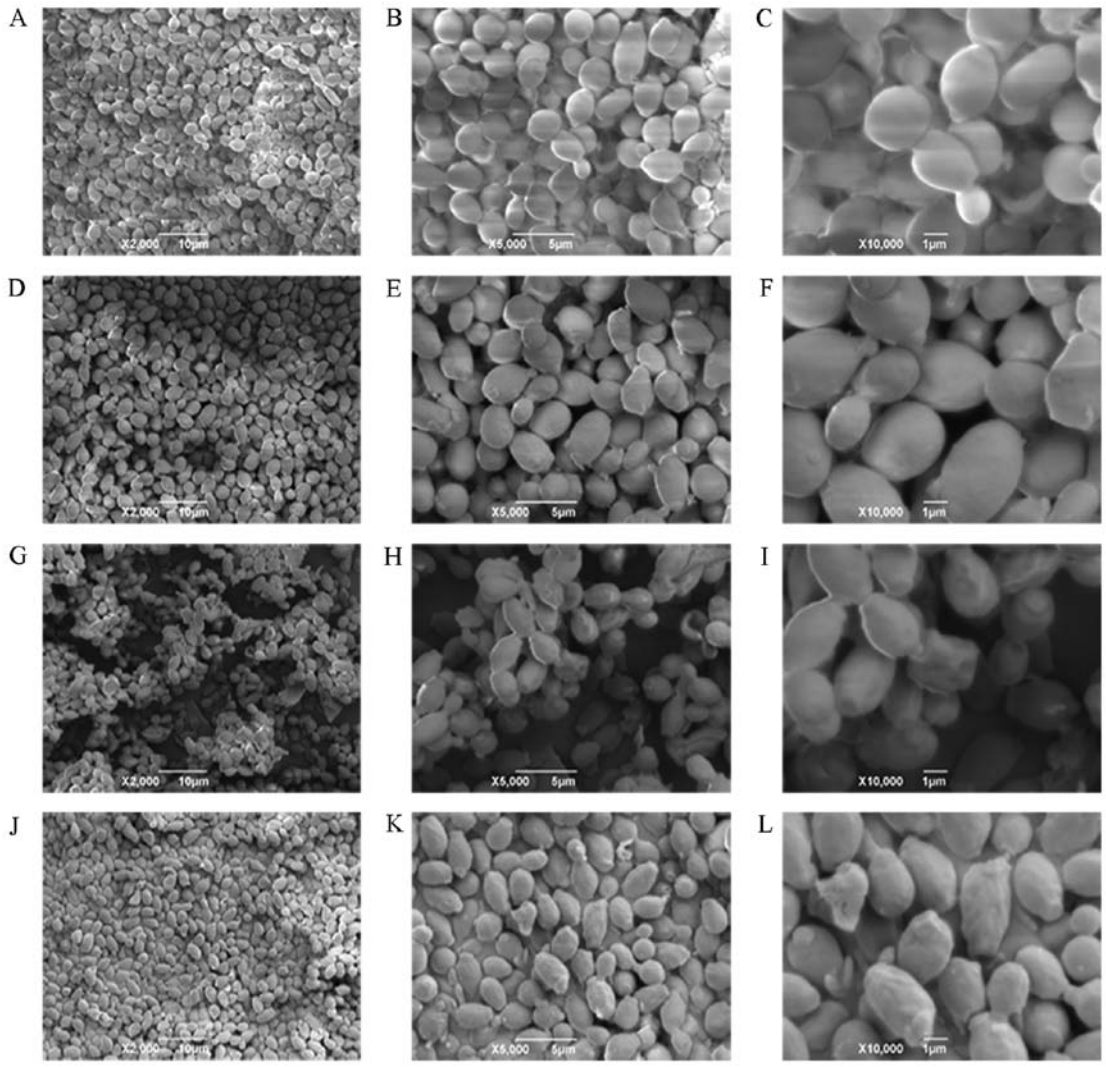

Figure 5. Scanning electron microscopy images of CA. (A-C) Images are of 2,000, 5,000, and 10,000 magnification; CA cells were treated for 5 h with 0X MIC sample I, respectively. (D-F) Images are of 2,000, 5,000, and 10,000 magnification; CA cells were treated for 5 h with 0.5X MIC sample I, respectively. (G-I) Images are of 2,000, 5,000, and 10,000 magnification; CA cells were treated for 5 h with 1.0X MIC sample I, respectively. (J-L) Images are of 2,000, 5,000, and 10,000 magnification; CA cells were treated for 5 h with 2.0X MIC sample I, respectively. CA, *Candida albicans*.

