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EXPERIMENTAL STUDY ON THE OPTIMIZATION OF EXTRACTION PROCESS OF GARLIC OIL AND ITS ANTIBACTERIAL EFFECTS

Yajie Guo

College of Materials and Chemistry and Chemical Engineering, Chengdu University of Technology, Chengdu, Sichuan 610059, China

E-mail: wngojiob@yeah.net

Abstract

Background: Garlic oil which is the main active constituent of garlic has a wide range of pharmacological activities, and a broad antibacterial spectrum. It also has a strong anti-cancer activity, and can significantly inhibit a variety of tumors such as liver cancer, gastric cancer and colon cancer. The objective is to study the extraction process of garlic oil and its antibacterial effects.

Materials and Methods: CO_2 Supercritical extraction was used to investigate the optimal processing conditions for garlic oil extraction; filter paper test and suspension dilution test were applied to determine the bacteriostatic action of garlic oil.

Results: In the CO_2 supercritical extraction experiment, factors influencing the yield of garlic oil were: extraction pressure > extraction temperature > extraction time in descending order. Range analysis showed that the optimal experimental conditions for CO_2 supercritical extraction of garlic oil were extraction pressure of 15 Mpa, temperature of 40 ℃, and duration of 1 h. Different concentrations of garlic oil could all inhibit the growth of *Staphylococcus aureus*, *Escherichia* coli and *Bacillus subtilis*, suggesting that garlic oil has an antibacterial effect.

Conclusion: The optimal experimental conditions for CO_2 supercritical extraction of garlic oil were: extraction pressure of 15 Mpa, temperature of 40 ℃, and duration of 1 h; garlic oil has an antibacterial effect.

Keywords: garlic oil, CO_2 , supercritical extraction; *Staphylococcus aureus; Escherichia coli, Bacillus subtilis*

Introduction

Garlic oil, whose chemical name is diallyl trisulfide, is the main active constituent of garlic. Garlic oil has a wide range of pharmacological activities, and a broad antibacterial spectrum (Yang et al., 2008; He et al., 2008). It also has a strong anti-cancer activity, and can significantly inhibit a variety of tumors such as liver cancer, gastric cancer and colon cancer (Hassan, 2004; Sundaram et al., 1996; Oltvai et al., 1993; Markos et al., 2008). Domestic scholars have done a lot of research (Zeng et al., 2006; Chen et al., 2008) on the preparation and extraction method of garlic oil, the majority of which adopted leaching method; relatively few studies have applied the supercritical extraction technology to extract garlic oil. In this paper, the optimal process parameters of garlic oil were determined by supercritical extraction method, meanwhile, the antibacterial effect of garlic oil was studied.

Materials and Methods

Drugs, reagents and instruments

Garlic, purchased from the market; beef extract peptone agar medium; supercritical extraction device (Joel High-Tech Co., Ltd., Dalian).

Tested bacterial strains

Staphylococcus aureus, Escherichia coli, Bacillus subtilis (China Medical University).

Optimization of extraction process of garlic oil

About 200 g of fresh garlic was weighed out, peeled, washed, and minced with a blender, followed by the extraction of garlic oil using CO_2 supercritical extraction method. Extraction column was filled based on the factors and levels of orthogonal experiment (see Tab. 1), after

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sealing, temperature and pressure were regulated, extraction time was selected according to the experimental requirements, at last, the yield of garlic oil was calculated.

Determination of factors of orthogonal experiment

Orthogonal experiment on three factors of temperature, pressure and extraction time was conducted based on the table of factors and levels of orthogonal experiment, and the optimal extraction process parameters were determined.

Table 1: Factors and levels of orthogonal experiment

Level	*Factor*		
	A Temperature (℃)	B Pressure (Mpa)	C Time (h)
1	30	10	1
2	40	15	2
3	50	20	3

Antibacterial experiment of garlic oil

Preparation of test solutions

After activating three test bacterial strains of *Staphylococcus aureus*, *Escherichia coli and Bacillus subtilis*, two loops of bacterial strains were picked separately with inoculating loop, diluted with sterile saline, oscillated, and prepared into bacterial suspension with bacterial count of about 1.5×10^8 cfu/mL. Garlic oil stock solution was taken, diluted under sterile conditions into three concentrations of 20%, 40% and 60% and set aside.

Filter paper test

Filter paper were taken, soaked sufficiently in different concentrations of garlic oil for 6 min and set aside. Taking sterile water as the control, bacterial suspensions were dipped with sterile cotton, and uniformly applied in the culture media, fully soaked filter paper was placed in the bacteria containing plates, 3 parallel pieces were set up for each culture dish in each experimental group. The dishes were placed in the 37℃ constant temperature incubator and cultured for 24 h, and then the diameter of inhibition zone was measured.

Determination of antibacterial effect by suspension dilution test

Sterile culture dishes were added with different concentrations of garlic oil test solutions as well as 10 mL of medium, shaken well, and then cultured at 37℃ for 24 h to allow the solutions to fully penetrate into the medium. After the medium was taken out, 1 mL of bacterial suspension was drawn and injected into it; the bacterial suspension was allowed to evenly spread on the medium. After culturing at 37℃ for 24 h, the number of bacterial colonies was observed and recorded.

Results

Optimization of extraction process of garlic oil

As can be seen from the experimental results, in the CO_2 supercritical extraction test, that the factors influencing the yield of garlic oil were: B>A>C, that is, the degrees of influence to the experimental results were: extraction pressure > extraction temperature > extraction time in descending order. Range analysis showed that the optimal experimental conditions for CO_2 supercritical extraction of garlic oil were B2A2C1, which means that the adoption of extraction pressure of 15 Mpa, temperature of 40℃, and duration of 1 h can maximize the extraction yield of garlic oil.

