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ORIGINAL PAPER

Antibacterial and antioxidant properties of *Ramulus Cinnamomi* using supercritical CO_2 extraction

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Abstract In this study, crude extracts of *Ramulus Cinnamomi* from supercritical carbon dioxide under various extraction conditions were examined for their antioxidant and antibacterial activity. The extractions were conducted in the range of 4,000–6,000 psi and 40–50 °C, and the solvent to feed ratio of the extraction was 30. The antibacterial activity was tested on the clinical drug-resistant strains, including 27 *Acinetobacter baumannii*, 20 *Pseudomonas aeruginosa* and 2 *Staphylococcus aureus* isolates by the disk diffusion method. The bioassay results indicated that *Ramulus Cinnamomi* showed obvious antimicrobial activity against the tested strains. This study also found that increasing the temperature and pressure would increase the yield of the supercritical fluid extraction (SFE), even though the best extraction conditions for antibacterial activity were found to be high pressure and low temperature. The minimum inhibitory concentration (MIC) was determined on the crude extract of *Ramulus Cinnamomi*, indicating that the crude extracts from supercritical extraction showed better antibacterial activity than those obtained by ethanol extraction. Based on the spectrophotometer and bioassay determination, the antimicrobial constituent was identified to be cinnamaldehyde. Time-kill kinetics and scanning electron microscopy (SEM) were employed to monitor the survival characteristics and the changes in morphologies, respectively, of the test microorganisms in the presence of herbal extracts. Moreover, antioxidant activity was evaluated by scavenging of the free radical DPPH. Extracts of *Ramulus Cinnamomi* provided 50% inhibition at 2 mg/ml concentration. This study will provide valuable information for extraction of the natural bioactive component, cinnamaldehyde, from *Ramulus Cinnamomi* by supercritical extraction.

Keywords Supercritical extraction · Nosocomial pathogens · Chinese herbal medicines · Antibacterial activity · Antioxidant activity · Cinnamaldehyde

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Introduction

In the past few years, numerous hospitals in Taiwan have isolated antibiotic resistant bacteria strains; the

infection ratio is already higher than 50%. Especially, the antibiotic-resistant clinical isolates of *S. aureus*, *A. baumannii* and *P. aeruginosa* are widespread [1]. Among them, the pan-antibiotic resistant *A. baumanni* and *P. aeruginosa* are increasingly common. The infections caused by antibiotic resistant strains not only complicate clinical therapy but also extend patients' hospital stays. Moreover, the mortality rate is increased and outbreaks hidden, which poses severe health risks. Therefore, in addition to strict control of antibiotics use, rapid development of new antibacterial substances or other therapy modes are urgent issues.

Over the years Chinese medicinal herbs or Chinese medical products have been applied increasingly. To date, a large number of research articles for the antibacterial and antioxidant activities of Chinese medicines have been published [2, 3]. Comparison with other antimicrobial substances, antibacterial components of Chinese medicine have the advantages of fewer side effects, a lower toxicity and a higher stability [2–4]. Currently, most antibacterial research articles on antibiotic-resistant bacteria are focused on *S. aureus* and *Mycobacterium* [4]. Few articles relating to research of clinical antibiotic-resistant bacteria strains of *A. baumannii* and *P. aeruginosa* can be found. Therefore, in our laboratory, we tried to isolate antibacterial substances from Chinese medicine and determined their efficiency against the clinical strains of *A. baumannii* and *P. aeruginosa*.

In our previous studies, we have collected and categorized more than a thousand antibiotic-resistant clinical *A. baumannii* and *P. aeruginosa* isolates from three hospitals in Taiwan. A large number (58) of ethanol extracts of Chinese herbs were also screened for their antimicrobial activities based on the disk diffusion method. Sixteen out of the 58 Chinese herbs were identified to possess high antimicrobial efficacies against the clinical organisms [5, 6]. In addition to ethanol extraction, among the 16 herbs, *Cortex Moutan*, *Radix Aucklandiae*, and *Ramulus Cinnamomi* were found to exhibit antibacterial activity after supercritical fluid extraction. The application of supercritical carbon dioxide possesses the advantages of non-toxic, high-yield and high-quality compounds, and cleaner downstream processes. The extraction process is also simpler than steam or solvent extraction [7–9]. However, few articles in the literature specifically explore the application of supercritical fluid extraction (SFE) with regard to the antibacterial activity [10, 11]. This study focused on investigating the effects of the extraction conditions on the antibacterial activity. In addition, the antibacterial properties and antioxidant activity of *Ramulus Cinnamomi* SFE extracts were also examined.

Experiments and materials

Preparation of crude extracts

Ramulus Cinnamomi was purchased from local herb suppliers, which were authenticated by Dr. Ching-Shen Liu, Department of Traditional Chinese Medicine, Kaohsiung Medical University Chung-Ho Memorial Hospital, Taiwan. The raw materials were washed, air-dried, grounded, and preserved in a refrigerator before extraction. In the case of ethanol extraction, the dried herb (1.5 kg) was extracted with 4.5 l of 95% ethanol overnight by shaking in an incubator set at 200 rpm and 37 °C as described in our previous studies [6]. The ethanol extraction was repeated three times and then filtered using Whatman filter paper No. 1 to remove insoluble debris. After filtration, the ethanol extract was dried by evaporation at a temperature of 40 °C. The dried extracts were stored at 4 °C until the assay. For SFE, the ground powders (5 g) were first statically immersed in the extractor for 5 min and then dynamically extracted until the weight ratio of the flowing CO_2 to the loaded samples was roughly equal to 30. Solvent flow rates were approximately 3.1–4.0 ml/min. The high pressure set-up from ISCO, model 260D, was employed to conduct the extraction. The CO_2 effluent from the extractor was depressurized through a heated capillary and trapped by ethanol. The ethanol solution was then vacuum dried to obtain the crude extract for the bioactivity assay.

