Comparison of antibacterial effects of compound plant essential oils

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Abstract. With the growing demand for alternatives to antibiotics, plant essential oils are becoming increasingly popular as green additives in the livestock industry due to their natural, safe, and versatile properties. This study investigated three essential oil compounds: anise, fennel, and coriander. Gas chromatography was used to analyze their composition, assess their thermal stability, and test their antibacterial activity against four pathogenic bacteria. The results showed that all three essential oils exhibited antibacterial activity, with cumin oil showing the most significant effect and excellent thermal stability.

1 Introduction

Although the widespread use of antibiotics has effectively curbed the occurrence and spread of animal diseases, the problems of increased drug resistance and environmental pollution caused by them have gradually emerged and attracted widespread attention. Plant essential oils, as a natural alternative to antibiotics, have attracted much attention because they are rich in bioactive ingredients and have fewer side effects. Plant essential oils are secondary metabolites of plants and are mainly composed of various chemical components such as alcohols, esters, ketones, phenols, and alkenes. These components each have unique functions, which not only give plant essential oils their unique aroma, but also enable them to have diverse biological functions. Specifically, the main components of plant essential oils are mixtures of terpenoid compounds such as monoterpenes, sesquiterpenes and their oxidized derivatives [1]. Among them, many components have been listed as GRAS (generally recognized as safe) substances by the US Food and Drug Administration (FDA) and are widely used in the field of food additives [2]. Research reports in recent years have shown that plant essential oils have multiple functions such as promoting animal growth, regulating the balance of animal intestinal microecology, and enhancing the antioxidant capacity and immune function of animal bodies [3-9]. The growth status of animals is closely

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related to their digestion and absorption capacity, and the activity of digestive enzymes in animals is an important indicator reflecting their digestion capacity [10]. At the same time, the balance of intestinal flora has a positive effect on improving the digestion and absorption capacity of animals. Cumin, fennel, and coriander are all traditional medicinal and edible plants. Their essential oils are rich in volatile components and have potential antibacterial properties. This study aims to compare and analyze the antibacterial effects and thermal stability of these three composite plant essential oils on common pathogens through in vitro experiments, in order to evaluate the antibacterial properties of different products.

2 Materials and Methods

2.1 Materials

2.1.1 Essential oils

Essential oils: Anise essential oils, fennel essential oils , and coriander essential oils.
The plant essential oils are commercially available in China and were purchased from a Chinese medicine company.

2.1.2 Test strains

Strains: *Staphylococcus aureus, Proteus mirabilis, Escherichia coli,* and *Bacillus cereus.*
Courtesy of the Belarusian Center for the Conservation of Non-Pathogenic Microorganisms (Institute of Microbiology, National Academy of Sciences of Belarus, Minsk, Republic of Belarus) and the Faculty of Biology of Belarusian State University (Minsk, Republic of Belarus).

2.2 Methods

2.2.1 Active Ingredient Analysis

Gas chromatography-mass spectrometry (GC-MS) was used to analyze the essential oils'main active ingredients of the three essential oils, focusing on trans-anethole, coriander alcohol, anise ketone, and limonene.

2.2.2 Thermal Stability Test

Equal amounts of each essential oil product were divided into two groups: a control group and a heat-treated group. The essential oils were heat-treated under conditions simulating feed pelleting (85°C, 40 seconds), and changes in the active ingredients before and after treatment were measured.

2.2.3 In vitro antibacterial assay

The experiment was divided into five groups. Each of the three essential oils was added to four opportunistic microbial culture dishes. A blank control group was set up. Another group was cultured with tetracycline. After a constant temperature of 37°C for 24 hours, analysis antibacterial effect.

2.2.4 Data Analysis

This experiment was designed with three replicates, and variance analysis was performed using Excel SPSS 19.0 to ensure efficient and accurate data processing.

