Chemical Significance of the Volatile Components of Spices in the Food Preservative Viewpoint II*

Antibacterial Activity of Volatile Components of Coriander-Seed

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(Tables 1-4)

INTRODUCTION

In his chemical studies on the volatile constituents of seaweeds the senior author of this paper found that some of the volatile constituents of seaweeds show antibacterial activity[1),2),3)]. This finding interested us and promoted us to institute the present work, although there had already been several studies[4),5),6),7)] on the antibacterial activity of those essential oils which are listed in pharmacopoeias as odoriferous antiseptic agents. As was mentioned in the preceding paper[8)], the present series of work was carried out in order to clarify the volatile components and antibacterial properties of those condiments which are used to season fish ham and fish-meat sausage. The volatile components of coriander-seed known to date are β-pinene, α-terpineol, γ-terpinene, p-cymene, terpinolene, geraniol, decylaldehyde and acetic acid[9)]. In the present work coriander-seed was submitted to steam distillation and the distillate was extracted with ether. This ether extract was fractionated into fatty acid, phenol, cabonyl, and terpene fractions by the same method as was used for the fractionation of volatile constituents of seaweeds[2),10),11)]. Antibacterial properties of each fraction were tested with *Bacillus subtilis, Salmonella enteritidis, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus morganii* and *Escherichia coli*. The fractions showed antibacterial activity at dilutions lower than 20～200 times.

EXPERIMENTAL

I. Collection and Fractionation of Volatile Components of Coriander-Seed

About 2 kg of coriander-seed were submitted to steam distillation and the distillate was saturated with sodium chloride and extracted with ether. The extract was dried, ether was distilled off, and the crude oil thereby obtained was fractionated[2),9),10)] into lower fatty acids, higher fatty acids, phenols, carbonyls and terpenes. Yield of each fraction is shown in Table 1.

The terpene fraction was further fractionated by distillation with the result shown in

* The previous report -I. Bull. Jap. Soc. Sci. Fish., **24**: 511 (1958).

Table 2.

Each fraction was subjected to chromatostripping[12),13),14),15),16),17)] in which 1:8-cineol was developed simultaneously as the reference standard for Rf. After it was con-

Table 1. Yield of volatile compounds from 2 kg of coriander-seed

Fraction	Yield (g)
1. Lower fatty acids fraction	0.6
2. Higher fatty acids fraction	2.7
3. Phenol fraction	1.2
4. Carbonyl fraction	0.5
5. Terpene fraction	6.7

Table 2. Result of fractional distillation of terpene fraction

Fraction	Pressure (mm)	b.p. (°C)	Yield (g)	N_D^{25}	Color
I	10	~42	1.2	1.4650	light yellow
II	〃	43~50	0.9	1.4659	〃
III	〃	51~55	0.9	1.4732	〃
IV	〃	56~60	0.5	1.4950	〃
V	5	~70	0.5	1.5003	〃
VI	3	~60	0.6	1.5010	〃
VII	〃	61~70	0.7	1.4951	〃
VIII	〃	71~90	0.5	1.4918	light brown

firmed that the Rf value of the simultaneously developed 1:8-cineol was within the range of error, Rf values were calculated for the sample. These value are listed in Table 3.

II. Assay of Antibacterial Activity

(a) Preparation of sample: The sample was dissolved in 5% ethanol to obtain solutions of 10, 20, 100, 200, 1000, 2000 dilutions.

(b) Preparation of test medium: The test medium was prepared by dissolving 10g of peptone, 5g of meat extract, 2.5g of sodium chloride and 25g of agar in distilled water and making up to 1 *l*. pH was adjusted to 7.4.

(c) Composition of bouillon for inoculation of test bacteria: The composition of the bouillon used for the inoculation of test bacteria was as follows: 10g of peptone, 5g of meat extract and 2.5g of sodium chloride, dissolved in distilled water to make up to 1*l*. pH was adjusted to 7.4.

(d) Test bacteria: The followlng six kinds of bacteria were preliminarily grown on the above-mentioned agar slant and exercised three times of subculture. The bacteria were then transferred to the bouillon medium and incubated at 37°C for 24 hours.

Test bacteria:

1. *Bacillus subtilis*
2. *Staphylococcus aureus*
3. *Proteus morganii*
4. *Salmonella enteritidis*
5. *Pseudomonas aeruginosa*
6. *Escherichia coli*

Table 3. Fractionation of neutral compounds by chromatostripping

Fraction	Pressure (mm)	b. p. (°C)	Rf values											
I	10	~42				0.38							0.73	β-pinene 0.91
II	〃	43~50				0.37				0.59			0.73	0.92 (pink)
III	〃	51~55		geraniol 0.20		0.38		0.48 (dark brown)	p-cymene 0.52	0.59 (pink)			0.72	
IV	〃	56~60		0.29 (yellow)	0.32	0.38 (red-orange)			0.52 (yellow)			0.68	0.73 (yellow-brown)	
V	5	~70	0.06 (brown)		0.31 (pink)		0.46 (orange)				0.64	0.68		
VI	3	~60									0.64	0.68		
VII	〃	61~70									0.64 (dark green)	0.68		
VIII	〃	71~90										0.68 (brown)		

(e) Test method: One cc of each of the diluted solutions of the sample was mixed with 20cc of agar medium maintained at 42～45°C and poured into a petri dish to form an agar plate. The test bacteria were inoculated on this agar plate in streaks[18] and incubated at 37°C for 48 hours. Antibacterial activity was judged by comparing the growths of test bacteria on the medium containing the sample and on the control plate.

The control plate was an agar medium into which 5% ethanol had been mixed to see the effect of the alcohol on the growth of test bacteria. It was streaked with test bacteria and was used as the basis for determining the antibacterial activity of samples.

(f) Judgement of antibacterial activity: The highest dilution of the sample at which growth of test bacteria was inhibited is listed in Table 4.

Table 4. Antibacterial activity of volatile compounds

Fraction	b. p. (°C)	Maximum dilution at which bacterial growth was inhibited					
		Bac. subtilis	*Esch. coli*	*Salm. enteritidis*	*Staph. aureus*	*Prot. morganii*	*Pseud. aeruginosa*
Higher fatty acid fraction	—	10	10	10	10	10	10
Phenol fraction	—	20	10	10	10	10	20
Terpene fraction	—	200	100	100	100	100	100
Terpene fraction I	～42 (10 mm.)	100	100	100	100	200	10
Terpene fraction II	43～50 (〃)	200	20	100	100	100	10
Terpene fraction III and VI	51～60 (〃)	200	20	100	100	100	10
Higher unsaturated fatty acid fraction	—	200	20	20	100	100	20
Higher saturated fatty acid fraction	—	20	10	10	20	10	10

As is shown in Table 4, each fraction showed antibacterial activity at dilutions lower than 20～200 times.

SUMMARY

1. The coriander-seed, which is used as a condiment in fish ham and fish sausage, was submitted to steam distillation, and the distillate was extracted with ether to collect volatile components. These components were fractionated into fatty acids, phenols and terpenes.

2. The terpene portion was refractionated, each fraction was subjected to chroma-

tostripping, and the presence of β-pinene, p-cymene, terpinolene and geraniol was proved.

3. Antibacterial activity of each fraction was tested with *Bacillus subtilis*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus morganii* and *Escherichia coli*. All the fractions inhibited bacterial growth at dillutions lower than 20～200 times.

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