Antimicrobial Activity of Aroma Chemicals and Essential Oils[1]

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ABSTRACT

Determination of the minimum inhibitory concentrations (MIC) of 212 common soap fragrance raw materials demonstrated that the paper disc-petri plate technique does not reflect the relative antimicrobial activity of these materials. Commonly used soap bacteriostats were shown to be 100 to 1000 times more effective than the most active fragrance materials. Of 521 fragrance materials initially screened by the petri plate method, 44% were inhibitory against one of the three test organisms, and 15% were effective against all three *(Staphylococcus aureus, Escherichia coli, Candida albicans)*. Of a selected number (212) of these positive materials, subsequently screened against a lipophilic diphtheroid organism *(Corynebacterium* sp.), 64 materials (30%) were positive against all four test organisms. However, only nine materials (4%) had a MIC as low as 50 ppm compared to the common soap bacteriostat TCC®, which had a MIC of 0.08 ppm (vs. *S. aureus)*. In hand-degerming tests, no reduction of bacterial counts was obtained with a soap containing the most active fragrance materials. These results demonstrate that creation of a practical antimicrobial soap fragrance does not appear to be possible.

INTRODUCTION

The antimicrobial activity of aromatic substances has been known for more than fifty years. Macht and Kunkel (1) in 1920 and Dyche-Teague (2) in 1924 described the antimicrobial effects of volatile oils or their vapors. More recently Maruzzella, et al. (3-8), reported rather extensive surveys of the antimicrobial action of many essential oils and perfumery chemicals in both the vapor phase and by direct contact of the liquids. Most of this previous work employed variations of the filter paper disc-petri plate method, similar to that used for evaluating antibiotics. However, a serious disadvantage of this method is that the data are, at best, semiquantitative and often not comparable from one laboratory to another because organisms are exposed to unknown concentrations of test chemicals. Based on these past results, considerable interest has developed concerning the possibility of using fragrance raw materials to create perfumes for soaps and other personal care products which would have bacteriostatic activity in addition to their primary function of providing a pleasant odor. This study was intended to provide a quantitative basis for critical evaluation of this concept. In this paper we report the results of a three-stage study of the bacteriostatic action of fragrance raw materials. First, a large number of materials were screened in a standardized petri plate procedure. Second, materials identified as having significant bacteriostatic activity were tested in liquid cultures to determine their minimum inhibitory concentrations (MIC). Third, a soap fragrance was created from the most active fragrance materials and tested in handwashing panel studies to determine its degerming efficacy. In all phases of this study, known antimicrobial chemicals were included as controls so that direct comparison of the efficacy of the fragrance materials vs. these bacteriostats could be made.

[1]Preliminary report presented at the 1976 national meeting of the American Chemical Society, Miami Beach, FL, September 1978.

MATERIALS AND METHODS

Test Organisms

The following microorganisms were used: *Staphylococcus aureus, Escherichia coli* and *Candida albicans,* obtained from Monmouth Medical Center, Long Branch, New Jersey; a lipophilic diphtheroid, probably a *Corynebacterium* species, obtained from the Department of Dermatology, University of Pennsylvania. These organisms, with the exception of *E. Coli,* are normal inhabitants of human skin (9,10), and two classes of organisms, Gram positive and diphtheroid, have been implicated in the production of body odor (9). Stock cultures were maintained on agar slants composed of tryptone (Difco), 0.3%; yeast extract (Difco), 0.3%; glucose, 0.3%; K_2HPO_4, 0.1%, and agar, 1% (referred to as TGY agar). Slants for the diphtheroid strain were supplemented with 0.5% Tween 80 (ICI America, Inc.).

Test Media

For the petri plate screening work, TGY agar was used and the pH was adjusted to 5.5 with H_3PO_4 to approximate the pH of human skin. The medium was sterilized (15 min, 121 C) and dispensed by a New Brunswick Scientific, Model AS-3, agar sterilizer. The diphtheroid medium was again supplemented with 0.5% Tween 80. For liquid cultures TGY medium was used without the agar, and the pH was not adjusted (pH 7.0) in order to optimize growth of all the test organisms. In this case the diphtheroid medium was supplemented with 0.1% Tween 80.

Fragrance Materials

Fragrance raw materials, including essential oils, absolutes, essences, extracts and synthetic chemicals were obtained from production inventory lots. Samples were prepared as 10% (w/v) solutions in 95% (v/v) ethanol except where solubility limitations occurred. In these cases 5% solutions were prepared or alternative solvents were used (e.g., diethyl phthalate, benzyl alcohol, benzyl benzoate). In all experiments appropriate solvent controls were included. Antimicrobial chemicals included as controls were: TCC® (3,4,4′-trichlorocarbanilide), Monsanto; Irgasan DP 300® (2,4,4′-trichloro-2′-hydroxydiphenyl ether), Ciba-Geigy; and hexachlorophene [2,2′-methylene bis (3,4,6-trichlorophenol)]. These chemicals were also prepared in alcoholic solutions at various concentrations depending on the final concentrations required in the growth media.

Petri Plate-Paper Disc Procedure

The growth medium (TGY agar) was maintained at 42 C during dispensing into plastic petri plates (8.5 cm diameter; Falcon Brand). The medium was seeded at 3.3% (v/v) with 24-hour shake cultures of the appropriate organisms and dispensed at 8 ml per plate. Paper discs (0.95 cm diameter; Schleicher & Schuell) were soaked with 20 µl of the 10% test solutions, and the discs were immediately applied to the center of the solidified seeded agar, one disc per plate. All materials were run in duplicate. It was found that

TABLE I

Composition of Soap Fragrance for Hand-Degerming Test

Ingredient	% in Fragrance	MIC (ppm)
Aldehyde, C-11, undecylenic	0.4	50 *(S. aureus)*[a]
Amyl cinnamyl alcohol	18.0	50 *(S. aureus)*
Anethol	10.0	100 *(C. albicans)*
p-tert-Butyl-m-cresol	0.5	50 *(S. aureus)*
Citronellol coeur	18.0	100 *(C. albicans)*
Cuminyl acetate	5.0	100 *(C. albicans)*
Isobutyl quinoline	0.1	50 *(C. albicans)*
Isocitral	0.5	100 *(Diphtheroid)*
Lemma (Schiff base)	5.0	100 *(S. aureus)*
Methyl isoeugenol	5.0	100 *(Diphtheroid)*
Methyl lavender ketone	7.0	100 *(S. aureus)*
Mousse Abs. Ess.	4.0	100 *(S. aureus)*
Musk ketone	5.0	100 *(Diphtheroid)*
Musk xylol	5.0	50 *(Diphtheroid)*
Ocmea (Schiff base)	8.0	50 *(S. aureus)*
Patchouli oil, dark	3.0	100 *(S. aureus)*
Rosalva	2.0	100 *(C. albicans)*
Sandalwood	0.5	50 *(S. aureus)*
Veramoss	3.0	100 *(Diphtheroid)*

[a]Indicates organism for which the MIC was determined. In some cases this MIC applies to more than one organism.