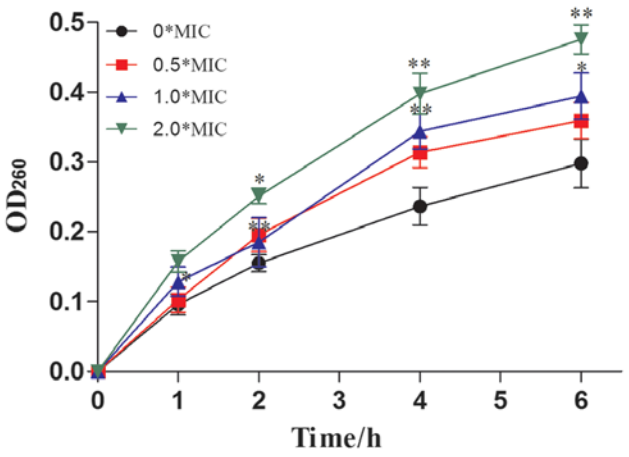

Figure 6. Effects of sample I at different concentrations on the cell permeability of *C. albicans*. Each value represents the mean ± standard deviation (n=3). A Tukey's post hoc test was applied to the experimental results. $^{*}P<0.05$ and $^{**}P\leq0.01$ vs. 0X MIC, no addition of sample I; 1.0X, MIC, sample I at 1.0 times the minimum inhibitory concentration; 2.0X MIC, sample I at 2.0 times the minimum inhibitory concentration. MIC, minimum inhibitory concentration; OD, optical density.

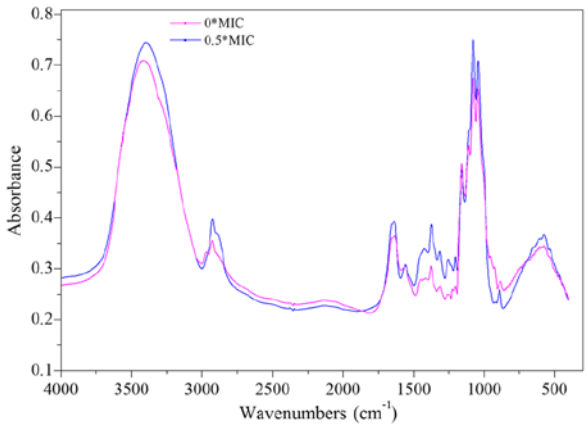

Figure 7. Infrared spectrum of β-(1-3)-D-glucan in CA cell wall. 0X MIC, no addition of sample I; 0.5X MIC, adding sample I at 0.5 times the minimum inhibitory concentration. Pretreatment for sample detection: KBr (pure spectrum) was dried at 70˚C for 12 h, and the same amounts of the β-(1-3)-D-glucan treated with 0X MIC polyphenol and 0.5X MIC polyphenol were prepared. The components were respectively mixed with KBr in an agate mortar, homogenized, reduced to powder, and compressed with a tablet press. MIC, minimum inhibitory concentration.

and '-CH_2-' groups (Fig. 7). Another plausible explanation could be that after treatment with sample I, glycosidic bond synthesis was inhibited (42), resulting in the formation of dextran molecules with an incomplete structure. The reduced number of glycosidic bonds inhibits the cross-linking of dextran, ultimately affecting the synthesis of CA cell walls. Abnormal cell wall structure leads to fragile cells that are easily ruptured (43,44).

Discussion

According to the literature, dandelion crude extract has notable bacteriostatic activity and is effective against a variety of bacteria, including *Bacillus subtilis*, *Staphylococcus aureus* (45), *Escherichia coli*, *Pseudomonas aeruginosa* (46), methicillin-resistant *Staphylococcus aureus*, *Bacillus cereus* (47), *Vibrio harveyi* (48), *Pseudomonas aeruginosa*, *Paracoccus bacillus* (49), and other microorganisms.