To test the effect of SFE extraction conditions on the antibacterial activity, the investigated parameters were pressure, temperature, and particle size of the powder. The ground powder was classified into two size groups, $D < 1$ mm and 1 mm $< D < 2$ mm. Nine extracts from small particle powders and one from a large particle powder were submitted to the MIC test for four particular bacterial strains, namely *A. baumannii* A10, *A. baumannii* A23, *P. aeruginosa* P1, and *P. aeruginosa* P5. Those extraction conditions are listed in Table 1.

Test organisms

A total of 27 *A. baumannii*, 2 *S. aureus* and 20 *P. aeruginosa* clinical strains were used in this study and were isolated from patients' blood or sputum during 2003–2004. The samples were provided by Chiayi Christian Hospital, Taiwan. Four reference strains, *S. aureus* ATCC6538P, *P. aeruginosa* ATCC19606, *P. aeruginosa* ATCC29260 and *P. aeruginosa* ATCC27853 were purchased from the Food Industry Research and Development Institute in Taiwan.

Table 1 The effect of extraction conditions on the antibacterial activity

No.[a]	*P* (psi)	*T* (°C)	MIC (mg/ml)			
			A10	A23	P1	P5
1	6,000	50	0.25–0.5	0.5–1.0	0.5–1.0	1.0–1.5
2	5,000	50	0.25–0.5	0.5–1.0	0.5–1.0	1.0–1.5
3	4,000	50	0.25–0.5	0.5–1.0	0.5–1.0	1.0–1.5
4	6,000	45	0.25–0.5	0.5	0.5	1.0
5	5,000	45	0.25–0.5	0.5	0.5	1.0
6	4,000	45	0.25–0.5	0.5–1.0	0.5–1.0	1.0–1.5
7	6,000	40	0.25–0.5	0.5	0.5–1.0	1.0–1.5
8	5,000	40	0.25–0.5	0.5	0.5–1.0	1.0
9	4,000	40	0.25–0.5	0.5–1.0	0.5	1.0
10	6,000	40	0.5	1	1	1.5

A Acinetobacter baumannii, *P Pseudomonas aeruginosa*

[a] Particle size of ground powder: 1–9 are <1 mm; 10 is 1–2 mm

Antibacterial activity of crude extract

The antibacterial sensitivity assay was performed by the standard disk diffusion method. Briefly, filter paper disks (8 mm in diameter) impregnated with sample solutions (30 µl/disk, g/ml) were placed on cation-adjusted Mueller–Hinton agar plates which had been inoculated with test organisms (5×10^6 CFU/ml) according to the standard protocol described by the National Committee of Clinical Laboratory Standards (NCCLS) [12]. The plates were incubated at 37 °C, and the diameters of the inhibition zones were measured after 18 h. Filter paper disks containing dimethyl sulfoxide (DMSO), without any test compounds, served as a control and no inhibition was observed. Additionally, for comparative purposes, tetracycline was included as a reference standard. Each assay was performed in triplicate and repeated at least two times. The analysis of variance was used to compare the means or averages. The computer program M-STAT was implemented to process the data and report significant differences at $P = 0.05$.

Determination of minimum inhibitory concentration (MIC)

The MIC of the crude extract was determined by the agar dilution method, according to NCCLS protocol with some modification [12]. Briefly, the sterilized cation-adjusted Mueller–Hinton agar media were allowed to cool to 50 °C, and 10 ml of the molten agar was added to test tubes which contained different concentrations of the test drugs (crude extract) and the control substance (DMSO). The concentrations of the extracts used in this test series ranged from 0.1 to 3.0 mg/ml. The media and the test drugs were thoroughly mixed and poured into pre-labeled sterile Petri dishes on a leveled surface. The suspensions of the respective microorganisms with their density adjusted to 5×10^6 CFU/ml were transferred onto each plate by spread-plating. The plates were then incubated at 37 °C for 18 h. The lowest concentration which inhibited the growth of the respective organisms was taken as the MIC. Each assay was performed in triplicate and repeated two times. The analysis of variance was used to compare the means or averages. The computer program M-STAT was implemented to process the data and report significant differences at $P = 0.05$.

Time-killing curve of the crude extracts

The time-killing curve was determined by Yu's method [13]. The concentration of each antimicrobial agent in the cation-supplemented Mueller–Hinton broth was set at a level equal to double the MIC level of the tested strain. Inoculates of ca. 5×10^5 CFU/ml of each individually tested strains harvested from the colonies grown overnight were used in these experiments. Aliquots of the cultures were taken every hour until an incubation time of 24 h was reached, and the aliquots were then serially diluted in the Mueller–Hinton broth and plated on Mueller–Hinton agar. Following 16 h of incubation, the number of colonies was counted to determine the total viable bacteria number. A cell culture with DMSO was assayed as the control.

