Pharmaceuticals **2004**, *1*, 1-30

Pharmaceuticals

ISSN 1424-8247
www.mdpi.net/pharmaceuticals/

Review

Specific Selection of Essential Oil Compounds for Treatment of Children's Infection Diseases

Alexander Pauli [a,*] and Heinz Schilcher [b]

[a] ReviewScience, 90513 Zirndorf, Fürther Str. 13, Germany
[b] Heinz Schilcher, 81737 München, Alfred Neumann Anger 17, Germany

* Author to whom correspondence should be addressed; e-mail: a.pauli@reviewscience.com

Received: 15 December 2003 / Accepted: 23 January 2004 / Published: 27 January 2004

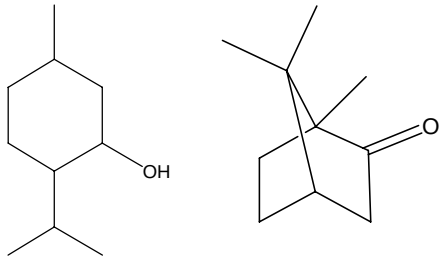
Abstract: Preparations with essential oils and their dosages applied in the therapy of children's infectious diseases are well documented. In contrast, information is only sparingly available about uses of isolated pure essential oil compounds for the treatment of such infections. To find out safe antimicrobials from essential oils, microbiological inhibitory data of children pathogens were combined with oral and dermal acute toxicity data to calculate oral and dermal therapeutical indices (TI). The superiority of antibiotic drugs became obvious following calculating oral TIs of antimicrobials from higher plants, which suggests that oral administrations of essential oil compounds are not suitable to cure severe infections. A few selected compounds from higher plants show moderate effectiveness against gram-positive bacteria, yeast and fungi, but not gram-negative bacteria. Topical application or inhalation of selected compounds for the treatment or additional treatment of mild infections is reasonable.

Keywords: Antibiotics, Antimycotics, Drugs Used in Dermatology, Essential Oils, Therapeutic Index

1. Introduction

Essential oils possess a wide spectrum of pharmacological activities, e.g. anti-inflammatory, hyperemic, spasmolytic, expectorant, diuretic, choleretic, carminative or antiseptic effects [1]. For this reason, essential oils became officinal drugs in many countries (Table 1), which is documented in their respective pharmacopoeias [2].

However, essential oils are not free of side effects and skin, respiratory- and gastrointestinal tract irritations, allergic reactions, phototoxicity, abortive as well as mutagenic and carcinogenic properties may be caused [1]. Even cases of poisoning of children by volatile oils, such as eucalyptus, sassafras, turpentine, wintergreen, chenopodium and citronella oil had occurred [3]. Moreover, care has to be taken with essential oils or preparations containing menthol **1** or camphor **2**.



1 **2**

In infants both compounds may cause respiratory depression or even death (Kratschmer Reflex), when given directly into the nose or close to the nose onto skin [4]. Adverse effects similar to the 'Kratschmer Reflex' may occur also with other compounds from essential oils and can not be predicted from animal experiments.

The use of essential oils for healing purposes is recommended especially in the treatment of catarrhal diseases. Their administration to children presupposes the selection of safe oils and the determination of appropriate doses to avoid side effects. Among officinal essential oils only a few oils come into question. Table 2 shows their appropriate doses that are calculated from adult doses and respect body weight, size and surface area of children [5].

Table 2. Essential Oils Used in the Treatment of Catarrhal Diseases of Children.

Aetherolum	**Common oil name**	**Administration route**	**Age of children in years**			
			0 – 1	**>1 – 4**	**>4 – 10**	**>10 – 16**
Eucalypti	Eucalyptus	Inhalation b	1 – 2 dr	4 – 6 dr	4 – 6 dr	4 – 6 dr
Foeniculi	Fennel	Oral	--------	--------	0,05 – 0,2 ml	0,1 – 0,6 ml
Menthae arvensis	Japanese peppermint	Inhalation c	--------	1 – 3 dr	2 – 4 dr	3 – 6 dr
Menthae piperitae	Peppermint	Inhalation c	1 dr	1 – 2 dr	2 – 3 dr	3 – 4 dr
Piceae	Norway spruce fir, Siberian pine needle	Inhalation a	2 dr/l	2 – 4 dr/l	3 – 4 dr/l	3 – 4 dr/l
Pini	Dwarf or Scots pine needle, Austrian or French turpentine	Inhalation c	1 – 2 dr	2 – 3 dr	3 – 4 dr/l	3 – 4 dr/l
Terebinthinae rect.	Rectified turpentine	Inhalation a	--------	--------	3 – 4 dr/l	3 – 4 dr/l

Notes: a) Inhalation with hot water, b) pure oil is given on a pillow, c) a) or b). Abbreviations used: dr = drops

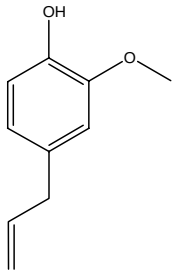
Table 1. Officinal Essential Oils Listed in Various Pharmacopoeias.

Essential Oil	Botanical Origin / Country	Austria	Europe	France	Germany	Italy	Japan	Switzerland	United Kingdom	USA
Anise (Staranise)	Pimpinella anisum L.; Illicium verum Hook		X	X	X	X			X	
Ambrose	Chenopodium ambrosioides L.					X				
Bitter-Orange-Flower	Citrus aurantium L. subspecies aurantium		X	X	X				X	
Camellia	Camellia japonica L.						X			
Caraway	Carum carvi L.	X			X				X	X
Cardamom	Elettaria cardamomum Maton var. minuscula Burkill								X	
Chamomile	Matricaria chamomilla L.	X			X					
Chia	Salvia lavandulifolia Vahl (Salvia hispanica)			X	X					
Cinnamon	Cinnamomum cassia Blume; C. ceylanicum Nees	X			X		X	X	X	
Citronella	Cymbopogon winterianus Jovitt	X						X		
Clove	Syzygium aromaticum Merril et L.M. Perry		X	X	X	X	X		X	
Coriander	Coriandrum sativum L.								X	
Dill	Anethum graveolens L.						X		X	
Eucalyptus	Eucalyptus globulus Labillardiere etc.		X	X	X	X	X		X	
Fennel	Foeniculum vulgare Miller var. vulgare	X			X		X	X		
Juniper	Juniperus communis L.	X						X		
Laurel	Laurus nobilis L.	X								
Lavender	Lavandula angustifolia Miller		X	X	X				X	
Lemon	Citrus limon (L.) Burman filius		X	X	X	X			X	
Mentha	Mentha arvensis L. var. piperascens						X			
Mint dementholized	Holmes ex Christy			X	X				X	
Norway spruce	Picea abies (L.) Karsten; Abies sibirica Ledebour			X	X					
Nutmeg	Myristica fragrans Houttuyn	X		X				X	X	
Orange	Citrus sinensis Osbeck			X		X	X		X	
Peppermint	Mentha piperita L.		X	X	X	X			X	X
Pine needle	Pinus silvestris L.				X					
Pumilio Pine	Pinus mugo Turra var. pumilio Zenari	X						X		
Rose	Rosa gallica L. etc.									X
Rosemary	Rosmarinus officinalis L.	X			X			X		
Sage	Salvia officinalis L.				X			X		
Spearmint	Mentha spicata L.; Mentha cardiaca Bak.			X	X				X	
Tangerine	Citrus reticulata Blanco (Citrus nobilis Andreus)			X		X				
Tea tree	Melaleuca alternifolia Cheel etc.				X					
Thyme	Thymus vulgaris L.		X		X				X	
Turpentine rectified	Pinus palustris Miller; Pinus pinaster Aiton	X			X		X	X	X	

Most essential oils consist of many, in part over 100 individual compounds, which are responsible as individuals or in their natural composition for beneficial and adverse effects of the respective entire

oil. In this paper some effort was done to select safe compounds from essential oils with simultaneously antimicrobial activity against children pathogens and to compare the selected compounds with medicinal antibiotics used for the therapy of children diseases.