20 µl was uniformly absorbed by the discs without excessive wetting. Total material on the disc was 2 mg for most samples. For those materials available in less than neat form, correspondingly less material was placed on the disc (e.g., a 50% absolute actually had only 1 mg on the disc, the remainder being the solvent used for that material). Control discs with 20 µl of 95% ethanol had no effect on growth of any of the organisms. Plates were incubated in an upright position at 37 C for 18 to 24 hr, during which time an adequate microbial lawn developed.

Liquid Culture Procedure

Minimum inhibitory concentrations were determined in TGY broth cultures (18 x 150 mm tubes; 10.0 ml/tube). Selected chemicals were tested initially at 0.1, 1.0 and 10 ppm to determine the approximate range of activity. When no positive materials were found, these were repeated at 10, 100, and 1000 ppm. Materials selected by the petri plate screen were then tested at 100, 500, and 1000 ppm. Those found positive at 100 ppm were repeated at 10, 50 and 100 ppm. All samples were run induplicate and that concentration at which no growth occurred in either tube was taken as the minimum inhibitory concentration. The effect being measured was bacteriostasis, since no attempt was made to subsequently plate out the inhibited cultures to determine if the inoculum had been killed.

Test materials were added to the tubes as 10% (w/v) solutions. In contrast to the petri plate screen, solutions of those materials available in less than neat form were adjusted accordingly so that the final solutions contained 10% of the test materials. Some materials incompletely soluble at 10% were prepared at 5% and double aliquots were added to the culture tubes. Control antimicrobials were tested in the range 0.02 to 200 ppm depending on the chemical and the organism. For example, TCC® was tested at 0.02 to 0.10 ppm against *S. aureus*, but up to 200 ppm against *E. coli*. Approximate effective ranges for the controls had been determined in preliminary experiments. All concentrations of ethanol added with test materials or controls were tested alone to correct for any solvent effects.

Tubes were inoculated with 50 µl of a 1:10 dilution in sterile 0.85% saline of a 24-hour shake culture (TGY broth). A minimum of 300,000 viable organisms were added to each tube. Tubes were mixed on a Vortex mixer and incubated at 37 C for 18 to 24 hr, sufficient time to obtain significant turbidity. Because poor growth was obtained with the diphtheroid in culture tubes, this organism was tested in 50 ml Erlenmeyer flask cultures (10 ml/flask) incubated at 37 C on a rotary shaker at 250 rpm.

TABLE II

Antimicrobial Activity of Fragrance Materials in Petri Plate Cultures

Organism	Number tested	Number positive	% Positive
S. aureus (Gram +)	521	162	31.1
E. coli (Gram -)	521	136	26.1
C. albicans (Yeast)	521	149	28.6
Diphtheroid	212	101	47.6
All four organisms	212[a]	64	30.2
Three organisms	212	89	42.0
Two organisms	212	118	55.7
One organism	212	144	67.9
Negative	212	68	32.1

[a]Only those materials tested against all four organisms are included in this total.

Handwashing Panels

The degerming efficacy of a bar soap containing 2% of a fragrance created from raw materials found to have antimicrobial activity in vitro (Table I) was tested in a modified Cade procedure (11). Comparison was made with a control soap containing 2% TCC®. The standard Cade procedure was shortened to include only a three-day "conditioning" period prior to use of the test soaps instead of the usual seven to ten day period. A standard "super-fatted" soap was used as the base soap in these panels. Base and test counts were taken by the "fifth basin" technique described by Kooistra, et al. (12). Aliquots and serial dilutions of the fifth basin were plated in TGY agar containing 1% Tween 80. Panels consisted of ten people, with equal numbers of males and females, selected from research personnel.

RESULTS AND DISCUSSION

Petri Plate Screen

A total of 521 fragrance raw materials were tested in this preliminary screen. Initially, chemicals were tested against *S. aureus, E. coli,* and *C. albicans*. Subsequently, 212 materials were retested against a diphtheroid organism isolated from human skin, since this group of organisms has been implicated in production of body odor (9). Summaries of the petri plate screen are presented in Table II. These data represent only qualitative comparison showing that the Gram positive *S. aureus* was only slightly more sensitive to these fragrance materials than the other types of organisms (Table II). Approximately 30% of the materials were effective against any one of the first three organisms. The proportion of positives for the diphtheroid (48%) reflects the biased selection process for the chemicals tested against this organism. These materials were selected from those found to be positive against one or more of the first three organisms, so that a higher percentage of "hits" was to be expected. From the second part of Table II, which includes data only from the restricted list of materials (diphtheroid test group) we can see that ca. 30% were positive for all four organisms, and 68% were positive for at least one of the four test organisms. Again, these data are based on a list of materials "weighted" toward positives. If we consider the total list of materials (521) in the petri plate screen and the original three organisms *(S. aureus, E. coli, C. albicans)*, we find that only 15% were effective against all three of these organisms, and only 44% were positive for at least one