Table 2 Comparison of *Ramulus Cinnamomi* antibacterial activity by SFE and ethanol extraction

Bacterial strains[a]	SFE		Ethanol extract	
	IZD (mm)	MIC (mg/ml)	IZD (mm)	MIC (mg/ml)
Acinetobacter baumannii	25.33 ± 0.58	1.28 ± 0.53	17.67 ± 8.26	3.07 ± 0.61
Pseudomonas aeruginosa	16.33 ± 4.16	2.56 ± 0.50	13.17 ± 2.51	4.00 ± 1.02

IZD inhibition zone diameter

[a] The total number of test strains was 27 and 20 for *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, respectively. Each assay was performed in triplicate and repeated two times

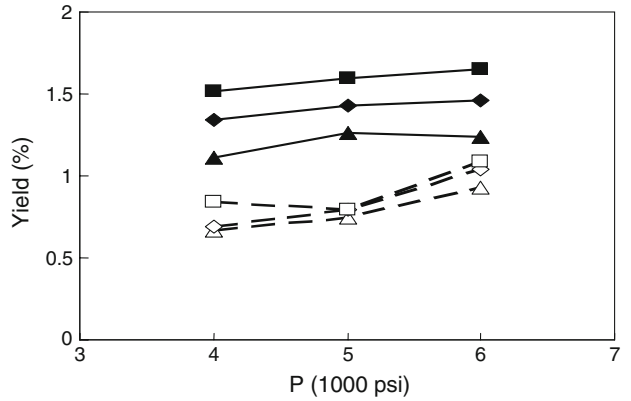

Fig. 1 The extraction yield of *Ramulus Cinnamomi* by SFE under different conditions. The symbols of *squares*, *diamonds*, and *triangles* represented large particles (1 mm $< D <$ 2 mm) extracted at 50, 45, and 40 °C, respectively. The *solid symbols* represent the same conditions but for small particles ($D <$ 1 mm)

Resistance to the active substances from the crude extract

Many literature reports indicate that microorganisms develop drug resistance due to the continual use of antimicrobial drugs in the treatment of infectious diseases [1]. Therefore, the ability of the test microorganisms to develop resistance to *Ramulus Cinnamomi* was investigated. The strains of the clinical isolates, *A. baumannii* (A6) and *P. aeruginosa* (P13), and the reference strain *S. aureus* ATCC6538P, were selected for a resistance test to the herbal extract. The tested organisms were sub-cultured in sub-MIC concentration of the crude extract for ten consecutive days in order to investigate their ability to develop resistance. During these 10 days, culture purity was assured by PFGE (pulsed-field gel electrophoresis), and the MIC of the sub-culture on day 11 was determined.

Combination effect of the crude extract with antibiotics

The strains of the clinical isolates *A. baumannii* (A6), *P. aeruginosa* (P13) and the reference strain *S. aureus* ATCC6538P were selected for agent combination analysis by the disk diffusion method as described previously. A disk impregnated with active herbal fractions was placed on an agar plate at a standard distance (30 mm) within the antibiotic disks. The plate was incubated at 37 °C and the pattern of inhibition zones was determined after 18 h. According to Matsuo's method [14], bridging or confluent zones of inhibition between the disks of the sample and the antibiotics was considered to indicate synergism. The eleven antibiotics used in this assay were ampicillin, tetracycline, kanamycin, gentamycin, Spectinomycin, amikacin, amoxillin, erythromycin, imipenem, Piperacillin G and clidamycin.

Table 3 The antibacterial susceptibility spectrum of the SFE extracts from *Ramulus Cinnamom*

Strains	MIC (mg/ml)		Strains	MIC (mg/ml)		Strains	MIC (mg/ml)		Strains	MIC (mg/ml)	
	Extracts	Cinnamaldehyde		Extracts	Cinnamaldehyde		Extracts	Cinnamaldehyde		Extracts	Cinnamaldehyde
A1	0.5	0.4	A14	0.5	0.4	A27	0.5	0.4	P13	0.5	0.4
A2	0.5	0.4	A15	0.5	0.4	P1	0.5	0.4	P14	0.5	0.4
A3	0.5	0.4	A16	1.0	0.4	P2	0.5	0.4	P15	1.0	0.4
A4	0.5	0.4	A17	0.5	0.4	P3	0.5	0.4	P16	0.5	0.4
A5	1.0	0.4	A18	0.5	0.4	P4	0.5	0.4	P17	0.5	0.4
A6	0.5	0.4	A19	0.5	0.4	P5	1.0	0.5	P18	0.5	0.4
A7	1.0	0.5	A20	0.5	0.4	P6	1.0	0.5	P19	1.0	0.5
A8	1.0	0.4	A21	0.5	0.4	P7	1.0	0.5	P20	1.0	0.5
A9	1.0	0.5	A22	0.5	0.4	P8	1.0	0.5	ATCC19606	0.5	0.4
A10	0.5	0.5	A23	1.0	0.4	P9	1.0	0.4	ATCC27853	0.5	0.5
A11	1.0	0.4	A24	0.5	0.4	P10	1.0	0.5	ATCC29260	0.5	0.5
A12	1.0	0.5	A25	0.5	0.4	P11	1.0	0.5	S1826	0.25	0.2
A13	1.0	0.5	A26	0.5	0.4	P12	1.0	0.5	S1827	0.5	0.4