Studies of the fungistatic activity in CA have revealed that the mechanism involves alterations in the gene encoding of the target enzyme lanosterol 14-α demethylase or overexpression of the efflux pump genes containing cerebellar degeneration related protein (CDR)1, CDR2, and multidrug resistance mutation 1 (50). Ding *et al* (51) reported that the molecular mechanism underlying the ATB-induced apoptosis of CA cells is based on the inhibition of tubulin polymerization, leading to G2/M phase cell cycle arrest. CA also has a strong effect on mycelial development and cell membrane morphology, properties that are related to abnormal actin skeleton and subsequent translational defects of hyphae-associated factors (52). The 20-polymer peptide Ib-AMP1 exhibits a specific fungistatic effect on CA by inhibiting different cellular processes rather than cell membrane ion channels or pores (53). Caspofungin hinders the growth of CA by restricting the synthesis of CA cell wall β-glucan (42).

Overall, existing research shows that the mechanism of antifungal activity in CA is very complex, and more extensive and comprehensive investigations are needed to properly elucidate this mechanism. At present, to the best of our knowledge, no studies have evaluated the antifungal activity of DAIS against CA.

In this study, the active ingredients in dandelion were extracted, isolated, and analyzed. The results demonstrated that among six different dandelion extracts, only sample I exhibited marked antifungal activity against CA, with an inhibition zone diameter of 10.38 mm recorded at sample I concentration of 53.2 mg/ml. HPLC-ESI-MS/MS analysis revealed that the main chemical components in sample I are 4-coumaric acid, ferulic acid, quercetin pentoside, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, luteolin, and two unknown components, with relative percentages of 11.45, 3.96, 10.48, 34.24, 3.91, 11.80, 3.65 and 4.21%, respectively. Therefore, 3,5-di-*O*-caffeoylquinic acid is the most abundant component.

The results of preliminary antifungal activity analyses show that the concentration of dandelion sample I is significantly correlated with the growth inhibition effect of CA. Comparing the absorbances of different concentrations of sample group, the MIC of DAIS was identified as 32.0 mg/ml. Kinetically, it was found that dandelion sample I mainly inhibits CA growth in the exponential phase. Furthermore, SEM images showed that DAIS are capable of damaging the membranes of CA cells, leading to cell surface depression, wrinkling, increased cell membrane permeability, macromolecule leakage, and ultimately, disordered cell metabolism. This suggests that inhibition of CA growth by DAIS may be related to the damaging effect of the latter on the cell membranes of the former. In addition, infrared spectrometry indicated that sample I destroy the glycosidic bond of β-(1-3)-D-glucan in CA cell walls, thereby changing its structure. In summary, dandelion sample I was proposed to increase the permeability of CA by destroying its cell wall and membrane, ultimately delaying cellular growth or leading to cell death. Sample I was reported to include eight chemical components; comparing the antifungal activity of these identified constituents with previous literature, these identified compounds did not have antifungal activities against CA. Thus, these two unknown compounds could exert antifungal activities against CA or there may be a synergistic effect between these unknown compounds with these identified compound; further investigation is required.

In the present study, we reported that dandelion sample I, composed of 4-coumaric acid, ferulic acid, quercetin pentoside, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid, luteolin, and two unknown components, exhibits good antifungal activity against CA. Our findings provide a basis for novel approaches to screen for anti-VVC drugs. However, further investigation is needed to determine the antifungal activities of the individual components, as well as the synergistic effects.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

KL was responsible for the design of this research. KL, HD, PZ, HH, FG, YL and ZX acquired and analyzed the data. ZX and HD drafted the manuscript. KL and HD wrote and revised the paper. All authors agreed with the final revision.

Ethics approval and consent to participate

Not applicable.

