Antimicrobial Activity of Flavor Components of Cardamom *Elattaria cardamomum* (Zingiberaceae) Seed

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The antimicrobial activity of the 10 most abundant volatile components of the cardamom *Elattaria cardamomum* (L.) Maton (Zingiberaceae) seed has been tested against 14 microorganisms. All of the compounds tested exhibited activity against at least one or more microorganisms.

INTRODUCTION

The control of troublesome microorganisms that cause problems with teeth, skin, and hair is increasingly important. By contrast to medicine which is used to heal ill people, the antimicrobial agents that control these microorganisms are often repeatedly and for long duration applied to healthy teeth, skin, and hair, so their safety is the first consideration. For example, the control of cariogenic bacteria such as *Streptococcus mutans* is important for preventing dental caries. Many attempts are being investigated to eliminate the cariogenic bacteria from oral flora. Although several antibiotics were found to effectively prevent dental caries in vitro and in vivo, many resulted in derangement of oral and intestinal bacterial floras which cause undesirable side effects (Fitzgerald, 1972). Therefore, edible plants, beverages, and food spices seem to be a superior source of antimicrobial agents (Himejima and Kubo, 1991).

The cardamom *Elattaria cardamomum* (L.) Maton (Zingiberaceae) seed has widely been consumed as a food spice worldwide since ancient time. Besides being used as a food spice, it has also been employed as a folk remedy to treat stomachache. The cardamom seed is also known as an aphrodisiac in India. It is sometimes employed for making mouthwash and soap as well. In addition, chewing the cardamom seed like tobacco is a common habit among many people in several Arabic countries such as Saudi Arabia. Thus, it would appear that the human oral toxicity of the cardamom seed is not serious or has been overlooked. This promoted us to investigate antimicrobial agents from the cardamom seed, especially considering the control of microorganisms that cause teeth, skin, and hair problems such as dental caries, acne, and dandruff.

In our preliminary screening, the *n*-hexane extract of the cardamom seed was found to exhibit rather broad antimicrobial activity including against *S. mutans*, *Propionibacterium acnes*, *Pityrosporum ovale*, and *Trichophyton mentagrophytes*. The *n*-hexane extract was divided into the distillate and residue fractions by steam distillation, and subsequent bioassays indicated that the distillate was the active fraction. Recently, we reported volatile flavor components from the essential oil of the cardamom seed (Abo-Khatwa and Kubo, 1987). Hence, we assayed antimicrobial activity against 14 selected microorganisms of the 10 major volatile compounds identified in the cardamom seed.

MATERIALS AND METHODS

Chemicals. The *n*-hexane extract of the *E. cardamomum* seed and the distillate and residue fractions used for the assay were from our previous study (Abo-Khatwa and Kubo, 1987). The authentic 1,8-cineole and α-terpinene were purchased from Sigma Chemical Co. (St. Louis, MO), α-terpinyl acetate and geraniol were obtained from Johnson Matthey (Ward Hill, MA), and linalyl acetate, safrole, eugenol, and methyleugenol were purchased from Aldrich Chemical Co. (Milwaukee, WI). In addition, linalool and limonene were gifts from Takasago International Corp. (Tokyo, Japan). All of them were used for the assay without further purification. *N,N*-Dimethylformamide (DMF) was purchased from EM Science (Gibbstown, NJ).

Microorganisms and Media. All microorganisms used for the antimicrobial assay were purchased from American Type Culture Collection (Rockville, MD). They are *Bacillus subtilis* ATCC 9372, *Brevibacterium ammoniagenes* ATCC 6872, *Staphylococcus aureus* ATCC 12598, *S. mutans* ATCC 25175, *P. acnes* ATCC 11827, *Escherichia coli* ATCC 9637, *Pseudomonas aeruginosa* ATCC 10145, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* ATCC 13315, *Saccharomyces cerevisiae* ATCC 7754, *Candida utilis* ATCC 9226, *P. ovale* ATCC 14521, *T. mentagrophytes* ATCC 18748, and *Penicillium chrysogenum* ATCC 10106.