The extraction conditions were 6,000 psi and 45 °C

A Acinetobacter baumannii, P Pseudomonas aeruginosa, S Staphylococcus aureus

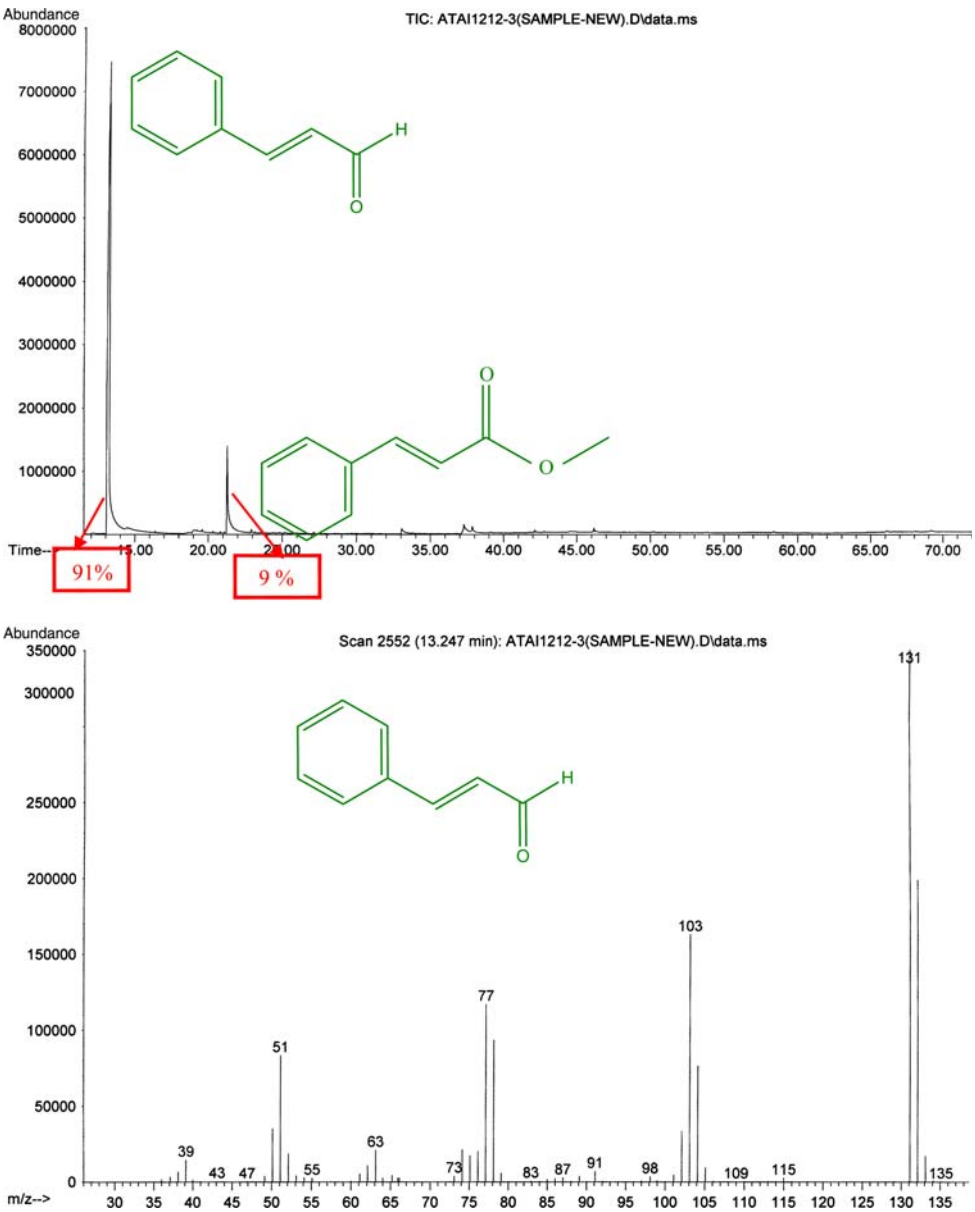

Fig. 2 GC–MS profile of the SFE extracts from *Ramulus Cinnamomi*

Scanning electron microscopy (SEM)

Samples were incubated at 37 °C for 16 h; afterward, bacterial suspensions were spun briefly at 5,000×*g* at room temperature, washed in PBS and finally resuspended in 0.5 ml of the same buffer. Cells were fixed with an equal volume of 5% glutaraldehyde (Sigma) in 0.1 M cacodylate buffer (pH 7.2) at 4 °C for 2 h. After being washed with the buffer, specimens were post-fixed for 1–16 h with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4) at 4 °C. Samples were dehydrated in graded acetone and dried by a CO_2 critical-point dryer (Hitachi Critical Point Dryer HCP-2). The dried samples were finally treated by gold coating and mounted on plates for SEM observation; samples were examined by a Leica Stereoscan (HITACHI S-2700) electron scanning microscope.

Spectrophotometer determination of antimicrobial components

The active components were identified by GC–MS (GC, Agilent 6890 N; MS, Agilent 5970; DB-5 column, 60 m × 250 μm × 0.25 μm) and FTIR (PERKIN ELMER SYSTEM 2000). For comparative studies, experiments were also conducted in a similar manner using cinnamaldehyde as a standard.