Patient consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Stockdale CK: Clinical spectrum of desquamative inflammatory vaginitis. Curr Infect Dis Rep 12: 479-483, 2010.
2. Theodoropoulos DS, Stockdale CK, Duquette DR and Morris MS: Inhalant allergy compounding the chronic vaginitis syndrome: Characterization of sensitization patterns, comorbidities and responses to sublingual immunotherapy. Arch Gynecol Obstet 294: 541-548, 2016.
3. Guaschino S and Benvenuti C; SOPHY Study Group: SOPHY project: An observational study of vaginal pH, lifestyle and correct intimate hygiene in women of different ages and in different physiopathological conditions. Part II. Minerva Ginecol 60: 353-362, 2008 (In English, Italian).
4. Minooeianhaghighi MH, Sepehrian L and Shokri H: Antifungal effects of Lavandula binaludensis and Cuminum cyminum essential oils against *Candida albicans* strains isolated from patients with recurrent vulvovaginal candidiasis. J Mycol Med 27: 65-71, 2017.
5. Yang S, Zhang Y, Liu Y, Wang J, Chen S and Li S: Clinical significance and characteristic clinical differences of cytolytic vaginosis in recurrent vulvovaginitis. Gynecol Obstet Invest 82: 137-143, 2017.
6. Liu YW, Li YL, Fang Q, Yang ZX and Bing AY: Advances in the study of treatment of candidiasis with traditional Chinese medicine. J Pathogen Biol 29: 620-622, 2009.
7. Wang PB, Peng C, Tang ZW, Wan F, Dai M and Cao XY: A study on the effect of Patchouli essential oil against mice model of candidal vaginitis. Lishizhen Med Materia Medica Res 35: 592-594, 2014 (In Chinese).
8. Zhang XF and Zhang CY: Treatment of recurrent candidal vaginosis with Pulsatilla decoction. Chin J Exp Traditional Med Formulae 18: 279-281, 2012 (In Chinese).
9. He W, Han H, Wang W and Gao B: Anti-influenza virus effect of aqueous extracts rom Dandelion. Virol J 8: 538, 2011.
10. Evensen NA and Braun PC: The effects of tea polyphenols on *Candida albicans*: Inhibition of biofilm formation and proteasome inactivation. Can J Microbiol 55: 1033-1039, 2009.
11. Gavanji S and Larki B: Comparative effect of propolis of honey bee and some herbal extracts on *Candida albicans*. Chin J Integr Med 23: 201-207, 2017.
12. Estevinho ML, Afonso SE and Feás X: Antifungal effect of lavender honey against *Candida albicans*, Candida krusei and Cryptococcus neoformans. J Food Sci Technol 48: 640-643, 2011.
13. Li J, Li XD, Yang LX and Jiang H: Experimental research of single herb medicine in vitro antibacterial activity. Chin J Exp Traditional Med Formulae 17: 283-286, 2011 (In Chinese).
14. Shi GX, Yan YY, Shao J, Zhang MX, Lu KQ, Wang TM and Wang CZ: Effect of andrographolide derivative Yanhuning on in vivo *Candida albicans* biofilms in rats. Zhongguo Zhong Yao Za Zhi 39: 2924-2929, 2014 (In Chinese).
15. Zhang GQ, Feng WR and Mi S: Therapeutic effects of ethanol extracts from Euphorbia humifusa on monilial vaginitis in rats. Chin J Exp Traditional Med Formulae 18: 191-194, 2012 (In Chinese).
16. Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. China Medical Science and Technology Press, I, 352, 2015.
17. Kim JJ, Park CM, Kim MJ, Cho CW and Song YS: Hypolipidemic effect of dandelion (Taraxacum officinale) extracts via fecal lipid excretion in C57BL/6 mice fed an atherogenic diet. Food Sci Biotechnol 23: 841-847, 2014.
18. Tahir K, Nazir S, Ahmad A, Li B, Khan AU, Khan ZU, Khan FU, Khan QU, Khan A and Rahman AU: Facile and green synthesis of phytochemicals capped platinum nanoparticles and in vitro their superior antibacterial activity. J Photochem Photobiol B 166: 246-251, 2017.
19. Song YM, Li DG, Zhang Y, Zhang WG, Jin YP, Zhou L and Tang JN: The bacteriostasis of the extracts from herba taraxaci on bacteria derived from bovine hidden mastitis. Acta Agriculturae Boreali Occidentalis Sinica 15: 55-57, 2006 (In Chinese).