To reactivate the freeze-dried microorganisms, *B. subtilis*, *S. cerevisiae*, *C utilis*, *P. ovale*, *T. mentagrophytes*, and *P. chrysogenum* were cultured with shaking at 30 °C. *B. ammoniagenes* and *E. aerogenes* were cultured stationarily at 30 °C; all other microorganisms were cultured stationarily at 37 °C.

The culture media for the bacteria consisted of 0.8% nutrient broth (BBL), 0.5% yeast extract (Difco), and 0.1% glucose except for the case of *S. mutans*. For the culture of *S. mutans*, 3.7% brain heart infusion broth (Difco) was utilized. The culture media for the fungi consisted of 2.5% malt extract broth (BBL) except for the case of *P. ovale* and *T. mentagrophytes*. For the culture of *P. ovale*, 1% bactopeptone (Difco), 0.5% yeast extract, 1% glucose, and 0.1% corn oil were used and for *T. mentagrophytes*, 1% bactopeptone and 4% glucose were used.

Antimicrobial Assay. The bioassay was performed by a broth dilution method (Taniguchi and Satomura, 1972). Thus, the test compounds were first dissolved in DMF, and 30 μL of each sample solution was added to 3 mL of the appropriate broth medium. Then, 30 μL of 2-day-old test microorganisms (5-day-old *T. mentagrophytes* and *P. chrysogenum*) was inoculated. The highest concentration used for the assay was 800 μg/mL because of the solubility limitation of the samples in DMF or the water-based media. The concentration of DMF in the broth media was always 1%, which did not affect the growth of any of the test microorganisms employed. The microorganisms were cultured stationarily except *T. mentagrophytes* and *P. chrysogenum*, which were cultured with shaking. After 2 days (5 days for *T. mentagrophytes* and *P. chrysogenum*), the growth of the microorganisms except *P. ovale*, *T. mentagrophytes*, and *P. chrysogenum* was examined as turbidity (OD at 660 nm) and that of the three other fungi was justified with the naked eye. The minimal inhibitory concentration (MIC) was measured by 2-fold serial broth dilution. The lowest concentration of the test compound in which no growth occurred was defined as the MIC.

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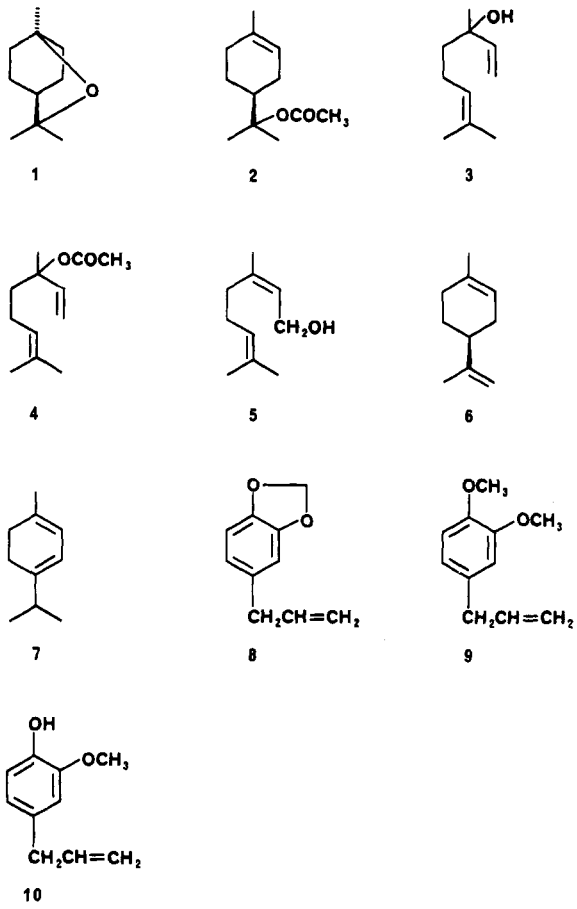

Figure 1.