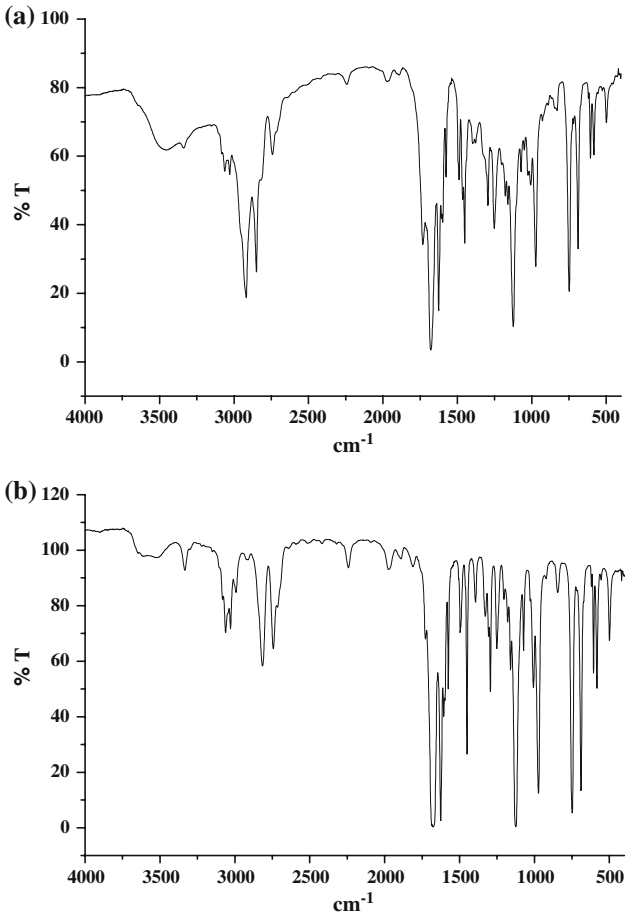

Fig. 3 FTIR chromatograms of the SFE extracts from *Ramulus Cinnamomi* and cinnamaldehyde, **a** the SFE extracts, **b** cinnamaldehyde

Antioxidation activity

Antioxidation activity was tested by 2,2-diphenyl-1-picrylhidrazyl (DPPH) radical assay [15] with little modification. Briefly, 1 ml of different concentrations of tested samples (crude extracts, cinnamaldehyde, or *o*-methoxy-cinnamaldehyde) was mixed with 1 ml of 0.5 mM DPPH in ethanol. Mixtures were vigorously shaken and left in dark for 30 min. Absorbance was measured at 517 nm using ethanol as a standard. One ml of 0.5 mM DPPH diluted in 2 ml of ethanol was used as control. Inhibition of the DPPH radical was calculated using the following equation:

$$I\% = \left[1 - \frac{X_1 - X_2}{X_3}\right] \times 100\%$$

where X_1 is absorbance of the tested sample (containing tested samples with DPPH solution), X_2 absorbance of the tested samples without DPPH, and X_3 absorbance of the control (containing all reagents except the tested samples). The IC_{50} value represents the concentration of the crude extract that caused 50% inhibition.

Results and discussion

Antimicrobial activity of the herbal extracts

The antibacterial activities of *Ramulus Cinnamomi* extracted from both extraction methods were compared. As shown in Table 2, comparing the results of the inhibition zone diameter and MIC test from the extracts of *Ramulus Cinnamomi* by SFE and ethanol extraction, it is observed that antibacterial activity of the SFE extract was much better than that of the ethanol extract.

The SFE extraction method was further used to test the effect of extraction conditions on the antibacterial activity. It was found that the yield of the extraction increased with increasing pressure and temperature, and with decreasing particle size as shown in Fig. 1. The results of MIC determination are shown in Table 1. The obtained results reveal that low temperature and high pressure extracts generally possessed a low MIC, although extraction at high temperature is favorable in order to obtain high yields. The MIC test found that increasing the size of the ground powders would also significantly reduce the antibacterial activity. This indicates that pretreatment of the raw materials is crucial.

Based on the optimal extraction condition (6,000 psi, 45 °C), the antibacterial activity of the SFE extracts against 49 clinical antibiotic resistant isolates and three reference strains were examined (Table 3). The extracts displayed varying degrees of inhibition against the tested bacterial species. In particular, they were active against the clinical strains, *S. aureus*, with a MIC in the range 0.5–0.25 mg/ml. For Gram-negative bacteria included in the clinical isolates and reference strains, the MIC was in the range 0.5–1.0 mg/ml. The obtained results indicate that the extracts possess a broad antibacterial spectrum against Gram-positive and Gram-negative bacteria. The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria could be due to a difference in the membrane structure. Gram-positive bacteria were more susceptible since have only an outer peptidoglycan layer which is not an effective permeability barrier. However, Gram-negative bacteria have outer phospholipids membranes that contain the structural lipopolysaccharide components. These components make the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to hydrophilic solutes [2].