Information about treatment of children infections with pure, individual compounds from essential oils is not much available from literature. A very early report described the rectal administration of eugenol **3**, the main constituent of clove oil (*Syzygium aromaticum*), in the treatment of systemic infections of several children. Eugenol decreased body temperature and reduced fever, however, this was not sufficient to prevent death among all treated patients [6].



3

Further, oral, topical and inhalative administration of camphor **2**, the main constituent of rosemary oil (*Rosmarinum officinale*), is recommended in supportive therapy of respiratory tract infection [7].

Because of the limited availability of pharmacological data obtained with living organisms, another selection strategy was necessary as a substitute in the selection of promising antimicrobials. In pharmacology the so-called 'Therapeutic-Index' (TI) is a measure for the effectiveness of pharmacological active drugs [8]. It is deduced from laboratory test results and the animal toxicity of compounds, respectively. The use of the TIs presupposes a collection of microbiological inhibitory and toxicological data. Such a collection is already available as a computer database [9], which was used for calculating TIs for children pathogenic microorganisms. The so-selected compounds were controlled for side effects as they are documented in materials safety data sheets (MSDS) or in 'Toxicology Reports' [10]. The effectiveness on the basis of TI-calculations of the selected compounds was then compared to antibiotics used in the therapy of children infections [11,16].

2. Material and Methods

The volume of the database used for the data analysis exceeds 153.000 data records [9]. It contains information about 6800 compounds and over 2500 species of bacteria, fungi and yeast, which is scheduled from almost 3000 microbiology and over 4500 toxicology references (Figure 1).

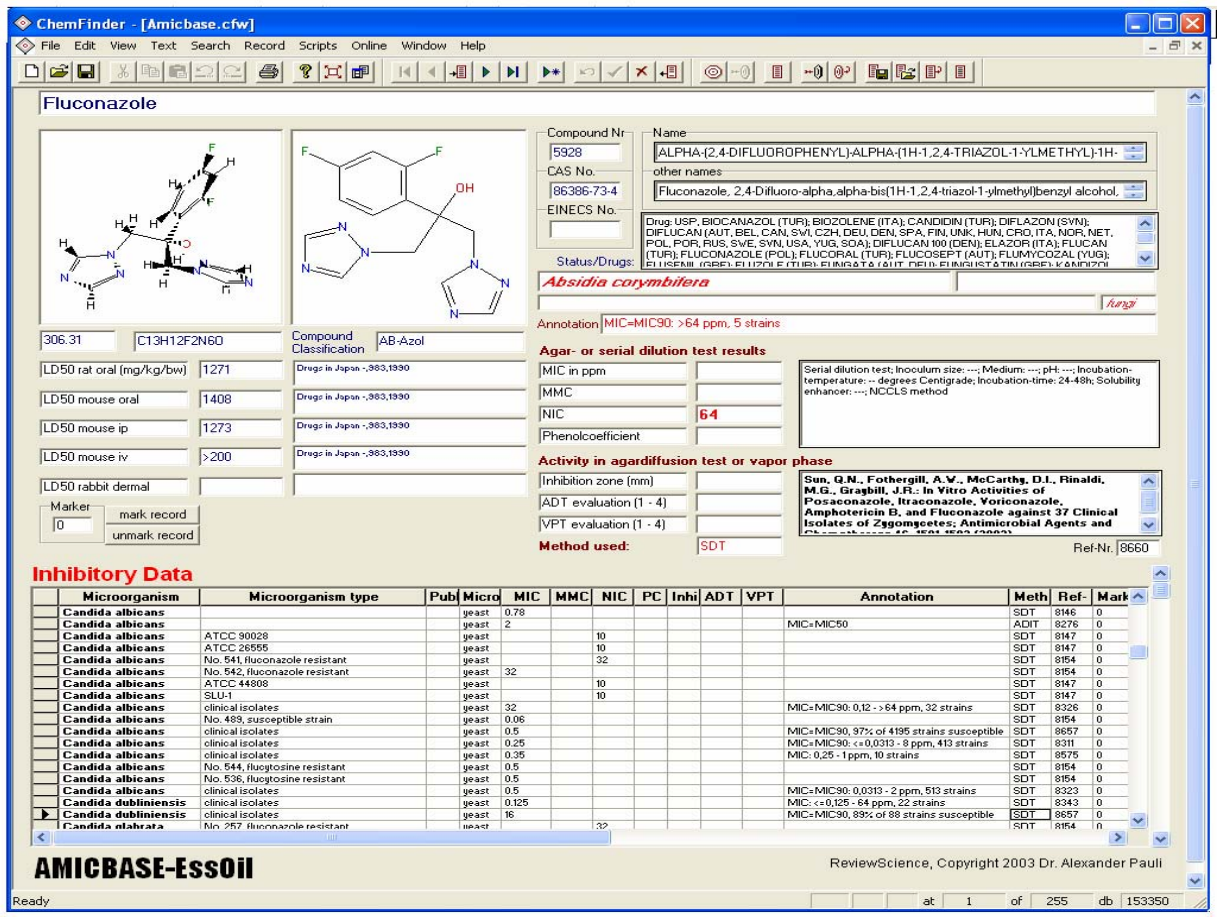


Figure 1. Example of one data record of the antimicrobial database.

Among constituents of higher plants, terpenoids (20.000 data records) and aliphatic compounds (16.000 data records) are the largest groups in the database. The antibiotics database comprises 84.000 data records, of which 53.000 data records relate to officinal drugs being available in Japan, Europe and USA.

The calculation of 'Therapeutic Indices' (TI) is done usually by combining ED_{50} and LD_{50}: the effective dose 50 (ED_{50}) is the amount causing 50% of a desired effect, and the lethal dose 50 (LD_{50}) is the amount causing 50% death of test animals [8].

TI calculation used for pharmaceutical products and substances: $TI = \frac{LD_{50}}{ED_{50}}$.

In the case of antimicrobials the concentration preventing microbial growth (minimal inhibitory concentration, MIC in ppm) is of interest and was used instead of ED_{50}.

Among toxicity data towards animals different types exist depending on the route of administration. Comprehensive toxicological data material exists following oral administration. In the case of skin affections the dermal toxicity of a compound is of interest. Therefore, oral 'Therapeutic Indices' (oral TI) and dermal 'Therapeutic Indices' (dermal TI) are given both.

TI calculation used for antimicrobials:

$$\text{oral TI} = \frac{\text{LD}_{50}\text{oral (mouse or rat)}}{\text{MIC}} \text{ and dermal TI} = \frac{\text{LD}_{50}\text{dermal (rabbit)}}{\text{MIC}}$$

The 1530 compounds presented in the database are characterized by their oral toxicity and 523 compounds by their dermal toxicity. Defined inhibitory concentrations (MIC) are necessary to calculate 'Therapeutic Indices' (TIs). About 40.000 data records of the entire database yield MIC-data of compounds from higher plants. The number of TIs calculated from combined antimicrobial and toxicological data exceeds 30.000 without antibiotics.

Typical children's diseases, infection sites and causative microorganisms were compiled together (Table 3) from the 'Merck Manual of Diagnosis and Therapy' [11].

Table 3. Diseases caused by Important Children's Pathogens.