TABLE III

Fragrance Materials with Antimicrobial Activity

Chemical[a]	*Staph. aureus*		*E. coli*		*C. albicans*		Diphtheroid	
	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm
Acetanilide	0[b]	NT[c]	13	NT	11	NT	NT	NT
N-Acetyl methyl anthranilate	0	>1000	0	>1000	0	>1000	0	>1000
Aldehyde, C-8	0	500	12	500	12	500	12[pd]	1000
Aldehyde, C-9	0	NT	0	NT	13	NT	NT	NT
Aldehyde, C-11 Undecylenic	0	50	0	>1000	22	50	17	500
Aldehyde, C-16	0	NT	15	NT	0	NT	NT	NT
Aldehyde, C-18	0	>1000	15	1000	20	500	15	1000
Allyl amyl glycolate	0	NT	0	NT	11P	NT	NT	NT
Amaryllide	14	>1000	13	1000	19	1000	17	1000
Ambrarome Absolute	13	NT	0	NT	0	NT	NT	NT
Amyl cinnamic aldehyde coeur	0	>1000	0	>1000	0	>1000	0	500
Amyl cinnamyl alcohol	0	50	0	>1000	0	500	12	500
Amyl salicylate	0	>1000	0	>1000	0	>1000	0	500
Amyris Oil	13	NT	0	NT	0	NT	NT	NT
Anethol, USP	0	500	0	500	0	100	0	500
Anisyl acetate	0	>1000	13	>1000	12P	1000	0	1000
Armoise Essence	0	1000	0	>1000	0	1000	0	>1000
Arras aldehyde, 50%	20	500	14	1000	17	500	26	500
Aubepine	0	>1000	18	>1000	11	1000	0	1000
Auralva (Schiff base)	11	NT	12	NT	12	NT	NT	NT
Balsam Copaiba, USP	0	>1000	0	>1000	0	>1000	0	>1000
Balsam Peru Oil	18	>1000	15	>1000	11	>1000	14	500
Basil Oil	0	500	0	500	0	500	0	1000
Bay Oil	18	500	13	1000	20	500	14	1000
Benzaldehyde	0	1000	0	1000	0	1000	0	>1000
Benzoic Acid	21	1000	28	1000	21	1000	28	1000
Benzoin coeur	18	>1000	16	1000	10	>1000	12	1000
Benzophenone	0	NT	0	NT	12	NP	NT	NT
Benzyl acetate	0	>1000	0	>1000	0	1000	0	1000
Benzyl alcohol	0	>1000	12P	>1000	0	>1000	0	500
Benzyl benzoate	0	>1000	0	>1000	0	>1000	0	500
Benzyl propionate	0	>1000	0	1000	0	500	0	1000
Benzyl salicylate	0	>1000	0	>1000	0	>1000	0	1000
Bergamot MPF	0	1000	0	>1000	0	500	0	1000
Beta gamma hexenyl formate	13	NT	17	NT	0	NT	NT	NT
Beta naphthyl anthranilate	16	1000	20	1000	22	500	15	1000
Beta pinene coeur	0	500	0	>1000	0	1000	0	>1000
Birch Tar rectified	14	500	14	>1000	17	1000	14	500
Boise de Rose filtered	0	>1000	17	1000	0	500	0	1000
Borneol	0	1000	0	1000	0	500	12	500
Camphene 46	0	1000	0	>1000	0	1000	0	>1000
Camphor Oil White	12	500	*[e]	>1000	0	500	12	>1000
Caraway Oil	0	500	0	>1000	0	500	0	500
Cardamom Oil, Guatemala	0	>1000	0	>1000	0	500	0	500
Carvone, dextro	10	1000	11	1000	0	500	0	1000
Carvone, laevo	0	>1000	11	1000	11	500	0	1000
Cashmeran	0	500	0	>1000	0	>1000	14	500
Castoreum Abs. 50%	11	NT	0	NT	0	NT	NT	NT
Cedarleaf Oil	0	1000	0	1000	0	500	0	>1000
Cedarwood, White	0	1000	0	>1000	0	>1000	0	500
Cedrone S	12	NT	0	NT	0	NT	NT	NT
Cedrus Atlantica coeur	11	500	0	>1000	0	>1000	11	1000
Celery seed oil	0	NT	0	NT	12	NT	NT	NT
Chamomile oil	0	1000	0	>1000	0	500	0	>1000
Cinnamalva	0	>1000	19	1000	13	500	0	1000
Cinnamic Alcohol	14	>1000	19	>1000	27	500	16	1000
Cinnamon leaf oil, Ceylon	18	500	17	1000	14	500	12	500
Ciste, colorless	12	NT	0	NT	0	NT	NT	NT
Citral, dimethyl acetal	0	500	0	>1000	33	500	14	500
Citral, refined	15	500	14	500	46	500	16	500
Citronama (Schiff base)	0	NT	0	NT	11	NT	NT	NT
Citronella, Formosa, Java	11	NT	0	NT	17	NT	NT	NT
Citronellal	0	500	0	>1000	12	500	0	500
Citronellol coeur	12	1000	10	1000	48	100	18	500
Citronellyl acetate	0	>1000	0	>1000	0	>1000	0	500
Citronellyl ethyl ether	11	NT	0	NT	0	NT	NT	NT
Citronellyl isobutyrate	0	>1000	0	>1000	0	>1000	0	>1000
Citrus oil, distilled	0	1000	0	>1000	0	500	0	>1000
Clove leaf oil	14	500	19	1000	19	500	15	500
Clove bud oil	16	500	16	1000	18	500	14	500
Cocal	15	500	14	>1000	11	1000	15	500
Coniferan	0	1000	0	>1000	0	>1000	0	500
Coriander oil	0	1000	11	1000	0	500	0	1000
Corn mint oil	10	NT	0	NT	0	NT	NT	NT
Coronal, beta	12	NT	0	NT	0	NT	NT	NT
Cortex Aldehyde, 50%	17	1000	21	500	16	>1000	16	500
Coumarin	13	>1000	15	>1000	16	1000	12P	1000
Cumin oil	0	500	0	1000	0	500	0	1000
Cuminyl alcohol	15P	1000	14	500	23	500	16	1000

TABLE III (contd.)