The major substance that possesses antibacterial activity against the nosocomial drug-resistant strains was identified as cinnamaldehyde by both GC and mass and IR spectrum

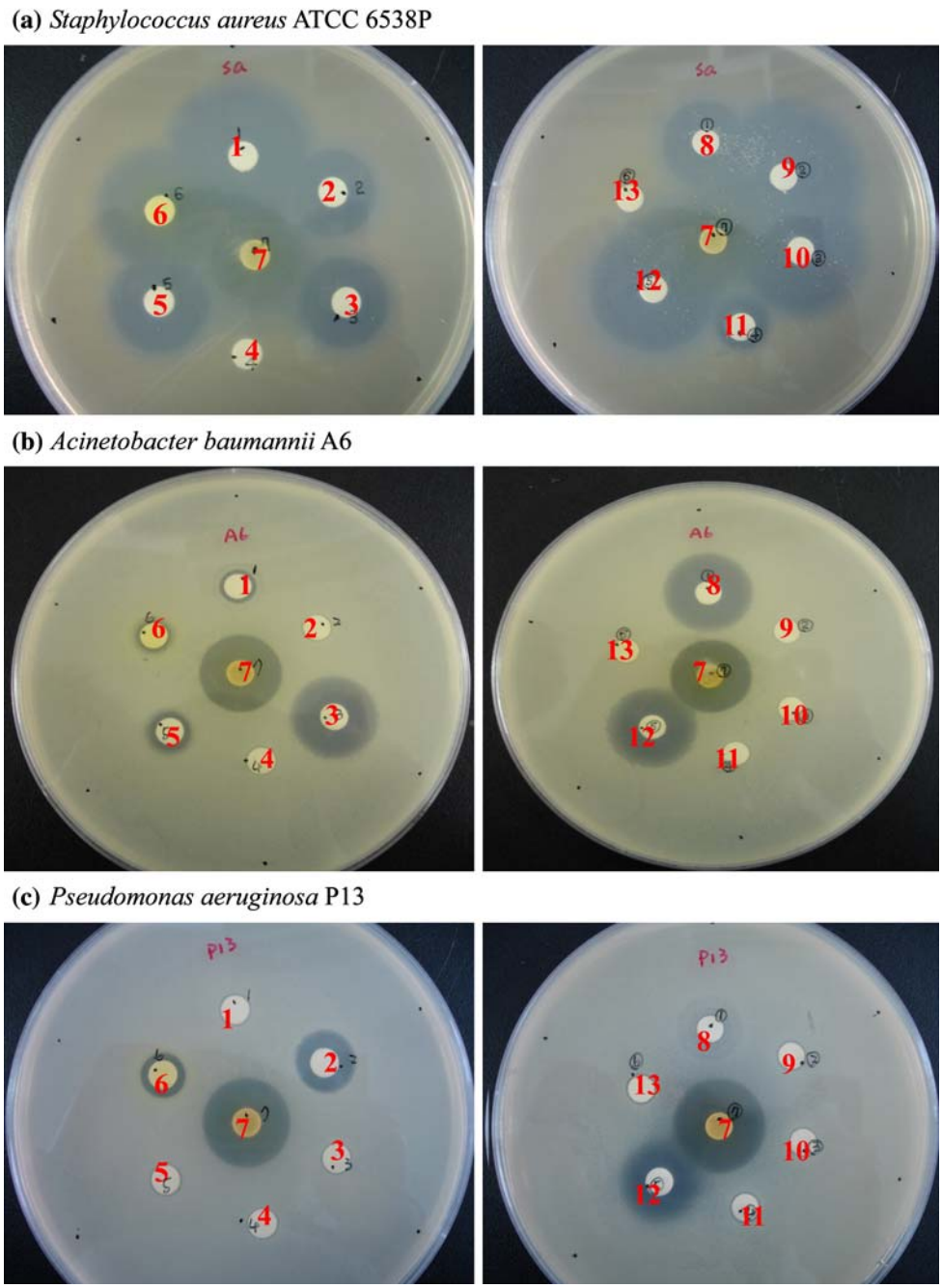

Fig. 4 Double disk diffusion synergy test of the SFE extracts and antibiotics. *1* Ampicillin, *2* Spectinomycin, *3* Kanamycin, *4* DMSO, *5* Gentamicin, *6* Tetracycline, *7* the SFE extracts, *8* Amikacin, *9* Piperacillin G, *10* Amoxillin, *11* Erytromycine, *12* Clindamycin, and *13* Imipenem

analysis (Figs. 2, 3). In addition, the standard cinnamaldehyde was also tested for its antimicrobial activity; the results evidenced that cinnamaldehyde possesses antibacterial activity with MIC values of approximately 0.2–0.5 mg/ml (Table 3). Comparing the extraction yield with the literature report [16], our results indicate that the cinnamaldehyde yield extracted by SFE (91%) is higher than the yield of the traditional alcohol extraction (83%).

Moreover, after exposure of the test strains to the *Ramulus Cinnamomi* extract for ten consecutive subcultures, the results showed that the MIC of each tested microorganism did not change. This indicates that the tested strains did not induce resistance to the herbal extract.

Combination effect of the herbal active fractions with antibiotics

By using the disk diffusion method, the drug combination effect was investigated for the active extracts and tested antibiotics. The results showed that none of the herbal extracts presented any synergistic interaction with the tested antibiotics for *A. baumannii* (A6) and *P. aeruginosa*

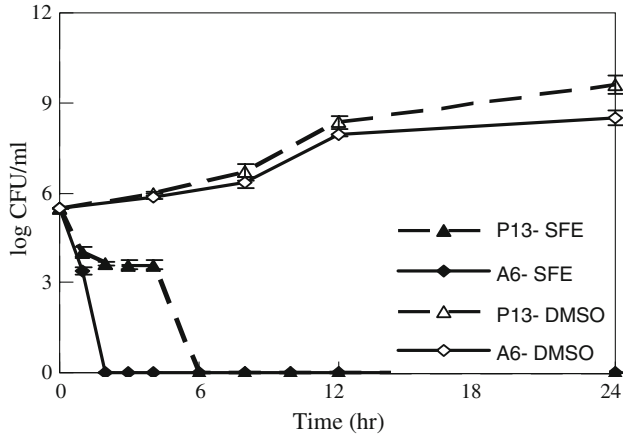

Fig. 5 Effect of the SFE extract from *Ramulus Cinnamomi* on growth of antibiotic-resistant nosocomial strains. The *curves* represent viable cell counts of the test organisms. The symbols of *triangles* and *diamonds* represent the test strains *Pseudomonas aeruginosa* P13 and *Acinetobacter baumannii* A6, respectively. The *solid symbols* represent the SFE extracts and the *open symbols* represent the solvent DMSO