20. Hu G, Wang J, Hong D, Zhang T, Duan H, Mu X and Yang Z: Effects of aqueous extracts of taraxacum officinale on expression of tumor necrosis factor-alpha and intracellsular adhesion molecule 1 in LPS-stimulated RMMVECs. BMC Complement Altern Med 17: 38, 2017.
21. Lans C, Turner N, Khan T, Brauer G and Boepple W: Ethnoveterinary medicines used for ruminants in British Columbia, Canada. J Ethnobiol Ethnomed 3: 11, 2007.
22. Li XH, He XR, Zhou YY, Zhao HY, Zheng WX, Jiang ST, Zhou Q, Li PP and Han SY: Taraxacum mongolicum extract induced endoplasmic reticulum stress associated-apoptosis in triple-negative breast cancer cells. J Ethnopharmacol 206: 55-64, 2017.
23. Duan HB and Liang YK: Extraction and antioxidant and antibacterial activity of dandelion sample I. China Food Additives 27: 80-86, 2017 (In Chinese).
24. Andrade JC, Morais Braga MF, Guedes GM, Tintino SR, Freitas MA, Quintans LJ Jr, Menezes IR and Coutinho HD: Menadione (vitamin K) enhances the antibiotic activity of drugs by cells membrane permeabilization mechanism. Saudi J Biol Sci 24: 59-64, 2017.
25. Chen HJ, Inbaraj BS and Chen BH: Determination of phenolic acids and flavonoids in Taraxacum formosanum Kitam by liquid chromatography-tandem mass spectrometry coupled with a post-column derivatization technique. Int J Mol Sci 13: 260-285, 2012.
26. Szczepaniak J, Cieślik W, Romanowicz A, Musioł R and Krasowska A: Blocking and dislocation of *Candida albicans* Cdr1p transporter by styrylquinolines. Int J Antimicrob Agents 50: 171-176, 2017.
27. Rad HI, Arzanlou M, Omid MR, Ravaji S and Doghaheh HP: Effect of culture media on chemical stability and antibacterial activity of allicin. J Functional Foods 28: 321-325, 2017.
28. Li JL, Yang XH, Wang TF, Hao ZC, Dai K and Wang RM: Studies on antibacterial mechanism of 10-HDA against Staphylococcus aureus. J Chin Institute Food Sci Technol 14: 73-79, 2014.
29. Wang HL, Hao LL, Wang P, Chen MM, Jiang SW and Jiang ST: Release kinetics and antibacterial activity of curcumin loaded zein fibers. Food Hydrocolloids 63: 437-446, 2017.
30. Moghayedi M, Goharshadi EK, Ghazvini K, Ahmadzadeh H, Ranjbaran L, Masoudi R and Ludwig R: Kinetics and mechanism of antibacterial activity and cytotoxicity of Ag-RGO nanocomposite. Colloids Surf B Biointerfaces 159: 366-374, 2017.
31. Chen B, Fan DQ, Zhu KX, Shan ZG, Chen FY, Hou L, Cai L and Wang KJ: Mechanism study on a new antimicrobial peptide Sphistin derived from the N-terminus of crab histone H2A identified in haemolymphs of Scylla paramamosain. Fish Shellfish Immunol 47: 833-846, 2015.
32. Memariani H, Shahbazzadeh D, Sabatier JM, Memariani M, Karbalaeimahdi A and Bagheri KP: Mechanism of action and in vitro activity of short hybrid antimicrobial peptide PV3 against Pseudomonas aeruginosa. Biochem Biophys Res Commun 479: 103-108, 2016.
33. Yi L, Dang J, Zhang L, Wu Y, Liu B and Lü X: Purification, characterization and bactericidal mechanism of a broad spectrum bacteriocin with antimicrobial activity against multidrug-resistant strains produced by Lactobacillus coryniformis XN8. Food Control 67: 53-62, 2016.
34. Ma Q, Davidson PM, Critzer F and Zhong Q: Antimicrobial activities of lauric arginate and cinnamon oil combination against foodborne pathogens: Improvement by ethylenediaminetetraacetate and possible mechanisms. LWT-Food Sci Technol 72: 9-18, 2016.
35. Tang QL, Huang GL, Zhao FY, Zhou L, Huang SQ and Li H: The antioxidant activities of six (1→3)-â-D-glucan derivatives prepared from yeast cells wall. Int J Biol Macromol 98: 216-221, 2017.
36. Kenny O, Smyth TJ, Walsh D, Kelleher CT, Hewage CM and Brunton NP: Investigating the potential of under-utilised plants from the Asteraceae family as a source of natural antimicrobial and antioxidant extracts. Food Chem 161: 79-86, 2014.