RESULTS AND DISCUSSION

On the basis of our previous paper (Abo-Khatwa and Kubo, 1987), the 10 most abundant volatile components of the essential oil obtained from the *n*-hexane extract of the cardamom seed by steam distillation, namely 1,8-cineole (**1**), α-terpinyl acetate (**2**), linalool (**3**), linalyl acetate (**4**), geraniol (**5**), limonene (**6**), α-terpinene (**7**), safrole (**8**), methyleugenol (**9**), and eugenol (**10**) (Figure 1) in decreasing concentration, were selected for the assay. The yield of the essential oil in large quantity (12% v/w) from the cardamom seed has been previously reported (Abo-Khatwa and Kubo, 1987). Despite having a relatively large yield, several compounds were unable to be assayed because of the following reasons: (1) myrcene was hardly soluble in DMF so that its necessary test solution was unable to be prepared; (2) sabinene and linalool oxide were available only in small quantity. Interestingly, this essential oil, possessing the characteristic odor of the cardamom seed, consisted of more than 57% oxygenated monoterpenoids and 28% monoterpene acetate but less than 3% aromatic volatiles. Two major components, 1,8-cineole and α-terpinyl acetate, constituted more than 66% of the essential oil (Abo-Khatwa and Kubo, 1987).

The above-mentioned 10 volatile components (**1–10**) were assayed against the 14 selected microorganisms. Throughout this experiment the broth dilution method was used despite the fact that it is time-consuming. This was because these compounds were all volatile and, more importantly, water insoluble. Thus, none of these compounds showed any activity by the paper disk diffusion method, one of the most common antimicrobial assay methods, since these water-insoluble substances might not diffuse into the media. Moreover, these volatiles were partially or even entirely evaporated from the paper disk when solvent was removed.

Table I. Antimicrobial Activity of the Components in the Distillate

	MIC against microorganisms[a] tested, μg/mL									
	1	2	3	4	5	6	7	8	9	10
Bs	>800	800	800	>800	400	800	>800	400	800	400
Ba	>800	100	800	400	400	>800	>800	>800	800	800
Sa	>800	>800	>800	>800	800	>800	>800	>800	800	800
Sm	>800	200	400	400	400	100	100	100	400	400
Pac	800	100	400	400	400	50	50	50	200	50
Pae	>800	>800	>800	>800	>800	>800	>800	>800	>800	>800
Ea	>800	>800	>800	>800	>800	>800	>800	>800	>800	>800
Ec	>800	>800	>800	>800	800	>800	>800	400	800	400
Pv	>800	>800	400	400	200	400	400	100	400	400
Sc	>800	>800	800	>800	400	25	50	200	800	800
Cu	>800	>800	400	>800	400	200	100	200	800	800
Po	>800	800	400	>800	200	200	800	100	100	200
Pc	>800	400	800	800	200	>800	>800	>800	800	200
Tm	>800	400	200	200	200	>800	>800	800	200	200

[a] Bs, *B. subtilis*; Ba, *B. ammoniagenes*; Sa, *S. aureus*; Sm, *S. mutans*; Pac, *P. acnes*; Pae, *P. aeruginosa*; Ea, *E. aerogenes*; Ec, *E. coli*; Pv, *P. vulgaris*; Sc, *S. cerevisiae*; Cu, *C. utilis*; Po, *P. ovale*; Pc, *P. chrysogenum*; Tm, *T. mentagrophytes*.

The antimicrobial activity of the individual compounds (**1–10**) is listed in Table I. Among the Gram-positive bacteria tested, *S. aureus* was the least sensitive bacterium. Thus, only geraniol, methyleugenol, and eugenol showed weak activity with MIC values of 800 μg/mL for each. By contrast, *P. acnes* was the most sensitive bacterium. Thus, all of the compounds tested showed activity against this bacterium with MICs ranging from 50 to 800 μg/mL. Among the compounds tested, limonene, α-terpinene, safrole, and eugenol were the most potent against this bacterium with MIC values of 50 μg/mL each, while 1,8-cineole was the least. *P. acnes* is one of the bacteria responsible for acne. This bacterium produces a lipase that hydrolyzes sebum triglycerides to free fatty acids and these can cause inflammation and comedones (Matsuoka, 1983). Interestingly, the major compound in the essential oil, 1,8-cineole, showed activity against only this bacterium among the microorganisms tested. The volatile components of the essential oil of the cardamom seed may be useful for protection from *P. acnes* infection. This result seems to make sense since cardamom is used as a fragrance in soap, perhaps to control acne.