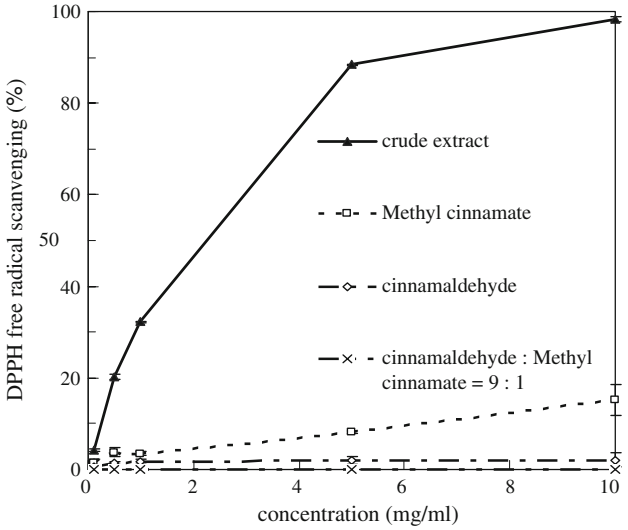

Fig. 7 Antioxidant activity for free radical scavenging activity by DPPH radical

(P13) strains (Fig. 4b, c). However, for the strain of *S. aureus* ATCC6538P, the crude extract of *Ramulus Cinnamomi* possessed a synergism effect with gentamycin, tetracycline, streptomycin, amikacin, amoxillin, penicillin, and clidamycin (Fig. 4a). The results may be explained by the fact that Gram-positive *S. aureus* was more susceptible to the antibacterial agents, due to the absence of an outer membrane that prevents the penetration of numerous antibiotic molecules and a periplasmic space where enzymes break down the foreign molecules which are introduced externally.

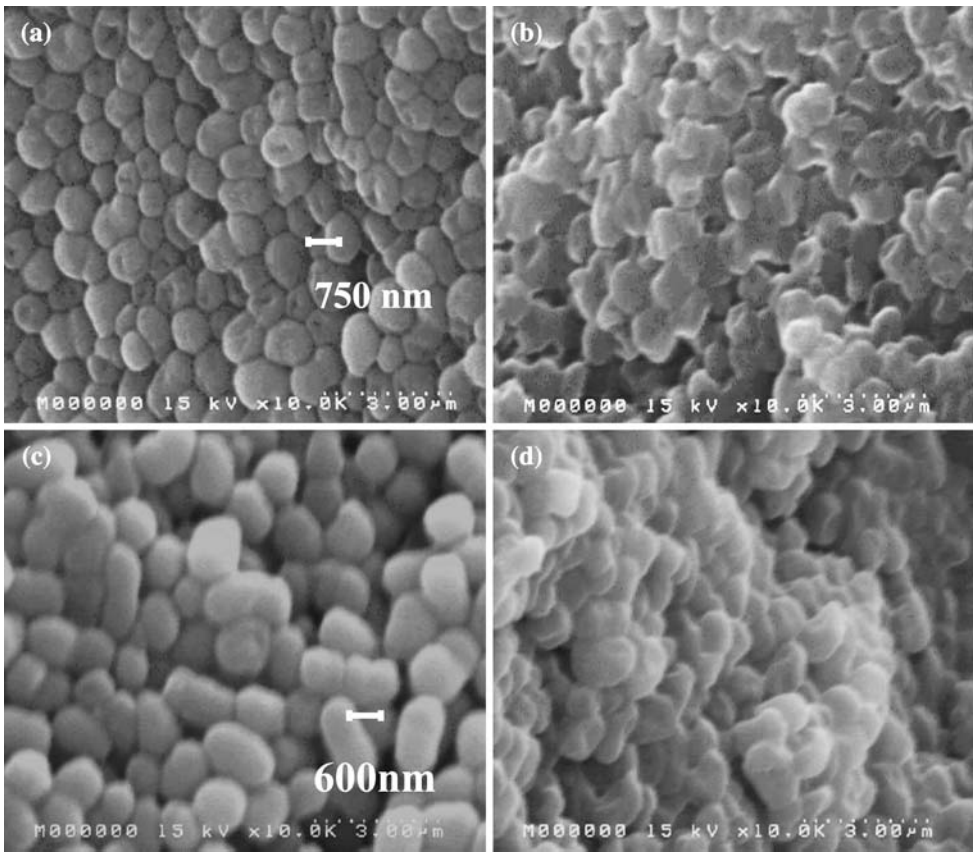

Fig. 6 Scanning electron micrographs of the clinical strains *Pseudomonas aeruginosa* P13 (*top*) and *Acinetobacter baumannii* A6 (*bottom*) cells, in the absence of the SFE extracts (**a**, **c**), and after 16 h incubation at 37 °C with concentrations of the extracts at 1/4 MIC value (**b**, **d**)

Time-kill assay

The strains of clinical isolates *A. baumannii* (A6) and *P. aeruginosa* (P13) were selected for this investigation. Figure 5 shows that when the isolates were exposed to double the MIC concentration, the crude extract of *Ramulus Cinnamomi* completely killed all of the tested microorganisms within 6 h and the inhibition effect lasted for more than 24 h. The crude extracts were considered to exhibit bactericidal activity for the test microorganisms.