3 Results and Analysis

3.1 Active Ingredient Analysis

Qualitative analysis by GC-MS revealed that the main components of the three essential oils were trans-anethole and coriander alcohol.Coriander essential oil's primary components are linalool (65%-75%) and geraniol (65%-80%). Other components include monoterpenes such as phellandrene, decanal, and α-pinene. Star anise oil primarily contains trans-anethole (80%-95%), while fennel oil contains approximately 35%-65% trans-anethole, along with other components such as fenchone. Minor components of star anise essential oil include eucalyptol, safrole, and anisaldehyde.
The active ingredient analysis of the test essential oils is in the Table 1.

Table 1. Active ingredients

Essential oil name	Ingredients	Content(mg/g)
Fennel	*Trans-anethole*	8.157
	Aniseone	0.403
	Anethole	0.352
	Limonene	0.308
Coriander	*Linalool*	5.876
	Geraniol	4.327
	Limonene	0.142
Anise	*Trans-anethole*	8.738
	Fenchone	1.149
	Limonene	0.297

3.2 Thermal Stability Analysis

The total loss of active ingredients after heat treatment is as follows:
The heat treatment revealed that the loss of different essential oils after treatment at 85°C for 40 seconds ranged from 5% to 30% (show Table 2).

Table 2. Heat treatment loss rate

Essential oil name	Heat treatment loss rate(%)
Fennel	20-30
Coriander	15-25
Anise	5-10

Coriander essential oil is mainly composed of linalool, and its monoterpene alcohols are easily volatile and oxidized, with a loss rate of 15%-25% after heat treatment. Star anise

essential oil contains a large amount of trans-anethole, and phenylpropanoids have the characteristics of structural stability and high temperature resistance, so the loss rate is only 5%-10%. In addition to containing cis-anethole, fennel essential oil also contains anisaldehyde, but aldehydes are easily oxidized, and the loss rate is as high as 20%-30%.

3.3 Comparison of In Vitro Antibacterial Effects

3.3.1 Antibacterial Effect against Escherichia coli

The comparison was based on the measured OD values. The OD values of the control group were indicated by "--"; when the OD value of the control group was 75% of that of the experimental group, it was indicated by "+"; when the OD value of the control group was 50% of that of the experimental group, it was indicated by "++"; when the OD value of the control group was 25% of that of the experimental group, it was indicated by "+++".

Table 3. Antibacterial effect

Group	***Escherichia coli***	***Proteus mirabilis***	***Staphylococcus aureus***	***Bacillus cereus***
Fennel	+	++	+	+++
Coriander	++	+++	++	++
Anise	+++	+++	+	+++
Control	--	--	--	--

Table 3 shows the antibacterial effects of three essential oils on four opportunistic microorganisms. Among them, star anise essential oil has an outstanding antibacterial effect, followed by coriander essential oil, and fennel essential oil shows strong antibacterial properties against some opportunistic microorganisms.

4 Discussion

Anise essential oils, the main component of star anise essential oil, exerts a broad-spectrum antibacterial effect by destroying bacterial cell membranes and inhibiting protein synthesis. Its antibacterial effect on *Escherichia coli*, *Bacillus cereus*, and *Proteus mirabilis* is significantly better than that of the other two essential oils. It also has a relatively stable structure during heat treatment. Coriander essential oil and fennel essential oil have outstanding antibacterial effects on *Proteus mirabili* and *Bacillus cereus*, respectively. However, due to its chemical composition, fennel essential oil is easily affected by factors such as light and temperature, and its antibacterial effect is not stable.

5 Conclusion

This study demonstrated that essential oils from fennel, anise, and coriander all possessed inhibitory activity against pathogenic *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, and Bacillus cereus*.Anise essential oils, due to their high trans-anethole content, exhibited the best antibacterial activity and excellent thermal stability. The trans-anethole content of fennel essential oil is slightly lower than that of star anise essential oil. Although

its thermal stability is slightly lower, it still has excellent antibacterial ability. Coriander essential oil, with its high coriander alcohol content, also exhibited good antibacterial activity against some strains. Although its antibacterial activity was slightly weaker, its rich olefins may play a positive role in regulating the intestinal microbiome. Thermal stability analysis indicated that the loss of active ingredients during heat treatment is not negligible, and the impact of heat treatment on the activity of essential oils should be considered in practical applications.

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