Fragrance Materials with Antimicrobial Activity

Chemical[a]	*Staph. aureus*		*E. coli*		*C. albicans*		Diphtheroid	
	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm
Cuminyl acetate	0	>1000	*	>1000	0	100	0	500
Cyclamal extra	0	NT	0	NT	23P	NT	NT	NT
2-Cyclohexyl cyclohexanone	0	NT	0	NT	10	NT	NT	NT
Cyclosia base	14	>1000	16	>1000	14	>1000	13	1000
Cymene coeur	0	1000	0	>1000	0	500	0	>1000
Cypress oil, French	0	1000	0	>1000	0	1000	0	>1000
Decalactone	15	>1000	0	>1000	12	1000	14	1000
n-Decanol	20	NT	0	NT	13	NT	NT	NT
Dibenzyl ether	0	>1000	0	>1000	0	>1000	0	500
Dibutyl sulfide, 10%	0	NT	16P	NT	0	NT	NT	NT
Diethyl phthalate	0	>1000	0	>1000	14P	500	12P	1000
Dihydro cuminyl alcohol	14	1000	16	500	23	500	19	500
Dimethyl anthranilate	0	1000	0	1000	14P	500	0	500
Dimethyl benzyl carbinol	0	>1000	16P	>1000	10	1000	0	>1000
Dimethyl octanol	0	NT	0	NT	12	NT	NT	NT
Dimethyl phenyl acetaldehyde	14	NT	0	NT	0	NT	NT	NT
Dimethyl phenyl ethyl carbinol	0	>1000	14	1000	21	1000	15	1000
Dimethyl phthalate	0	NT	11	NT	12	NT	NT	NT
Dimethyl sulfide	0	1000	0	>1000	0	500	0	1000
Diphenyl oxide	0	>1000	0	>1000	0	500	0	500
Dipropylene glycol	0	>1000	0	>1000	0	>1000	0	>1000
p-Ethyl acetophenone	0	NT	0	NT	13P	NT	0	NT
Ethyl benzaldehyde	0	500	11	500	11	500	0	500
Ethyl benzoate	0	1000	0	1000	0	500	0	1000
Ethyl-3-hydroxy-3-phenyl propionate	0	NT	13	NT	12	NT	NT	NT
Ethyl linalool	18	500	14P	1000	11	500	20	500
Ethyl methacrylate	0	>1000	0	>1000	0	>1000	0	>1000
Ethyl phenyl glycidate	0	NT	0	NT	11	NT	NT	NT
Ethyl vanillin	14	>1000	18	1000	19	1000	15	1000
Eucalyptus oil, 70-75%	0	>1000	0	>1000	0	1000	0	>1000
Eugenol, USP	16	500	21	500	22	500	15	500
Fennel oil, Sweet	0	500	0	500	0	500	0	500
Fir balsam, Abs.	12	500	15P	1000	0	>1000	0	1000
Fraistone	0	NT	0	NT	26P	NT	NT	NT
Furfural	12	>1000	11	>1000	0	1000	0	>1000
Galaxolide	0	1000	0	>1000	0	>1000	0	500
Galbanum coeur	0	1000	0	>1000	0	>1000	0	500
Geraniol coeur	14	1000	16	500	38	500	20	500
Geraniolene, light	13	NT	0	NT	11P	NT	NT	NT
Geranium, African	12	NT	0	NT	19	NT	NT	NT
Geranoxy acetaldehyde, 50%	0	NT	0	NT	13	NT	NT	NT
Geranyl benzoate	0	>1000	0	>1000	0	>1000	0	>1000
Geranyl methyl tiglate	0	NT	0	NT	14	NT	NT	NT
Geranyl propionate	0	>1000	0	>1000	0	>1000	0	500
Grapefruit oil	0	500	0	>1000	0	500	0	>1000
Guaiene	0	500	0	>1000	0	>1000	12	500
Guaicwood oil	13	500	0	>1000	0	>1000	0	500
Hay Abs.	12	NT	0	NT	0	NT	NT	NT
Hedione	0	>1000	0	>1000	0	1000	12	500
Helional	0	500	14	1000	14	500	16	500
Heliotropyl acetate	0	NT	13P	NT	12P	NT	NT	NT
n-Hexanol	0	NT	11	NT	0	NT	NT	NT
Hexyl cinnamic aldehyde	0	>1000	0	>1000	0	>1000	0	500
Hydratropal acetone	18	NT	0	NT	21	NT	NT	NT
Hydratropic alcohol, white	12	>1000	14	1000	13	1000	11	>1000
Hydroxy citronellal dimethyl acetal	0	NT	0	NT	11	NT	NT	NT
Hydroxy citronellal	20	>1000	16	>1000	13	1000	14	>1000
Hyssop oil	11	NT	0	NT	0	NT	NT	NT
Indisan	12	500	0	>1000	0	>1000	10	500
Indole	18	1000	23	500	17	500	22	500
Iralia	11	NT	0	NT	0	NT	NT	NT
Iritone	10	NT	0	NT	0	NT	NT	NT
Isoamyl pentenoate	12	NT	0	NT	0	NT	NT	NT
Iso beta gamma hexenyl acetate	18	>1000	18	1000	12	1000	12	>1000
Isoborneol	0	>1000	0	500	0	500	0	1000
Isobutyl benzyl carbinol	11	500	12	1000	15	500	14	500
Isobutyl cinnamate	11	NT	0	NT	0	NT	NT	NT
Isobutyl furyl propionate	0	NT	0	NT	10	NT	NT	NT
Isobutyl quinoline	29	100	13	>1000	26P	50	17	100
Isocitral	19	500	11	1000	20	500	18	100
Isoeugenol	23	500	18	500	14	500	14	500
Isoeugenyl benzoate	0	>1000	0	>1000	0	>1000	0	>1000
Isojasmone	12	NT	0	NT	12	NT	NT	NT
Isomuguet aldehyde, 50%	25	NT	0	NT	11	NT	NT	NT
Isopropyl cyclohexyl propanol	15	NT	0	NT	18	NT	NT	NT
Isopropyl quinoline	29	500	13	500	16	100	20	500
Isopulegol M Extra	10	1000	12	1000	0	1000	0	1000
Jasmonate	0	NT	0	NT	11	NT	NT	NT

TABLE III (contd.)