Morphological changes induced by SFE extracts on target bacteria

To visualize the effect of SFE extracts on the test microorganisms, scanning electrographs (at 8,000–20,000× magnification) were obtained from the clinical strains P13 and A6, after treatment with the extracts (Fig. 6). In the case of both strains, control cells incubated in a broth in the absence of the extracts exhibited a regular, smooth surface (Fig. 6a, c), whereas cells incubated with extracts at concentrations corresponding to 1/4 of the MIC value revealed severe membrane damage consistent with disruption of the membrane integrity (Fig. 6b, d). These findings demonstrate that lysis occurs when susceptible organisms are exposed to the extracts, and suggest that the active components perform the action mainly against cell membranes or disturb cell division.

Antioxidation activity

Results of the DPPH radical-scavenging assay revealed that crude extracts showed substantial antioxidant activity. Scavenging of DPPH radicals was concentration dependent, with $IC_{50} = 2$ mg/ml. However, neither cinnamaldehyde nor *o*-methoxy-cinnamaldehyde showed obvious antioxidant activity (Fig. 7). Although, the ethanol extracts of the *Cinnamomui* species have been shown to possess antioxidant effects [17], nothing has been reported on its antioxidant components. In this study, we have also evidenced that the crude extracts of *Ramulus Cinnamomi* possess antioxidant activity, but none of the major components in the SFE extracts of *Ramulus Cinnamomi*, namely cinnamaldehyde and *o*-methoxy-cinnamaldehyde, showed antioxidant activity. We presume that other components of the crude extracts contribute to its antioxidant activity.

Conclusions

The results of this investigation on the antibacterial properties of *Ramulus Cinnamomi* suggest its therapeutic potential for the treatment of drug-resistant bacterial infections. The isolated compound that showed antibacterial activity was identified as cinnamaldehyde. These findings correlated with the observations of the previous reports [16, 18]. Although, no previous reports concerning the antibacterial activity of *Ramulus Cinnamomi* against large amounts of clinical drug-resistant isolates, our results supported that the active herbal extract was uniformly active against all the tested strains. This clearly indicates antibiotic resistance does not interfere with the antimicrobial action of the *Ramulus Cinnamomi* extracts. In accordance with literature data, the ethanol extracts of *Cinnamomum cassia* have been shown to exhibit antioxidant effect [17]. Our results also evidenced that the crude extracts of *Ramulus Cinnamomi* exhibited substantial antioxidant activity. Further studies are needed in order to isolate and identify the compounds responsible for the antioxidant activity. Overall, the results of this study provide valuable information for further phytochemical extraction reference and characterization studies of active compounds, which are necessary for the development of new drugs for the drug-resistant strains.

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References

1. Hsueh PR, Tseng SP, Teng LJ, Ho SW (2005) Clin Microbiol Infect 11:670–673
2. Arias ME, Gomez JD, Cudmani NM, Vattuone MA, Isla MI (2004) Life Sci 75:191–202
3. Soylu EM, Soylu S, Kurt S (2006) Mycopathologia 161(2):119–128
4. Daud A, Gallo A, Riera AS (2005) J Ethnopharmacol 99(2):193–197
5. Li ST, Liang MT, Chen CH, Lo CH, Chuang LY (2005) Symposium on phytochemicals: bioactivity, genomics, and proteomics. Taiwan, p 27
6. Liu CS, Yang CH, Chang CW, Chen CH, Chuang LY (2007) Am J Chin Med 35(6):1047–1060
7. Lee W, King P, Furm IV (2002) Chinese Medicine Technical Publication, p 75
8. Brunner G (1994) Gas extraction—an introduction to fundamentals of supercritical fluids and the application to separation processes. Steinkopff/Springer, Darmstadt/New York
9. Johnston KP, Penninger JM (1989) Supercritical fluid science and technology. ACS symposium series 406
10. Mi XH, Gong ZN, Zhang WM, Zhang XR (2004) J Nan Jing Normal University (Natural Science), vol 27. pp 63–66
11. King DS, Lee KH, Wung KT, Liu I (2003) Chem Res Appl 15:678–679
12. National Committee of Clinical Laboratory Standards (1993) Document M7-A3. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd edn. Approved Standard
13. Yu HH, Kim KJ, Cha JD, Kim HK, Lee YE, Choi NY, You YO (2005) J Med Food 8(4):454–461
14. Matsuo K, Uete Y (1993) Jpn J Antibiot 46(3):222–233

15. Dordevic S, Petrovic A, Dobric S, Milenkovic M, Vucicevic D, Zizic S, Kukic J (2007) J Ethnopharmacol 109:458–463
16. Ooi SM, Li Y, Kam SL, Wang H, Wong EY, Ooi VE (2006) Am J Chin Med 34(3):511–522
17. Lin CC, Wu SJ, Chang CH, Ng LT (2003) Phytother Res 17:726–730
18. Kwon JA, Yu CB, Park HD (2003) Lett Appl Microbiol 37:61–65