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Table 2: Orthogonal experimental results for optimization of extraction process of garlic oil

No.	A	B	C	Garlic oil yield (%)
1	1	1	1	0.275
2	1	2	2	0.359
3	1	3	3	0.244
4	2	1	2	0.336
5	2	2	3	0.394
6	2	3	1	0.312
7	3	1	3	0.219
8	3	2	1	0.388
9	3	3	2	0.275
k1	0.878	0.830	0.975	
k2	1.042	1.141	0.970	
k3	0.882	0.831	0.857	
K1	0.293	0.277	0.325	
K2	0.347	0.380	0.323	
K3	0.294	0.277	0.286	
R	0.054	1.03	0.039	

Filter paper test results

The experimental results showed that different concentrations of garlic oil could all inhibit *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* these three tested bacterial strains, and a clear dose-effect relationship was observed between inhibitory effect and drug concentration. (Figure 1).

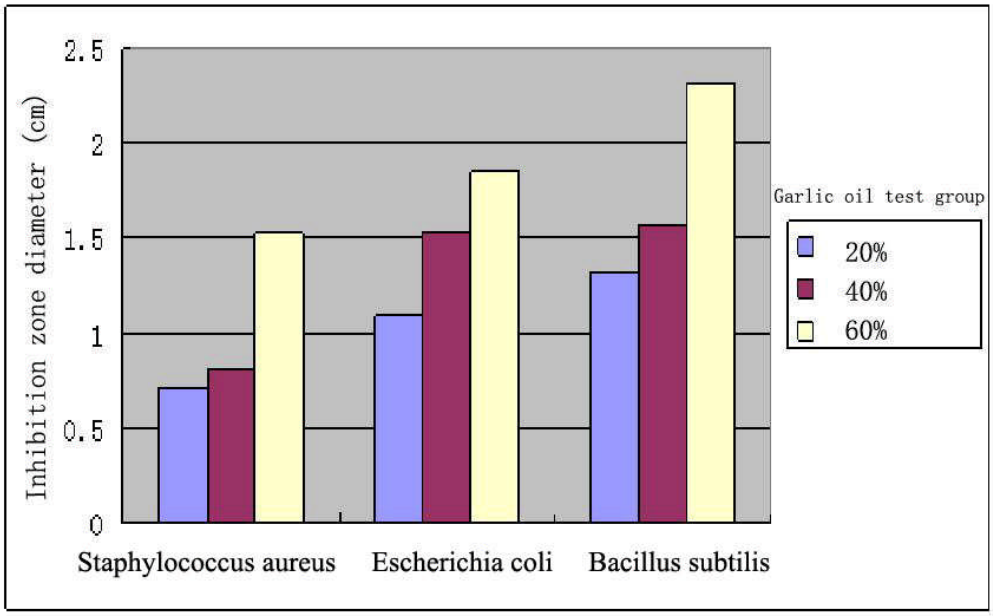

Figure 1: Results for determination of antibacterial effect of garlic oil by filter paper test

Suspension dilution test results

After 24 h of cultivation, different concentrations of garlic oil inhibited the three kinds of bacterial strains. No significant colony of the three bacterial strains were observed in the 60% garlic oil culture dish, indicating that high concentrations of garlic oil has a significant inhibitory effect on *Staphylococcus aureus, Escherichia coli* and *Bacillus* subtilis (Table 3).

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Table 3: Number of colonies in each concentration of garlic oil (24 h later)

Group	Concentration (%)	Number of colonies		
		Staphylococcus aureus	*Escherichia coli*	*Bacillus subtilis*
Garlic oil test group	20	35	18	44
	40	7	4	16
	60	No significant colony formation	No significant colony formation	No significant colony formation

Discussion

Supercritical fluid extraction is a new-form of extraction and isolation technology. Its working principle is to cause quick dissolution of fluid taking advantage of subtle changes in the temperature and pressure of the fluid at the critical point, thereby extracting active constituents or effective parts in the raw material, then proceed to the isolation and purification in a low pressure environment, thus achieving the isolation or purification of mixture. This technology has the advantages of simple process, energy efficiency, safety and reliability, and is thus widely applied (Teng et al., 2008; Jiao et al., 2007).

Garlic oil has a broad-spectrum antibacterial effect. It has different degrees of inhibitory and killing effects on pathogenic bacteria such as *Staphylococcus aureus, Staphylococcus albus, Staphylococcus saprophyticus, Shigella flexneri, Shigella sonnei, Salmonella typhi and Escherichia coli*, its inhibitory and killing effect is especially preferable against *Staphylococcus aureus* (pathogens for summer skin infections), *Shigella flexneri* (primary pathogens for dysentery) and *Salmonella typhi.*

The results of this experiment showed that the factors influencing the yield of garlic oil in the CO_2 supercritical extraction test were: extraction pressure > extraction temperature > extraction time in descending order. Range analysis showed that the optimal experimental conditions for CO_2 supercritical extraction of garlic oil were the extraction pressure of 15 Mpa, temperature of 40 ℃, and duration of 1 h.

It can be seen from the antibacterial test of garlic oil that different concentrations of garlic oil could all inhibit the growth of *Staphylococcus aureus*, *Escherichia coli* and *Bacillus subtilis*, suggesting that garlic oil has an antibacterial effect. Its antibacterial mechanism may be the occurrence of competitive inhibition between sulfur containing active group in garlic and mercapto group in cysteine molecules needed for the growth of target bacteria, thereby inhibiting the growth of target bacteria (Su, 2007).

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