Fragrance Materials with Antimicrobial Activity

Chemical[a]	*Staph. aureus*		*E. coli*		*C. albicans*		Diphtheroid	
	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm
Jasmone, cis	19	1000	13	1000	21	500	15	500
Jasmutone	11	500	12	>1000	13	500	14	500
Labdanax	0	NT	13	NT	12	NT	NT	NT
Labdanol	11	NT	0	NT	0	NT	NT	NT
Labdanum resin, Abs.	14	500	0	>1000	0	>1000	12	1000
Lactone HB	17	1000	12	500	16	500	14	500
Lauryl alcohol	0	1000	0	>1000	0	>1000	10	500
Laurine extra	17	>1000	12	>1000	13	1000	0	1000
Lavandin abrialis	0	NT	12	NT	0	NT	NT	NT
Lavandulol	11	1000	12	1000	13	500	0	500
Lavender Abs., Camilli	0	>1000	0	>1000	0	1000	0	1000
Lemma (Schiff base)	18	100	11	>1000	12	500	12	500
Lemon oil, Cal.	0	500	0	>1000	0	500	0	>1000
Lemongrass	13	500	14	500	15	500	17	500
Lime oil, washed	0	1000	0	>1000	0	500	0	1000
Limonene	0	>1000	0	>1000	0	500	0	>1000
Linalool oxide	0	NT	10	NT	0	NT	NT	NT
Linalool	0	1000	18	1000	0	500	0	500
Linalyl acetate	0	>1000	0	>1000	0	>1000	0	500
Linalyl cinnamate	0	>1000	0	>1000	0	>1000	0	>1000
Lovage oil	14	NT	0	NT	14	NT	NT	NT
LRG No. 182 (Ethoxycyclohexanone)	11	NT	0	NT	0	NT	NT	NT
LRG No. 1181 (Neo-, isomenthones)	13	NT	12	NT	0	NT	NT	NT
Lyral	16	1000	13	>1000	12	1000	14	1000
Mace, whole extract	12	>1000	0	>1000	0	>1000	0	500
Maltol	0	>1000	16	>1000	16	1000	14	>1000
Mandarin oil	0	1000	0	>1000	0	500	0	>1000
Menthol, USP	11	500	11	500	10	500	0	500
Methallyl pentenoate	14	NT	0	NT	0	NT	NT	NT
p-Methoxy hydrotropic aldehyde	18	1000	26	1000	15	500	16	500
Methyl anthranilate	12	>1000	14	>1000	16	1000	0	1000
p-Methoxy phenoxy acetaldehyde	18	1000	20	>1000	16	1000	19	500
Methyl benzoate	0	>1000	0	>1000	0	1000	0	1000
p-Methoxy phenoxy acetaldehyde dimethyl acetal	0	>1000	0	>1000	11	1000	0	1000
Methyl cinnamate	11	1000	12	>1000	13	500	0	500
α-Methyl cinnamic aldehyde	20	1000	17	500	18	500	0	500
Methyl cyclocitrone	10	NT	0	NT	0	NT	NT	NT
Methyl eugenol	12	1000	11	1000	16	500	12	500
Methyl heptenol	12	NT	13	NT	0	NT	NT	NT
Methyl hexyl acetaldehyde	34	>1000	22	>1000	15	1000	16	500
Methyl isoeugenol	0	>1000	0	>1000	0	>1000	0	100
Methyl lavender ketone	13	100	13	1000	18	500	15	500
Methyl β-naphthyl ketone	0	NT	0	NT	15	NT	NT	NT
Methyl octin carbonate	0	>1000	0	>1000	0	500	0	500
Methyl octyl acetaldehyde	18	NT	0	NT	0	NT	NT	NT
2-Methyl-2-pentenoic acid	19P	1000	35	1000	18	1000	25	1000
Methyl-p-cresol	0	>1000	0	1000	0	500	0	>1000
Methyl phenyl ethyl alcohol	13	>1000	19	>1000	16	1000	0	>1000
Methyl p-toluate	0	1000	0	1000	0	500	0	1000
Miel Blanc, Delaire	11	NT	17P	NT	0	NT	NT	NT
Mousse Abs., Verte Maroc	21	50	15	1000	18	500	18	500
Muguet aldehyde	40	NT	0	NT	14	NT	NT	NT
Muscagene	12	NT	0	NT	0	NT	NT	NT
Musk ambrette	0	>1000	0	>1000	0	>1000	13P	500
Musk ketone	0	>1000	0	>1000	0	>1000	12P	100
Musk xylol	0	>1000	0	>1000	0	>1000	11P	50
Myrac aldehyde	17	NT	0	NT	33	NT	NT	NT
Myrrh coeur	13	1000	0	>1000	0	>1000	12	1000
Myrtenal	13	NT	10	NT	0	NT	NT	NT
Myrtle oil, Charabot	12	NT	11	NT	0	NT	NT	NT
Naame (Schiff base)	12	NT	0	NT	0	NT	NT	NT
Narcisse ketone	0	NT	0	NT	13	NT	NT	NT
Narcitol	14	NT	18	NT	13	NT	NT	NT
Nerol	14	500	13	1000	41	500	12	500
Nerolidol	15	NT	0	NT	0	NT	NT	NT
Neroly blanc	13	NT	10	NT	0	NT	NT	NT
Nortonkalactone	29	>1000	13	>1000	17	>1000	0	>1000
Nutmeg oil	0	500	0	>1000	0	500	0	1000
Oakmoss essence	22	50	0	>1000	12	1000	14	500
Ocimene	22	500	14	1000	13	500	16	500
Ocmea (Schiff base)	13	50	10	500	13	50	13	100
Opoponax oil	10	NT	0	NT	0	NT	NT	NT
Orange oil, Fla.	0	500	20	>1000	0	500	22	500
Orange, terpeneless Abs.	11	NT	0	NT	0	NT	NT	NT
Orange terpenes	12	NT	16	NT	0	NT	NT	NT
Orenyle	14	NT	0	NT	0	NT	NT	NT
Origanum oil, Spanish	33	500	24	500	13	500	16	500
Orivone	11	NT	0	NT	39	NT	NT	NT

TABLE III (contd.)