Causative Microorganism	Children disease	Localization
Streptococcus pneumoniae	Pneumonia	Respiratory tract
	Meningitis	Nasopharynx
	Rhinitis, Sinusitis	Nose, nasal cavity
	Otitis media	Ear
	Conjunctivitis, Orbital cellulitis	Eye
	Bacteremia, Sepsis	Bloodstream
Staphylococcus aureus	Rhinitis, Sinusitis	Nose, nasal cavity
	Conjunctivitis, Orbital cellulitis	Eye
	Infectious gastroenteritis	Gastrointestinal tract
	Sepsis	Bloodstream
	Impetigo, Staphylococcal skin infections	Skin
	Staphylococcal scalded skin syndrome	Skin
Escherichia coli	Pneumonia	Respiratory tract
	Meningitis	Nasopharynx
	Urinary tract infections	Urinary tract
	Infectious gastroenteritis	Gastrointestinal tract
Salmonella spec.	Bacteremia, Sepsis	Bloodstream
	Infectious gastroenteritis	Gastrointestinal tract
Mycobacterium tuberculosis, M. bovis, M. africanum	Pulmonary tuberculosis Lymphadenopathy	Respiratory tract Lymph nodes
Microsporum audouinii, M. canis, M. gypseum, Trichophyton tonsurans, T. violaceum	Tinea capitis	Skin, hair
Candida albicans	Pneumonia	Bloodstream, respiratory tract
	Nappy (diaper) rush	Skin
	Oral candidiasis	Mouth
Bordetella pertussis	Pertussis	Respiratory tract
Haemophilus influenzae	Epiglottis	Respiratory tract
Clostridium tetani	Tetanus	Skin, wounds

The most hazardous infectious for children - according to the statistics of the World Health Organization dating from the year 2001 [12] - are shown in Table 4. Among children's diseases caused

by bacteria in particular respiratory infections followed by diarrhea, pertussis, syphilis, meningitis and tetanus are most frequent causes of death.

Table 4. WHO Statistic on Causes of Death among Children in the Year 2001.

	No. cases of death in age group 0 – 4 years		
	Male	Female	Both
Lower respiratory infect.	1070739	963096	2033836
Diarrhoeal diseases	685979	667149	1353128
Malaria	454970	502027	956997
Other infectious diseases	308522	310123	618645
Measles	276372	277156	553527
HIV/AIDS	178049	174069	352118
Pertussis	142430	142111	284541
Tetanus	100657	100784	201441
Syphilis	78084	64292	142376
Meningitis	42999	36473	79471
Tuberculosis	27979	21351	49330
Upper respiratory infect.	16179	18672	34851
Dengue	2730	6393	9123
Leishmaniasis	4833	4040	8872
Japanese encephalitis	2133	4490	6623
Diphtheria	2470	1976	4446
Trypanosomiasis	2237	1245	3482
Hepatitis B	1668	1782	3450
Otitis media	1420	997	2417
Ascariasis	1059	1094	2153
Hepatitis C	646	831	1476
Other intestinal infections	517	540	1057
Leprosy	206	169	375
Other STDs	184	140	324
Trichuriasis	227	95	323
Schistosomiasis	110	21	131
Poliomyelitis	46	34	80
Gonorrhoea	0	33	33
Trachoma	31	0	31
Lymphatic filariasis	31	0	31
Hookworm disease	5	3	7
Onchocerciasis	6	0	6
Chagas disease	4	1	5
Chlamydia	0	0	0

	No. cases of death in age group 5 – 14 years		
	Male	Female	Both
Measles	86435	87434	173868
Other infectious diseases	62495	53268	115763
Lower respiratory infect.	57849	43601	101450
Diarrhoeal diseases	54967	39016	93983
Malaria	54388	29391	83779
Tuberculosis	17257	17584	34842
HIV/AIDS	15102	13904	29006
Tetanus	12840	12746	25587
Meningitis	12623	10605	23229
Trypanosomiasis	6183	11125	17309
Leishmaniasis	7135	9663	16797
Dengue	1904	1171	3075
Hepatitis B	1180	1500	2680
Upper respiratory infect.	1087	835	1922
Trichuriasis	878	907	1785
Ascariasis	869	739	1608
Japanese encephalitis	1130	357	1487
Hepatitis C	563	736	1299
Otitis media	622	222	843
Schistosomiasis	159	659	818
Diphtheria	518	212	730
Pertussis	254	252	506
Other intestinal infections	111	115	226
Leprosy	68	52	120
Poliomyelitis	45	52	97
Chlamydia	74	0	74
Syphilis	10	15	25
Other STDs	3	6	9
Hookworm disease	9	0	9
Chagas disease	4	1	5
Gonorrhoea	0	1	1
Trachoma	0	1	1
Lymphatic filariasis	0	0	0
Onchocerciasis	0	0	0

Tables 5 to 17 summarize data about the effectiveness of antimicrobials from higher plants and antibiotic drugs against children pathogens. The selected compounds possess ‘Therapeutic Indices’ equal and higher than 100 units. Thus, the toxic dose towards animals is in minimum over 100 - fold higher than the inhibitory dose towards microorganism.

The results of natural antimicrobials are compared to results of antibiotic drugs obtained with resistant and non-resistant microbial strains. Because of the data variety additional information was included, which is the reported minimum and maximum MIC, the minimum and maximum TI, the number of tested strains. Of antibiotic drugs the MIC-breakpoints have frequently been determined [13]. This allows to distinguished between strains that are regarded as normal or as resistant. The minimum MIC of resistant strains is given as MIC-res for each antibiotic drug. When the quality data range for antibiotic drugs is given only [14], the double amount of the highest concentration was taken as MIC-res. TI-res is the 'Therapeutic Index' calculated from MIC-res for resistant strains, respectively. Side effects are characterized and annotated in the status field, which indicates whether a compound is used as drug ('Drug') or is listed by the Unites States Food and Drug Administration as compound that may occur in food ('Food') [15].

3. Results

The results of effective compounds against causative microorganisms of children's diseases are scheduled according to Table 3.

3.1. Streptococcus pneumoniae

Streptococci are human parasites, which colonize skin and mucous membranes and can be isolated from alimentary, respiratory and genital tracts. Among Pneumococci several types exist, of which capsulated strains are regarded as pathogen. Antibiotic resistances of Pneumococci isolated from children in Germany are as follows: penicillin G 8,6%, cefotaxime 3,1%, erythromycin 27,4%, tetracycline 10,7% [16]. In addition, resistances of Pneumococci towards trimethoprim, sulfonamides and chloramphenicol are reported [17]. Table 5 shows the calculations on pharmaceutical drugs used for the treatment of Pneumococcus infection and the calculations of most successful compounds from higher plants in comparison. The data is sorted first by TI-res and if not available by minimum TIs.

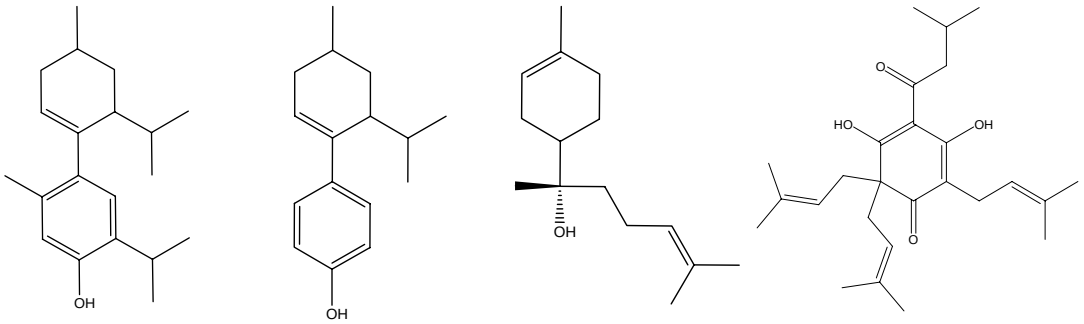
When Pneumococci strains without resistance mechanisms were tested, the approved antibiotics dominate the list of oral TIs (Table 5), of which penicillin G is preferably be used in the therapy of Pneumococcus infections in children. The TIs of compounds from higher plants including essential oils are inferior to such therapeutically used drugs.