In the case against *S. mutans*, the major compound, 1,8-cineole did not show any activity up to 800 μg/mL, but all other compounds exhibited some activity with MICs in the range 100–400 μg/mL. Since the yield of essential oil from the cardamom seed is in large quantity, the total activity of the essential oil may be enough to control this bacterium, especially if it is continuously chewed. Although 1,8-cineole did not exhibit activity against *S. mutans*, because of its fresh odor, it is used in large quantities in fragrances as well as in flavors such as in mouth care products (Bauer et al., 1990). The result indicates that, in addition to the use of fragrance and flavor, the volatile components of the cardamom seed seem to possess an additional function, namely "anticavity" activity.

None of the compounds tested exhibited any activity against Gram-negative bacteria, *P. aeruginosa* and *E. aerogenes*, although three aromatic compounds, safrole, methyleugenol, and eugenol, and one monoterpene, geraniol, exhibited some activity against other Gram-negative bacteria tested, *E. coli* and *P. vulgaris*. However, the activity of each substance was weak, with MICs in the range 100–800 μg/mL. Nevertheless, few phytochemicals show activity against Gram-negative bacteria, especially against *Pseudomonas* species.

Table II. Antimicrobial Activity of the Essential Oil of the Cardamon Seed

microorganisms tested	MIC, μg/mL
B. subtilis ATCC 9372	1600
B. ammoniagenes ATCC 6872	1600
S. aureus ATCC 12598	1600
S. mutans ATCC 25175	400
P. acnes ATCC 11827	100
P. aeruginosa ATCC 10145	>1600
E. aerogenes ATCC 13048	>1600
E. coli ATCC 9637	>1600
P. vulgaris ATCC 13315	800
S. cerevisiae ATCC 7754	>1600
C. utilis ATCC 9226	1600
P. ovale ATCC 14521	>1600
P. chrysogenum ATCC 10106	1600
T. mentagrophytes ATCC 18748	800

In the case of fungi, the major volatile, 1,8-cineole, did not show any activity up to 800 μg/mL, but all nine other compounds tested exhibited antifungal activity against the five fungi assayed. Besides 1,8-cineole and linalyl acetate, all other compounds tested exhibited antifungal activity against *P. ovale* with MICs in the range 100–800 μg/mL. This fungus occurs on human skin and causes pityriasis versicolor (tinea versicolor), which is a mild, chronic, superficial human dermatomycosis. Again, besides the function as a fragrance for soap, the volatiles of the cardamom seed seem to have another function, namely "antidandruff" activity. Similarly, the essential oil of the cardamom seed appears to control another dermatomycotic fungus, *T. mentagrophytes*, primarily a parasite of the hair. Thus, use of the cardamom seed as a fragrance for making skin and hair care products seems to make sense.

All of the volatile components used for the assay are common flavor substances, found in many sources, and widely used for fragrances and flavors (Bauer et al., 1990). Furthermore, most of them have also been used as flavor ingredients in foods such as ice cream, candy, and chewing gum (Furia and Bellanca, 1975). For example, 1,8-cineole occurs in many terpene-containing essential oils, sometimes even as the main component such as in eucalyptus oil and laurel leaf oil. In addition to flavor and fragrance, the volatiles of the cardamom seed seem to possess additional functions that have been overlooked. Furthermore, they are all biodegradable and, more importantly, renewable. Needless to say, the efficient use of renewable natural resources is becoming increasingly important worldwide. In addition, 1,8-cineole was recently reported as a skin penetration enhancer of drugs (Williams and Barry, 1991). It may play an additional important role in increasing penetration of the active substances through the skin.

The antimicrobial activity against the same microorganisms of the essential oil of the cardamom seed is listed in Table II. As expected, it exhibited a moderate but broad spectrum.

In conclusion, the essential oil of the cardamom seed and/or each individual substance described herein may be considered constituents for cosmetic products, particularly for use as an antimicrobial agent in addition to a fragrance and flavor. As far as the antimicrobial activity of the purified substances is concerned, it should be noted that, in general, the combination of more than two compounds may be superior than the use of a single compound to avoid the development of resistance mechanisms of microorganisms. Moreover, the activity of each natural substance is usually not potent enough to be considered for practical use, so enhancement and broadening of the total activity by combining two or more compounds are advisable (Kubo and Taniguchi, 1988). However, further study is needed since the rationale for the combination is still in an embryonic stage.

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