Fragrance Materials with Antimicrobial Activity

Chemical[a]	*Staph. aureus*		*E. coli*		*C. albicans*		Diphtheroid	
	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm
Oxyphenylon	0	>1000	12	>1000	13	>1000	12	>1000
Para-cresol	20	>1000	29	1000	16	1000	22	1000
Para-cresyl acetate coeur	0	>1000	0	>1000	12P	1000	0	>1000
Para-isopropyl hydratropic aldehyde	13	NT	0	NT	12	NT	NT	NT
Para-methyl benzyl acetate	11	NT	0	NT	0	NT	NT	NT
Para-methyl dimethyl benzyl carbinol	14	>1000	14	1000	13	1000	14	1000
Para-tert butyl cyclohexanone	11	1000	0	>1000	27P	500	0	500
Para-tert butyl-meta-cresol	85	50	26	500	85P	50	22	100
Para-tolyl alcohol	12	>1000	16	>1000	17	>1000	12	>1000
Patchouli oil, dark	12	100	0	>1000	0	>1000	13	500
Patchouli oil, light	0	500	0	>1000	0	>1000	13	500
Peach aldehyde coeur	12	NT	0	NT	14	NT	NT	NT
Pepper oil, black	0	1000	0	>1000	0	>1000	0	>1000
Peppermint	0	NT	0	NT	10	NT	NT	NT
Persicol (γ-undecalactone)	0	NT	0	NT	11	NT	NT	NT
Petinerol	0	1000	12	1000	14	500	0	1000
Petitgrain S.A.	0	>1000	0	>1000	0	500	0	1000
Petitgrain terpenes	14	500	11	>1000	10	500	12	>1000
Phellandrene	18	NT	18	NT	0	NT	NT	NT
Phenoxy ethyl propionate	0	>1000	0	>1000	12	500	0	1000
Phenyl acetaldehyde	21	100	40	1000	33	500	18	100
Phenyl ethyl acetate	0	NT	25P	NT	0	NT	NT	NT
Phenyl ethyl alcohol	0	>1000	16	>1000	0	>1000	0	>1000
Phenyl ethyl cinnamate	0	>1000	0	>1000	0	>1000	0	>1000
Phenyl ethyl phenyl acetate	13	>1000	0	>1000	0	>1000	0	500
Phenyl propyl alcohol	12	>1000	18	>1000	16	1000	14	1000
Phenyl propyl aldehyde	12	500	27	500	14	1000	0	500
Phixia	16	>1000	15	>1000	12	1000	14	1000
Piconia	13	NT	0	NT	0	NT	NT	NT
Pimento berry oil	16	500	17	1000	18	500	14	500
Pine needle oil, Siberian	0	500	0	>1000	0	1000	0	1000
Pine oil	12	1000	14	>1000	11	500	12P	1000
Propylene glycol, USP	0	>1000	0	>1000	0	>1000	0	>1000
Rosacene	14	1000	12	1000	29	500	0	1000
Rosalva	16	1000	0	>1000	16	100	14	500
Rosemary oil, Span. Tunis.	0	1000	0	>1000	0	1000	0	>1000
Rosetone	NT	>1000	0	>1000	0	>1000	0	>1000
Rosin gum	12	NT	0	NT	0	NT	NT	NT
Sandalwood	11	50	0	>1000	*	>1000	11	500
Santalol	13	500	0	>1000	0	>1000	13	500
Sauge sclaree Abs.	12	500	0	>1000	0	>1000	11	500
Sesquiterpenes PC	13	500	12P	>1000	0	>1000	12	500
Spearmint oil	0	1000	0	>1000	0	500	0	1000
Spruce oil	0	500	0	>1000	0	1000	0	1000
St. Guaiol	13	500	0	>1000	0	>1000	12	500
St. John's bread conc. 10%	0	NT	15P	NT	0	NT	NT	NT
Styrax alva essence	21	NT	0	NT	0	NT	NT	NT
Styrax clarified, extra	11	>1000	0	>1000	0	500	11	>1000
Surfleurs Hay	11	NT	0	NT	0	NT	NT	NT
Tabac absolute	10	NT	0	NT	0	NT	NT	NT
Tangerine oil, Fla.	0	500	0	>1000	0	500	0	>1000
Terpineol	12	1000	19	1000	20P	1000	0	1000
Thuja oil	0	500	0	>1000	0	500	0	1000
Thyme, white	25	500	27	500	14	500	16	500
Tiglyl piperidide	0	NT	13	NT	11	NT	NT	NT
Tolu resin abs. 50%	17	>1000	17	>1000	11	1000	13	1000
Tonalid	0	>1000	0	>1000	0	>1000	0	500
Tonka abs.	11P	>1000	14	>1000	14P	>1000	0	>1000
Trans-decahydro beta naphthol	15	1000	15	1000	22P	500	16	500
Trans-3-pentenyl acetone	0	NT	18	NT	0	NT	NT	NT
Treemoss abs., French, 50%	18	100	0	>1000	0	1000	14	500
Trimethyl cyclohexanol	0	NT	11	NT	0	NT	NT	NT
Trimethyl cyclohexenone	0	>1000	12	1000	10	1000	0	>1000
Trimethyl cyclohexenol	15	NT	13	NT	0	NT	NT	NT
Undecylenic acid	21	NT	0	NT	13	NT	NT	NT
Vanillin	16	>1000	22	>1000	19	1000	12P	>1000
Veltol plus	0	>1000	20	>1000	12	>1000	0	>1000
Veramoss	0	500	0	>1000	12	500	12	100
Verdural extra	13	NT	13	NT	0	NT	NT	NT
Violettone A, colorless	16	NT	12	NT	0	NT	NT	NT
Wintergreen oil	0	>1000	*	>1000	0	500	0	1000
Yaracetal	0	NT	13	NT	0	NT	NT	NT
Ylang concrete	0	>1000	0	>1000	0	1000	0	1000
Zingerone	11	>1000	12	>1000	11	>1000	0	>1000
CONTROLS								
Hexachlorophene	13	0.10	12	50	0	50	0	100

TABLE III (contd.)

Fragrance Materials with Antimicrobial Activity

Chemical[a]	*Staph. aureus*		*E. coli*		*C. albicans*		Diphtheroid	
	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm	Zone diam. mm	MIC ppm
Trichlorocarbanilide (TCC®)	11	0.08	0	>200	0	100	0	50
Trichlorohydroxy diphenyl ether (Irgasan DP300®)	25	0.04	22	0.06	12	6.0	0	50

[a]The majority of materials in this table are identified by trivial or trade names commonly used in the fragrance industry. Chemical names for all materials (where applicable) can be obtained from fragrance handbooks.

[b]O indicates no inhibition of growth was detected.

[c]NT indicates chemical was not tested against this organism.