Pneumococci resistant to penicillin G are characterized by MIC-res >=1 ppm, however, even at this concentration the TI-calculation for penicillin G is much better than for compounds from higher plants. Tetracycline, cotrimoxazol and chloramphenicol are not much different at their lowest level of effectiveness (TI-res) from meta-menthene-thymol **4**, meta-menthene-phenol **5**, alpha-bisabolol **6**, a component from chamomile (*Matricaria recutita*) essential oil, or lupulone **7**, a component present in hop extract (*Humulus lupulus*). However, only a small number of strains of Pneumococci have been tested with these compounds, which impairs the drawing of general conclusions.

Table 5. Effective Inhibitors of *Streptococcus pneumoniae*, oral TI $_{max.}$>= 100.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC-range in ppm	TI max. - TI min.	MIC-res	TI-res	Status
Cefotaxime	Mou-o	>20000	>100	0,004 – 4	>5000000 - 5000	>2	<10000	Drug
Erythromycin	Mou-o	2580	>100	0,0008 – 64	3225000 - 80	>0,5	<5160	Drug
Penicillin G	Mou-o	>5000	>100	0,008 –16	625000 – 312	>1	<5000	Drug
Linoleic acid	Mou-o	>50000	2	13	>3846	---	---	Food*
n-Dodecanol	Rat-o	>12800	2	13	>984	---	---	Food*
m-Menthene-phenol	Mou-o	2900	1	3	966	---	---	---
Lauric acid	Rat-o	12000	2	13	923	---	---	Food*
Lauric aldehyde	Rat-o	23000	2	25	920	---	---	Food*
Lupulone	Mou-o	1500	2	3,3	454	---	---	Food
Sulfamethoxazole	Mou-o	2300	5	1 – 8	2300 - 340	---	---	Drug
Tetracycline	Mou-o	678	>100	0,12 – 4	5650 - 170	>2	<340	Drug
Cotrimoxazol	Mou-o	3740	>100	0,12 – 32	31000 - 233	>32	<233	Drug
alpha-Bisabolol	Rat-o	14850	>4	32 - 64	464 - 232	---	---	---
Myristic acid	Rat-o	>10000	2	50	>200	---	---	Food
Chloramphenicol	Mou-o	1500	>100	1 – 32	1500 - 46	>8	<184	Drug
m-Menthene-thymol	Mou-o	1925	1	12	160	---	---	----
Lauramine HCl	Mou-o	1160	2	10 – 12,5	116 - 93	---	---	Food*
Palmitic acid	Rat-o	>10000	3	7 – 125	>1428 - >80	---	---	Food
Trimethoprim	Mou-o	2764	>100	<0,06 – 128	>2750 - 80	---	---	Drug
Oleic acid	Rat-o	74000	4	4 – 1000	1850 - 74	---	---	Food*
Undecylenic acid	Mou-o	8150	2	50 – 1000	163 - 8	---	---	Drug
Stearic acid	Rat-o	4600	3	7 – 1000	657 - 4,6	---	---	Food

Notes: * may cause digestive tract irritation. Abbreviations used: -o = orally administered, Mou = mouse



4 **5** **6** **7**

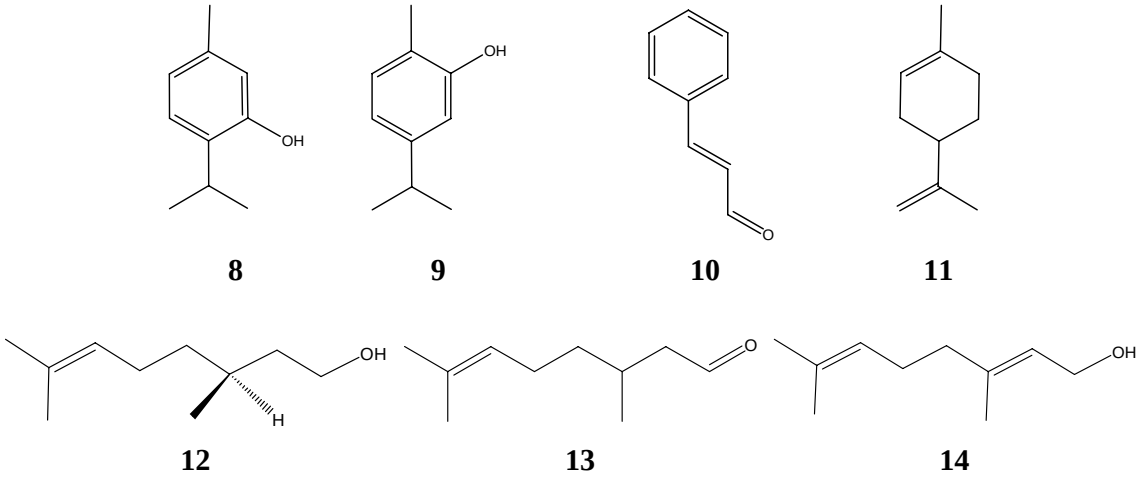
The *in vitro* activity of fatty acids like oleic, linoleic and linolenic acid is inactivated by addition of blood serum [18]. Such compounds might have no practical significance in systemic treatment of infectious diseases, and therefore, they are not discussed in the following tables. The low toxicity of oleic acid and related long-chain aliphatic compounds indicate a possible use as topical antiseptics.

Treating respiratory tract infections by inhalation of volatile compounds lies near by hand. Very active compounds against *Streptococcus pneumoniae* in the vapor phase are shown in Table 6.

Table 6. Strong Inhibitors of *Streptococcus pneumoniae* in the Vapor Phase.

Trivial name	Microorganism	Activity evaluation	Status
Thymol 8	Streptococcus pneumoniae type II	++++	Food *
Thymol	Streptococcus pneumoniae type III	++++	Food *
Thymol	Streptococcus pneumoniae type VI	++++	Food *
Carvacrol 9	Streptococcus pneumoniae type III	++++	Food *?
Cinnamic aldehyde 10	Streptococcus pneumoniae type VI	++++	Food *
Limonene 11	Streptococcus pneumoniae	++++	Food *
Citronellol 12	Streptococcus pneumoniae	++++	Food *
Citronellal 13	Streptococcus pneumoniae	++++	Food *
Geraniol 14	Streptococcus pneumoniae	++++	Food *

Notes: * may cause respiratory tract irritation, ++++: strong inhibitory activity



8 **9** **10** **11**

12 **13** **14**

Almost all compounds listed in Table 6. may cause irritation of the respiratory system, and therefore, their use as therapeutics via inhalation is questionable, although thymol **8** superimposes the germicidal activity of phenol, which has a long history as disinfecting agent, about 5 to 30 times towards gram-positive bacteria. The antimicrobial properties of alpha-bisabolol are unexamined in the vapor phase. No toxic signs were observed in animal experiments following inhalation (7h exposure in a highly enriched and/or saturated atmosphere at 20 °C, Worksafe Australia). In toxicology studies alpha-bisabolol **6** was found to be safe [19]. Alpha-bisabolol strongly inhibits *Streptococcus pneumoniae* in the serial dilution tests (MIC = 32 - 64 ppm). This may explain why inhalation of vapors of chamomile flowers is recommended in German traditional medicine.

3.2. Staphylococcus aureus

Staphylococci are found mainly on human skin, skin glands and mucous membranes and sometimes in the mouth, blood, mammary glands, intestinal, genitourinary and upper respiratory tracts. Pathogenic strains are known to form toxins. Antibiotic resistance is reported towards ß-lactam antibiotics (oxacillin, ampicillin), aminoglycosides (gentamycin), macrolides (erythromycin), quinolones (ciprofloxacin), clindamycin, chloramphenicol, tetracycline, trimethoprim [17] and the reserve antibiotic vancomycin in 1999 [20].