37. Dias MI, Barros L, Alves RC, Oliveira MB, Santos-Buelga C and Ferreira IC: Nutritional composition, antioxidant activity and phenolic compounds of wild Taraxacum sect. Ruderalia. Food Res Int 56: 266-271, 2014.
38. Agregán R, Munekata PES, Franco D, Dominguez R, Carballo JM and Lorenzo JM: Phenolic compounds from three brown seaweed species using LC-DAD-ESI-MS/MS. Food Res Int 99: 979-985, 2017.
39. Xie CL, Tang HK, Song ZH, Qu SS, Liao YT and Liu HS: Microcalorimetric study of bacterial growth. Thermochimica Acta 123: 33-41, 1988.
40. Sun D, Zhang W, Lv M, Yang E, Zhao Q and Wang W: Antibacterial activity of ruthenium(II) polypyridyl complex manipulated by membrane permeability and cells morphology. Bioor Med Chem Lett 25: 2068-2073, 2015.
41. Ajiboye TO, Mohammed AO, Bello SA, Yusuf II, Ibitoye OB, Muritala HF and Onajobi IB: Antibacterial activity of Syzygium aromaticum seed: Studies on oxidative stress biomarkers and membrane permeability. Microb Pathog 95: 208-215, 2016.
42. Deresinski SC and Stevens DA: Caspofungin. Clin Infect Dis 36: 1445-1157, 2003.
43. Georgopapadakou NH: Update on antifungals targeted to the cells wall: Focus on beta-1,3-glucan synthase inhibitors. Expert Opin Investig Drugs 10: 269-280, 2001.
44. Meng S, Hu SB, Xie WA, Ding XZ, Sun YJ and Xia LQ: Antifungal effects and mechanism of bioactive components of Allinm chinense on *Candida albicans*. Food Sci: 26, 2005 (In Chinese).
45. Qian L, Zhou Y, Teng Z, Du CL and Tian C: Preparation and antibacterial activity of oligosaccharides derived from dandelion. Int J Biol Macromol 64: 392-394, 2014.
46. Gao DA: Analysis of nutritional components of taraxacum mongolicum and its antibacterial activity. Pharmacognosy J 2: 502-505, 2010.
47. Kenny O, Brunton NP, Walsh D, Hewage CM, McLoughlin P and Smyth TJ: Characterisation of antimicrobial extracts from dandelion root (Taraxacum officinale) using LC-SPE-NMR. Phytother Res 29: 526-532, 2015.
48. Tan X, Sun Z, Chen S, Chen S, Huang Z, Zhou C, Zou C, Liu Q, Ye H, Lin H, *et al*: Effects of dietary dandelion extracts on growth performance, body composition, plasma biochemical parameters, immune responses and disease resistance of juvenile golden pompano Trachinotus ovatus. Fish Shellfish Immunol 66: 198-206, 2017.
49. Li LS, Shi WJ, Guan Ming, Li XJ, Fang T and Zheng JL: Study on active component and bacteriostasis of tetraploid plant of herba Taraxaci. Chin J Exp Traditional Med Formulae 14: 55-58, 2008 (In Chinese).
50. White TC, Marr KA and Bowden RA: Clinical, cellsular, and molecular factors that contribute to antifungal drug resistance. Clin Microbiol Rev 11: 382-402, 1998.
51. Ding Y, Li Y, Li Z, Zhang J, Lu C, Wang H, Shen Y and Du L: Alteramide B is a microtubule antagonist of inhibiting *Candida albicans*. Biochim Biophys Acta 1860: 2097-2106, 2016.
52. Yu QL, Zhang B, Ma F, Jia C, Xiao C, Zhang B and Li M: Novel mechanisms of surfactants against *Candida albicans* growth and morphogenesis. Chem Biol Interact 227: 1-6, 2015.
53. Lee DG, Shin SY, Kim DH, Seo MY, Kang JH, Lee Y and Hahm KS: Antifungal mechanism of a cysteine-rich antimicrobial peptide, Ib-AMP1, from Impatiens balsamina against *Candida albicans*. Biotechnol Lett 21: 1047-1050, 1999.