[d]P indicates the zone of inhibition around the paper disc was only partially cleared.

[e]* Indicates a general reduction of growth was evident, but no measureable zone of inhibition was observed.

organism.

While these petri plate data are poor indicators of the relative antimicrobial activity of the test materials, the method is a practical way of screening large numbers of materials. The zones of inhibition ranged from 10 mm diameter (just barely larger than the paper disc) to a clearing of the entire plate (85 mm), but these zone sizes do not accurately reflect relative antimicrobial effectiveness. For example, TCC® produced a cleared zone of 11 mm against *S. aureus* but was subsequently shown to be much more bacteriostatic than any of the fragrance materials. The size of the cleared zone in this method is dependent on the solubility and rate of diffusion of the sample in the aqueous medium. The very low solubility of TCC® is responsible for its apparently poor bacteriostatic activity in this type of assay. Some materials produced no definite cleared zones, but obvious reduction of growth over the entire plate compared to control plates indicated either rapid diffusion of the material or an antimicrobial effect of the vapor. An attempt to minimize vapor effects and evaporative loss of volatile materials by using a double agar layer technique proved to be unworkable for this large number of samples.

Minimum Inhibitory Concentration

The petri plate screen identified 309 materials with significant antimicrobial activity against at least one of the test organisms. Because of the large numbers of tubes involved in determining a minimum inhibitory concentration (minimum 24 tubes per sample in our procedure), the number of materials to be tested was reduced to 212. This list of materials (the same materials as those tested against the diphtheroid organism in the petri plate assay) included those that showed relatively strong antimicrobial activity in the petri plate screen or were suspected of having significant antimicrobial activity because of structural considerations (e.g., phenolics).

The original range of concentrations to be tested (0.1 to 10 ppm) was selected to be 100-fold higher than the approximate MIC for TCC® (0.1 ppm). When no positives were found among 40 of the antimicrobial fragrance materials producing large zones of inhibition, even at 100 ppm, further preliminary experiments were conducted on 12 selected materials to determine the appropriate range of concentrations. Since most of the materials tested were insoluble or very poorly soluble above 1000 ppm (0.1%), this was selected as the maximum concentration to be tested even though many of the test materials were ineffective at this concentration. The final levels tested were 100, 500 and 1000 ppm, with 10 and 50 ppm tested for all those found positive at 100 ppm. This broad range was necessary to encompass the wide variety of materials tested.

Petri plate and MIC results are summarized in Table III which includes all materials found to be positive against at least one organism in the petri plate assay. For completeness, all other materials tested, but for which no antimicrobial activity was found, are listed in Table IV. Twenty-three materials were found to be effective at 50 or 100 ppm (none at 10 ppm) against at least one organism. These include a wide variety of structural types: phenolics, terpenoids, heterocyclics, esters, alcohols, etc. Five of these materials are essential oils or absolutes including two of the oakmoss type which are complex mixtures of phenolics, depsides, resinoids, and other compounds. *E. coli* (Gram negative) was least senstivie in the liquid cultures, with *C. albicans*(yeast) and the diphtheroid being somewhat more sensitive than *S. aureus* (Gram positive). This is somewhat surprising since previous work had indicated that the Gram positive organisms are usually more sensitive than other types to bacteriostatic action of fragrance materials (3,5). This result is also in contrast to the findings of our own petri plate screen in which *S. aureus* was most sensitive (Table II). This again emphasizes that qualitative petri plate screening methods and quantitative minimum inhibitory concentration methods are not necessarily comparable. The results for antimicrobial activity against the diphtheroid organism may have been affected by the inclusion of Tween 80 in the growth medium. This surfactant may have increased the solubility of some of the fragrance materials or aided in their penetration of the bacterial cells walls and membranes. This question can only be answered by retesting the other organisms in the presence of Tween 80.

The results for all three antimicrobial compounds (TCC®, Irgasan DP 300® and hexachlorophene) are in close agreement with accepted industry values ($\leq$0.1 ppm) against *S. aureus*. However, *E. coli, C. albicans* and the diphtheroid were all somewhat resistant to these antimicrobials with the exception of Irgasan DP 300® against *E. coli*. No MIC for TCC® vs *E. coli* was obtained. At the highest level tested (200 ppm), growth still occurred, and testing of higher concentrations was not attempted since the practical use limit of TCC® had already been far exceeded. The apparent resistance of the diphtheroid may have been due to a neutralizing effect of the Tween 80 in the growth medium. Use of Tween 80-containing-medium for the other test organisms increased the apparent MIC of these antimicrobials in each case. No correlation between type of organisms or chemical structure of test materials and bacteriostatic activity was evident from these data. Some materials were effective at relatively low concentration against one organism and negative against one or more of the other organisms (e.g., amyl cinnamyl alcohol, 50 ppm for *S. aureus;* 1000 ppm for *E. coli*). Furthermore, chemical with related structures were not always equally effective against the same organisms (e.g., amyl cinnamyl alcohol, 50 ppm for *S. aureus;* amyl cinnamic aldehyde, 1000 ppm for the same organism). Several compounds

TABLE IV

Fragrance Materials Showing No Antimicrobial Activity in Petri Plate Screen[a]