In the antibiotic therapy first choice drugs are flucloxacillin (methicillin susceptible strains, MSSA), vancomycin and teicoplanin (methicillin resistant strains, MRSA) and vancomycin + flucloxacillin or vancomycin + gentamycin (glycopeptide intermediate resistant strains, GISA). Recommendations as second choice antibiotics are: cefaclor, cefuroximaxetil, and loracarbef (MSSA), quinopristin + dalfopristin, linezolid and further alternatives: vancomycin + rifampicin, cefazolin + vancomycin + netilmycin, imipenem + vancomycin + netilmycin, fusidic acid + rifampicin, Cotrimoxazol + fusic acid or rifampicin, minocycline, fosomycin + cefotaxime, and cotrimatzol + nitrofurantoin (MRSA), and quinopristin + dalfopristin, ampicillin + sulbactam, and linezolid (GISA). No differences were made in the choice of antibiotic treatment between grownups and children [16].

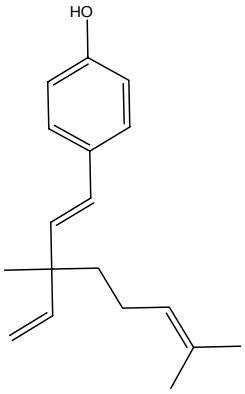
Table 7. Effective Inhibitors of *Staphylococcus aureus*, oral $TI_{max.} > 100$.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. – TI min.	MIC-res	TI-res	Status
Netilmicin	Rat-o	Su: >10000	>100	0,125 – 32	>80000 – 312	>1	>10000	Drug
Cefazolin	Mou-o	Na: >11000	>100	0,01 - >256	>110000 - <43	0,25 – 1	**<5500	Drug
Flucloxacillin	Mou-o	Na: 7600	>100	0,125 – 32	60800 – 238	***0,125 – 1	3800	Drug
Minocycline	Mou-o	3100	>100	<0,015 - 12,5	>206666 – 248	0,06 - 0,5	**<3100	Drug
Fusidic acid	Mou-o	1500	>100	0,032 – 16	46875 - 94	>0,5	<3000	Drug
Cefotaxime	Mou-o	Na: >20000	>100	0,12 - >60	>166666 - <333	0,5 - 4	**<2500	Drug
Cefaclor	Mou-o	>20000	>100	0,25 – 128	>80000 - 156	1 – 4	**<2500	Drug
Vancomycin	Mou-o	5000	>100	0,125 – 8	40000 – 625	>2	<2500	Drug
Bakuchiol	Mou-o	2560	1	1,4	1828	---	---	---
Oleic acid	Rat-o	74000	3	15 – 90	4933 - 822	---	---	Food
Mupirocin	Mou-o	5000	>100	0,05 – 512	100000 – 10	>8	625	Drug
Gentamicin	Mou-o	>10000	>100	0,035 - >256	>285714 - <39	>16	<624	Drug
Cefuroxime	Mou-o	>10000	>100	0,13 - >60	>76923 - <167	>32	<624	Drug
Rifampicin	Mou-o	500	>100	0,002 - 3,1	250000 - 161	>1	<500	Drug
Loracarbef	Dog-o	>2000	>100	0,5 – 16	>4000 - 125	0,5 – 2	**<500	Drug
Fosfomycin	Mou-o	Ca: >3500	>100	0,5 - >128	>7000 - <27	0,5 - 4	**<440	Drug
n-Tridecanol	Rat-o	17200	3	20 – 50	860 – 344	---	---	Food*
Imipenem	Mou-o	>5000	>100	0,006 – >256	>833333 – <20	>16	<312	Drug
Teicoplanin	Mou-o	1000	>100	0,125 - 6,3	8000 - 160	>4	<250	Drug
Linoleic acid	Mou-o	>50000	10	4 - >200	>12500 - <250	---	---	Food*
Lupulone	Mou-o	1500	7	2 - 6,25	750 – 240	---	---	Food
Cotrimoxazol	Mou-o	3740	>100	0,015 - 2,86	249333 – 1300	>16	<234	Drug
Lauricidin	Rat-o	****53400	11	10 – 250	5340 – 213	---	---	Food
3-Heptylacrolein	Rat-o	5000	3	25	200	---	---	Food
Shikonin	Mou-o	>1000	2	4 – 5	>250 – 200	---	---	---
Swartziadione	Mou-o	*****>300	1	1,56	>192	---	---	---

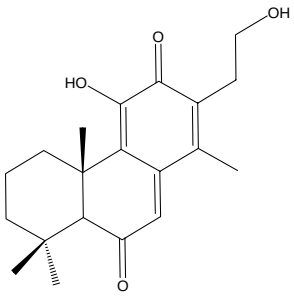
alpha-Bisabolol	Mou-o	11350	9	32 – 100	354 - 113	---	---	---
m-Menthene-phenol	Mou-o	2900	1	28	103	---	---	---
Sorbic acid	Rat-o	7360	50	50 – 100	147 - 73	---	---	Food
Butylparaben	Mou-o	13200	20	60 – 270	220 - 49	---	---	Food
Ampicillin-sulbactam	Mou-o	>6000	>100	<=0,06 – 128	>100000 - 47	---	---	Drug
o-Methoxy-cinnamaldehyde	Rat-o	>5000	3	50 – 200	>100 - 25	---	---	Food
Dimethyl fumarate	Rat-o	2240	2	10 – 100	224 - 22	---	---	---
Diethyl fumarate	Rat-o	1780	2	10 – 100	178 - 18	---	---	---
Lauric aldehyde	Rat-o	23000	8	50 - >2000	460 - <11,5	---	---	Food*
ortho-Phenylphenol	Rat-o	2000	10	15,6 – 200	128 - 10	---	---	Food
Nitrofurantoin	Mou-o	360	>100	0,75 – 32	480 - 11	>32	<11	Drug
n-Dodecanol	Rat-o	>12800	15	12,5 - >2000	>1024 - <6,4	---	---	Food*
Linolenic acid	Mou-o	>3200	8	5 – 500	640 – 6,4	---	---	Food*
Undecylen aldehyde	Rat-o	>5000	5	50 – 1000	>100 - 5	---	---	Food
Lauric acid	Rat-o	12000	22	22 – 2500	545 - 4,8	---	---	Food*
Falcarindiol	Mou-o	ca. 100	3	1 – 25	100 – 4	---	---	---
Hinokitiol	Mou-o	760	51	0,2 – 200	3800 -3,8	---	---	---
Capric alcohol	Mou-o	6500	16	40 – 2000	162 - 3,2	---	---	Food
n-Tetradecanol	Rat-o	32,5 ml/kg	4	40 - >10000	812 - <3,2	---	---	Food
Farnesol	Mou-o	7400	16	25 – 2000	123 - 3,7	---	---	Food
Isoborneol	Rat-o	5200	4	50 – 2000	104 - 2,6	---	---	Food
Hyacinthin	Mou-o	3890	6	32 - >2000	121 – 2	---	---	Food
Nerolidol	Mou-o	15000	25	25 - >10000	600 - <1,5	---	---	Food
Eugenol	Mou-o	3000	49	25 – 2100	120 - 1,4	---	---	Food
n-Undecyl alcohol	Rat-o	3000	13	25 - >2000	120 - <1,3	---	---	Food
Citral	Mou-o	6000	49	12,5 – 5000	480 - 1,2	---	---	Food
Diacetyl	Rat-o	1580	11	<9,75 – 1000	>162 - 0,16	---	---	Food
Linezolid	---	---	>100	0,5 – 8	---	>4	---	Drug
Quinupristin-dalfopristin	---	---	>100	0,06 – 32	---	>2	---	Drug

Notes: * may cause digestive tract irritation, ****** calculated with double MIC $_{max.}$ of quality data range [14], ******* range of 87 clinical isolates [21] assumed as quality data range, ******** 53,4 ml/kg calculated with density = 1, ********* LDL = lethal dose lowest. Abbreviations used: Na = Na-salt, Ca = Ca-salt, Su = sulfate-salt,

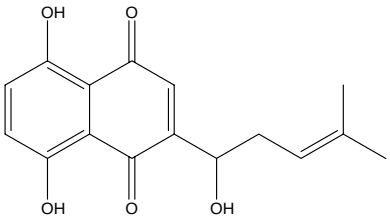
The list of effective inhibitors of *Staphylococcus aureus* is dominated again by therapeutics in use (Table 7). According to TI calculations, bakuchiol **15**, a natural long-chain phenol occurring in Malaya tea *(Psoralea corylifolia)* was the most successful compound from higher plants and it superimposes antibiotics towards which Staphylococci strains had developed a high level of resistance, e.g. mupirocin, fosfomycin or nitrofurantion. Bakuchiol was further examined on its activity against dental plaque forming microorganisms and the authors concluded that the compound is of value as food additive or as additive in mouth washes [22]. Promising *in vitro* results were again obtained with lupulone **7**. Swartziadione **16**, a constituent of the African tree *Swartzia madagascariensis*, was patented for treatment or inhibition of microbial infections. Shikonin **17**, another patented constituent of higher plants [23], occurs in the extract of Groomwell root (*Arnebia euchroma)*, which is used traditionally in China as topical wound healing therapeutic.