Abalyn
Abitol
Acetate, C-9
Acetophenone
Agrumea (Schiff base)
Alcohol, C-12
Aldehyde, C-10
Aldehyde, C-12, Lauric
Allyl caproate
Allyl cyclohexyl propionate
Allyl ionone
Ambrain ex gum labdanum
Amyl acetate
Amyl vinyl carbinyl acetate
Amyl vinyl carbinol
Aprol 100
Astratone
Badiane Oil, Fringhian
Benzyl isoeugenol
Benzyl phenyl acetate
Bergamal
Besabolene
Beta gamma Hexenyl acetate
Beta gamma Hexenol
Borneol
n-Butyl pentenoate
Butyl benzoate
Butyl methacrylate
Butyl undecylenate
Cabreuva oil
Carbitol
Carrot oil
Caryophyllene
Caryophyllene acetate
Cassie Essence Abs.
Castor Oil
Cedrenyl acetate
Cedramber
Celestolide
Citralva
Citrindol
Citroflex No. 2
Citron, CI Chauvet
Citronellyl crotonate
Citronellyl formate
Citronellyl propionate
Citroviol
Civet, Artificial
Cognac oil
Copaiba oil
Cubeb oil
Cyclacet
Cycloctal
Cyclohexyl ethyl acetate
Cyclotene
Cyclotropal
4-Damascol
Decanyl acetate
Dimethyl malonate
Dihydro floralate
Dihydro cyclacet
Dihydro pseudo ionone
Dihydro terpinyl acetate
Diisobutyl ketone
Dimethyl benzyl carbinyl acetate
Dimethyl benzyl carbinyl butyrate
Dimethyl octanyl acetate
Dimetol
Dimyrcetol
Dipentene
Dodecalide
Dodecyl nitrile
Elemi oil
Estragon oil
Ethyl acetate
Ethyl acetoacetate
Ethyl amyl ketone
Ethyl pentenoate
Ethyl butanol
Ethyl butyl ketone
Ethyl butyrate
Ethyl geranate
Ethyl isovalerate
Farnesol
Farnesyl acetate
Fleuramone
Floralozone
Flouve oil
Fructone
Galbanol
Gamma terpinene coeur
Gelsone
Geranyl acetate
Geranyl acetone
Geranyl phenyl acetate
Geranyl tiglate
Glycolierral
Grisalva
Grisavan
Hay oil
Hay oil, High Alps
Helycrisum oil
Herbac
Hercolyn D
Hexylene glycol
cis-3-Hexenyl salicylate
Hexyl pentenoate
n-Hexyl isopentenoate
Hexyl methacrylate
Hexyl salicylate
Hyacinth body
Hydratropic aldehyde, dimethyl acetal
Indolene
Isoborneol
Isobornyl acetate
Isobutyl isobutyrate
Isobutyl pentenoate
Isobutyl phenyl acetate
Isobutyl salicylate
Isohexyl pentenoate
Isolongifolene
Isomenthone
Isopropyl myristate
Isopropyl palmitate
Jasmal
Jessemal
Labdalva
Lavandulyl acetate
Leaf acetal
Lemon terpenes
Lilial
Linalyl benzoate
Linalyl propionate
Linseed oil, abs.
Lolitol
Longifolene
Lyrame (Schiff base)
Maraniol
Marjolaine Essence
Mate Abs.
Melonal
Mentha citrata
Menthanyl acetate
Menthone
Menthyl pentenoate
Methyl acetophenone
Methyl chavicol
Methyl diphenyl ether
Methyl heptenone
Methyl-n-hexyl ether
Methyl hexyl ketone
Methyl ionantheme
Methyl ionone, gamma
Methyl isohexyl carbinyl acetate
Methyl nonyl acetaldehyde
3-Methyl pentanol
Moskene
Mugyl acetone
Musk 36A
Myrcenyl acetate
Neoindisan
Nerolin
Neryl acetate
Octea cymbarum
Oenanthic ether
Olibanol
Olibanum Olearome
Orange, bitter
Para cresyl caprylate
Para cresyl isobutyrate
Parsley seed oil
Pennyroyal
Phenyl acetaldehyde, dimethyl acetal
Phenyl ethyl chloride
Phenyl ethyl isobutyrate
Phenyl ethyl salicylate
Picol formate
Pinocarvyl acetate
Proflora
Pseudo linalyl acetate coeur
Raldeine Omega
Reseda body
Rhodinol
Rhodinol residue
Rhodinyl formate
Rose oxide
Shimus Oil
Sinpine
Styralyl acetate
Talia
Terpinolene
Terpinyl acetate
Tetrahydro linalool
Tetrahydro muguol
Tolpine
Triethylene glycol
Trimethyl nonanone
Trimethyl undecyl aldehyde
Trimofix R
Triplal
Turfurol acetate
Turpentine
Vanilla concentrate (20%)
Vanitrope
Vanoris
Verdox coeur
Vertenex
Vertofix coeur
Vetiveryl acetate
Vionex acetate
Wormwood Abs., terpeneless
Ylang concrete

[a]The majority of materials in this table are identified by trivial or trade names commonly used in the fragrance industry. Chemical names for all materials (where applicable) can be obtained from fragrance handbooks.

were inadvertantly tested more than once because of reliance on trade names in selection of test materials. For example, hydroxycitronellal was also tested under the names cyclosia base, laurine, and phixia. Results were comparable for all four materials (see Table III), emphasizing the reproducibility of these methods.

Using the data accumulated in vitro, those materials identified as having the strongest antimicrobial activity were tested in hand-degerming experiments. The two soaps tested as described in METHODS contained, respectively, 2% (w/w) of TCC® (control) or 2% (w/w) of a fragrance whose composition is given in Table I. This fragrance was created to maximize antimicrobial efficacy within the limits of a reasonably pleasant soap aroma. No skin-degerming was achieved with the test soap (Table V), whereas the control soap (TCC®) showed significant reduction of bacterial counts. The failure to achieve the usual count reduction with TCC®, i.e. 90-99%, was probably due to the somewhat shortened test period in this modified Cade procedure. Considerable individual variation was encountered, due, in part, to insufficient "conditioning" with a blank soap to allow skin flora to "normalize" prior to the start of the actual test period. Nevertheless, the reduction observed with the control soap (TCC®) was found to be statistically significant at a 99.5% confidence level, indicating that this modified procedure would have shown degerming if it had occurred with the fragranced soap.

CONCLUSIONS

The purpose of this study was to determine if fragrance raw materials could be demonstrated to have antimicrobial activity comparable to well known bacteriostatic agents. It is apparent from the data presented here that in terms of bacteriostasis, the best fragrance material is 100 to 1000 times less effective than common soap antimicrobials against one of the major types of skin organism. Thus, the creation of a practical fragrance with significant antimicrobial activity appears highly unlikely.

TABLE V

Hand-Degerming Efficacy of Test Fragrance Soap

Soap	Base count[a]	Test count[a]	% Difference[b]
Experimental fragrance	3.03×10^6	3.90×10^6	+ 19.2[c]
TCC® (Control)	2.23×10^6	3.66×10^5	- 72.0[d]

[a]Mean of 10 subjects, 5 male, 5 female.

[b]Mean of % difference for each of ten subjects.

[c]Not statistically significant.

[d]Statistically significant reduction at a 99.5% confidence level.

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