15

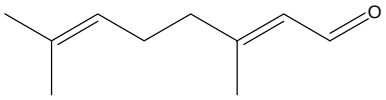


16

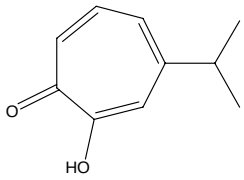


17

Further compounds having in part good results in the TI calculation are citral **18** from e.g. lemongrass (*Cymbopogon citratus*), hinokitiol **19** from the heartwood of Thuja trees, which possesses an extraordinary tropolone-ring substructure, and eugenol **3**. The wide range of activity of these compounds points to the existence of microbial resistance mechanisms and to a natural variability towards antimicrobials, which can not be explained alone with variations of the testing methods used in the examinations of the respective compounds.

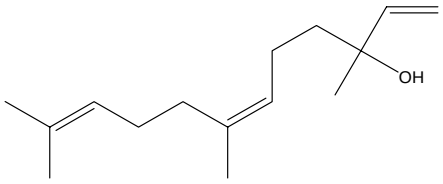


18

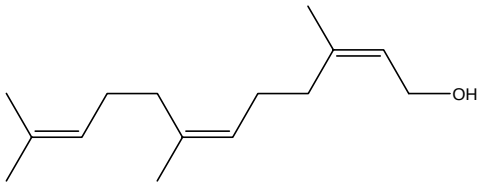


19

Highly calculated compounds from essential oils are the sesquiterpenes nerolidol **20** and farnesol **21**, which are both methyl-substituted long-chain C-12 alcohols, and further primary alcohols having a carbon chain of 12 to 14 atoms and the monocyclic sesquiterpene alpha-bisabolol **6**.



20



21

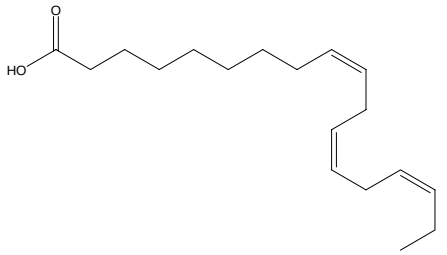
Because *S. aureus* is also prominent for skin and wound infections, a second list on the basis of dermal ‘Therapeutic indices’ was calculated (Table 8).

Table 8. Effective Inhibitors of *Staphylococcus aureus*, dermal TI $_{max.}$ >= 100.

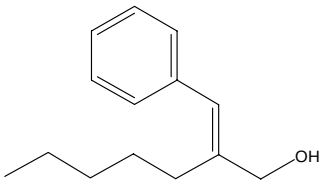
Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Triclosan	Rabbit-dermal	9300	10	0,01 - 0,03	930000 - 310000	Drug*,**
Haloprogine	Rabbit-dermal	1625	6	1,56 - 3,12	1041 - 520	Drug*,**
Hexetidine	Rabbit-dermal	1,86 ml/kg	1	5	372	Drug
Linolenic acid	Rabbit-dermal	>20000	2	5 – 100	>4000 - 200	Food*
Hexachlorophene	Rabbit-dermal	>600	10	0,3 - 3	>2000 - 200	Drug*
Hinokitiol	Rabbit-dermal	>2000	14	0,2 - 12,5	>10000 - 160	---
3-Heptylacrolein	Rabbit-dermal	3400	2	25	136	Food*
Oxolinic acid	Rabbit-dermal	>2000	9	0,4 - 25	>5000 - 80	Drug
Hydroquinone	Mammal-dermal	5970	11	10 – 90	597 - 66	Drug*,**
o-Methoxy-cinnamaldehyde	Rabbit-dermal	>5000	3	50 – 200	>100 - 25	Food*,**
Dimethyl fumarate	Rabbit-dermal	1250	2	10 - 100	125 - 12,5	---*
beta-Naphthol	Rabbit-dermal	>10000	2	100 – 1000	>100 - 10	---*,**
Isobornyl acetate	Rabbit-dermal	>20000	6	200 - >2000	>100 - 10	Food
n-Tridecanol	Rabbit-dermal	5600	3	20 - >800	280 - 7	Food*
Diacetyl	Rabbit-dermal	>5000	11	9,75- 1000	>512 - 5	Food*
Hyacinthin	Rabbit-dermal	>5000	6	32 - >1000	>156 - 5	Food*
Isoborneol	Rabbit-dermal	>5000	5	50 - >1000	>100 - 5	Food*
Undecylen aldehyde	Rabbit-dermal	>5000	5	50 – 1000	>100 - 5	Food*
alpha-Amylcinnamyl alcohol	Rabbit-dermal	>5000	6	50 - >2000	>100 - 2,5	Food
n-Undecyl alcohol	Rabbit-dermal	4,76 ml/kg	13	25 - >2000	190 - 2,4	Food*
n-Dodecanol	Rabbit-dermal	>10 ml/kg	16	12,5 - >10000	>800 - 1	Food*
n-Tetradecanol	Rabbit-dermal	7,13 ml/kg	25	40 - >10000	173 - <0,7	Food*
Nerolidol	Rabbit-dermal	>5000	8	25 - >10000	>200 - 0,5	Food*

Notes:* may cause skin irritation ** mutagen

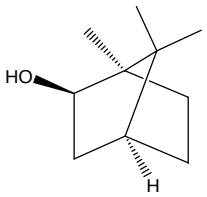
The most effective inhibitors of *Staphylococcus aureus* are again approved drugs used to treat skin infections. Almost all compounds listed in Table 8 are classified as skin irritants. The dermal toxicity of alpha-bisabolol **6** has not been determined in numbers, however, it is regarded as safe and allergic reaction is not reported although humans are exposed to it to a large extend, because it is widely used in cosmetics. Interestingly, (+)-epi-alpha-bisabolol from *Peperomia galioides* turned out to be as active wound healing agent, when a series of commercial available terpenoids was studied [24]. A good result in the dermal TI calculation was obtained with linolenic acid **22**. It is the main constituent of the fatty oil of 'evening primrose' (*Oenanthera biennis*), which is used internally for the adjuvant treatment of neurodermitis in Germany.



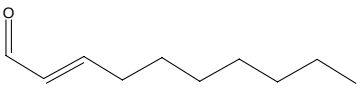
22



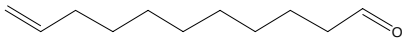
23



24



25



26

Other active compounds are long-chain alcohols (C11 – C14), nerolidol **20**, alpha-amylcinnamyl alcohol **23**, isoborneol **24** and its acetate ester, unsaturated aldehydes (3-heptylacrolein **25**, undecylen aldehyde **26**) and hinokitiol **19**.

3.3. Escherichia coli

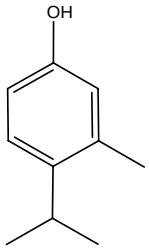
Escherichia coli belongs to the normal intestinal microflora of humans. Pathogenic strains are characterized by the ability to form toxins. Diarrhea is caused by enteropathogenic *Escherichia coli* strains. Antibiotic resistance is reported towards ß-lactam antibiotics (ampicillin), aminoglycosides, quinolones, chloramphenicol, tetracycline (doxycycline), and trimethoprim [17]. Diarrhea is treated with orally administered colistin in children and infants [16].

Table 9. Effective Inhibitors of *Escherichia coli*, oral $TI_{max.}$ >= 100.

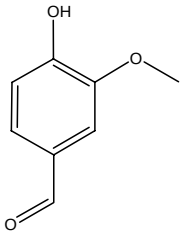
Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Colistin	Mou-o	LD >2000	3	0,5 – 10	>4000 –200	Drug
trans-Nerolidol	Mou-o	***15000	1	****125	120	Food
para-Thymol	Mou-o	6280	2	60 – 96	104 - 65	---
Diethyl fumarate	Rat-o	1780	2	10 – 100	178 – 18	---
Hinokitiol	Rat-o	>500	12	1,56 – 120	>320 – 6	---
Sorbic acid	Rat-o	7360	11	50 - <2000	147 - <3,7	Food*, **
Butylparaben	Mou-o	13200	6	120 – 4000	110 – 3,3	Food
Vanillin	Mou-o	3925	5	25 – 2000	157 – 2	Food
Eugenol	Mou-o	3000	23	<25 – 2100	>120 – 1,4	Food*
Linoleic acid	Mou-o	>50000	2	90 - >100000	>555 - <0,2	Food*
Citral	Mou-o	6000	16	12,5 – 6660	480 – 0	Food**
Nerolidol	Mou-o	15000	4	400 – 9500	37,5 – 1,6	Food
Thymol	Mou-o	640	31	100 – 1000	6,4 – 0,6	Food**

Notes: * may cause digestive tract irritation ** mutagen ******* calc. with nerolidol toxicity, ******** minimal bactericidal conc.

Only a few compounds are effective inhibitors of *Escherichia coli* (Table 9). The most interesting one, a synthetic isomer of thymol **8** with the name para-thymol **27**, is about 10 times less toxic than thymol ($TI_{max.}$ = 6,4) itself. Not much data is available for para-thymol, e.g. spectrum of antimicrobial activity, side effects. The trans-isomer of nerolidol had strong inhibitory properties, but further testing is necessary, because stereochemically undefined nerolidol **20** was much less active. The results of hinikitiol **19** vary and were influenced by different test conditions. Variation of data is given also with citral **18**, vanillin **28** and eugenol **3**.



27



28

3.4. Salmonella species

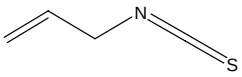
Salmonella species have been isolated from humans and almost all animals throughout the world and they constitute another genus being prominent for causing diarrhea in humans all kind of age. Humans present the only known reservoir for *Salmonella typhi* and *paratyphi* serotypes. Resistances of *Salmonella* species towards antibiotics are reported from tetracycline, trimethoprim, quinolones and chloramphenicol [17]. In diarrhea treatment caused by *Salmonella* infections the drug of first choice is amoxicillin. Alternatively, quinolones, ceftriaxone and cotrimoxazol can be used [16].

Table 10. Effective Inhibitors of *Salmonella* sp., oral $TI_{max.}$ >= 100.

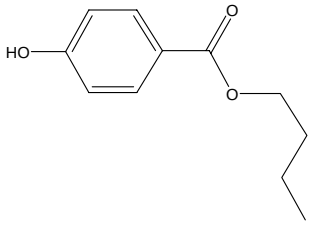
Trivial name	**Animal test**	**LD_{50}-oral mg/kg bw**	**n-strains**	**MIC range in ppm**	**TI max. - TI min.**	**Status**
Ceftriaxone	Mou-o	>10000	90	0,031 – 0,25	322000 - 40000	Drug
Amoxicillin	Mou-o	>25000	>100	0,01 - >100	>2500000 - <250	Drug
Isoborneol	Rat-o	5200	1	50	104	Food
Ciprofloxaxin	Mou-o	5000	>100	0,008 – >64	625000 - <78	Drug
Cotrimoxazol	Mou-o	3740	>100	<0,015 - >100	250000 - <37	Drug
Butylparaben	Mou-o	13200	6	125 – 1000	105 – 13	Food
p-Benzylphenol	Mou-o	>20000	2	*****160 – 2170	>125 – 9	---
Allyl isothiocyanate	Mou-o	308	5	1 – 47	308 – 6,5	Food

Notes: * bactericidal after15 min. exposure

Similar to *Escherichia coli*, not many compounds are selected (Table 10). Allyl isothiocyanate **29** or with its more common name ‘oil of mustard’ (German “Senföl”) is a very irritant compound with pungent taste. Its inhibitory data as well as the data of the preservative butylparaben **30** is not conclusive due to the small number of tested *Salmonella* strains, respectively. Nevertheless, butylparaben was shown previously as effective compound against *E. coli*. Only one data record exists with isoborneol **24**, which occurs together with borneol as main constituents of the essential oil of annual wormwood (*Artemisia annua*), a plant growing in India.



29



30

3.5. Mycobacterium tuberculosis

Most species of Mycobacteria are living in soil or water in contrast to microorganisms belonging to the *Mycobacterium tuberculosis* complex (*M. bovis, M. microti,* and *M. africanum, M. tuberculosis*), which are found in diseased tissues of humans and warm-blooded animals suffering on tuberculosis. Resistances are found in particular towards all antibiotic drugs (ethambutol, rifampicin, streptomycin, isoniazid, and pyrazinamide), which represents a global problem [17]. The calculation of TIs resulted in a new ranking favoring compounds from higher plants (Table 11).

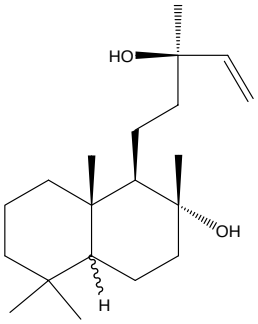
Table 11. Effective Inhibitors of *Mycobacterium tuberculosis*, oral $TI_{max.}$ >= 100.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	MIC-res	TI-res	Status
cis-Phytol	Rat-o	**>5000	1	2	>2500	---	---	---
Ethambutol	Mou-o	8700	>10	0,95 – 7,5	9157 – 1160	***10	870	Drug
Sclareol	Rat-o	>5000	1	6	>833	---	---	---
Farnesol	Mou-o	7400	1	8	825	---	---	Food
Propylparaben	Mou-o	6332	1	8	791	---	---	Food
Phenyl salicylate	Rat-o	3000	1	4	750	---	---	Drug
trans-Phytol	Rat-o	**>5000	4	2 – 8	>2500 – 625	---	---	---
Streptomycin	Rat-o	9000	>30	0,1 – 100	5000 - 5	***16	560	Drug
Rifampicin	Mou-o	500	>100	0,01 – 16	50000 – 31	***2	250	Drug
beta-Naphthol	Rat-o	1960	1	8	245	---	---	Drug
alpha-Naphtol	Rat-o	1870	1	8	233	---	---	---
Methylparaben	Mou-o	>8000	1	40	>200	---	---	Food
Pyrazinamide	Mou-o	>3000	>20	4 - 4000	>750 – 0,7	***16	187	Drug
asym.-m-Xylenol	Rat-o	3200	1	40	160	---	---	---
Retinoic acid	Rat-o	1960	3	13	150	---	---	Food
Isoniazid	Mou-o	133	>50	0,01 – 100	13300 – 1	***1	133	Drug
4-Hexylresorcinol	Mou-o	1040	2	* 1 - 8	1040 - 130	---	---	Drug
Bakuchiol	Mou-o	2560	1	10 - 20	256 – 128	---	---	---
Retinol	Mou-o	4000	3	40 - 50	100 - 80	---	---	Food
Lupulone	Mou-o	1500	13	10 – 110	150 – 14	---	---	---
Thymol	Rat-o	980	7	8 – 100	122 – 10	---	---	Food

Notes: * bactericidal conc., ** calc. both with toxicity of pythol, *** estimated MIC for resistant strains

The results indicate that several constituents from higher plants seem to be superior to antibiotic drugs being used for therapy of tuberculosis, however, only a small number of strains have been tested

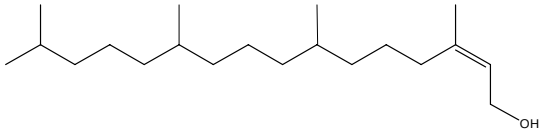
so far. Beside sclareol **31** from Clary Sage (*Salvia sclarea*) the hop (*Humulus lupulus*) constituent lupulone **7** was selected among most effective inhibitors. The latter was once tested in tuberculosis infected mice, which had received intramuscularly injections of 60 mg/kg body weight over a 4 weeks period, but the renal toxicity of lupulone prevented its development as pharmaceutical drug [25].



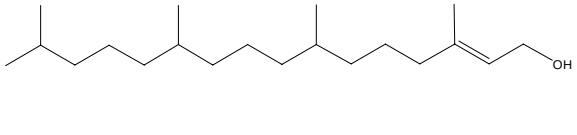
31

Other compounds of Table 11 can be classified in three groups: long-chain aliphatic compounds, aromatic acid esters and phenols.

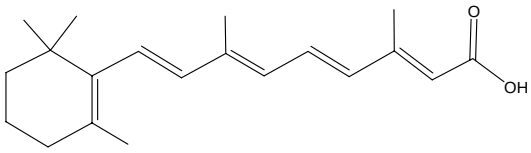
The selected compounds of group 1, cis- **32** and trans-phytol **33**, farnesol **21**, retinoic acid **34** and retinol **35** possess structural similarities, which are in particular a long side chain consisting of 3 to 4 isoprene-units plus a terminal polar group. Similarly, fatty acids were strong inhibitors *in vitro* (data not shown). However, the effectiveness *in vivo* of all selected lipophilic compounds seems to be questionable due to their possible adsorption to lipophilic lung tissue and inactivation.



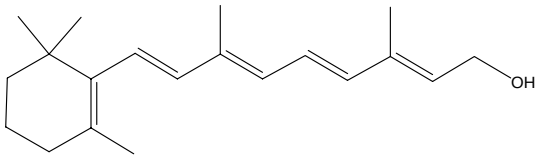
32



33

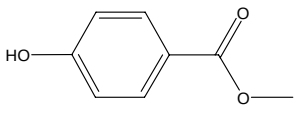


34

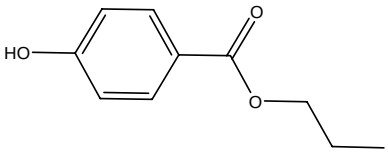


35

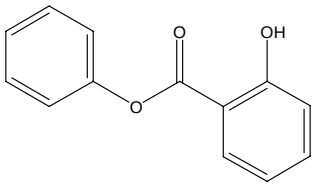
Surprisingly, preservatives like methyl- **36** and propylparaben **37** are very successful in the TI-calculations. A further ester of aromatic acids, phenyl salicylate **38**, is well known as anti-infective drug for a long time.



36

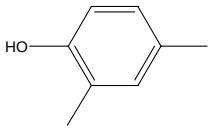


37

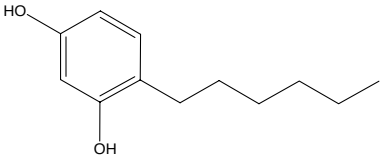


38

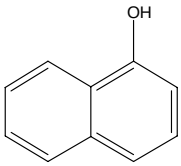
The third group consists of phenols (asym.-m-xylenol **39**, 4-hexylresorcinol **40**, alpha- **41** and beta-naphtol **42,** bakuchiol **15**) having a similar range of lipophilicity, of which bakuchiol is the most fat-soluble representative.



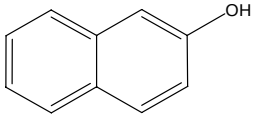
39



40



41

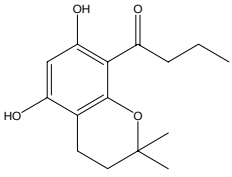


42

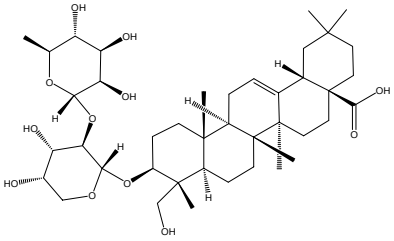
In comparison with the water solubility of antitubercular drugs, all investigated compounds are less soluble in water, which might prevent their use as lung therapeutics.

3.6. Dermatophytes

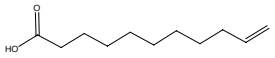
Skin and hair infections of children (*Tinea capitis*) are caused by dermatophytes belonging to the genera *Trichophyton* and *Microsporum*. They occur either in soil or possess host preference (humans or animals), e.g. *Microsporum canis* infections are often result of contact between susceptible children and stray kittens [17]. The calculations made with *Microsporum* species, compounds from higher plants and orally administered antifungal drugs are shown in Table 12.



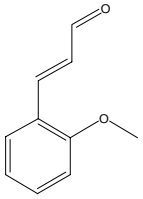
43



44



45



46

Interestingly, a series of 1’-oxo-substituted phloroglucinols **43** turned out to be as successful and non-toxic inhibitors of *Microsporum* species. Good results were obtained again with bakuchiol **15** and alpha-hederin **44**, which originates from hederasaponine C, the main saponine of Common Ivy (*Hedera helix*). Alpha-hederin was found to be non-toxic following oral administration, but it was

rather toxic when given intravenously. Undecylenic acid **45**, a naturally occurring compound, has a long history as antifungal drug. When compared with the lowest level of activity of antibiotics, the effectiveness of alpha-bisabolol **6** was similar to griseofulvin, nystatin, fluconazole, itraconazole and ketoconazole. Other compounds seem be to of minor importance, e.g. o-methoxycinnamaldehyde **46**, due to their mutagenic or tumorigenic properties.

Table 12. Effective Inhibitors of *Microsporum* Species, oral $TI_{max.}$ >= 100.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Terbinafine	Mou-o	4000	>40	0,002 - 1	2000000 – 4000	Drug
Oenanthic acid	Mou-o	6400	2	2	3200	Food
Amphotericin B	Mou-o	>8000	>100	0,125 - 8	>64000 - 1000	Drug
Decanoic acid	Rat-o	>10000	2	12,5	>800	Food*
o-Methoxycinnamaldehyde	Mou-o	4430	2	3,13 - 6,25	1415 - 708	Food***
Pelargonic acid	Rat-o	3200	2	2 – 5	1600 – 640	Food*
1-(2,4,6-Trihydroxy-3-isobutyl-phenyl)-hexan-1-one	Mou-o	>1000	1	1,6	>625	---
Bakuchiol	Mou-o	2560	5	0,5 - 5	27777 - 512	---
1-(3-Butyryl-2,4,6-trihydroxy-phenyl)-butan-1-one	Mou-o	>1000	1	3	>333	---
4-(1,1-Dimethylethyl)phenol	Rat-o	3250	2	10	325	---**
alpha-Hederin	Mou-o	>4000	1	12,5	>320	---
Thiabendazole	Mou-o	2400	10	0,2 - 7,8	12000 – 307	Drug
alpha-Bisabolol ****	Rat-o	14850	1	50	297	Food
Griseofulvin	Mou-o	>5000	>100	0,25 - 25	>20000 – 200	Drug
4-Methyl-1-(2,4,6-trihydroxy-3-isobutyl-phenyl)-pentan-1-one	Mou-o	1000	1	6	166	---
Cyclohexyl-(2,4,6-trihydroxy-3-isobutyl-phenyl)-methanone	Mou-o	>1000	1	6	>166	---
1-(3-Allyl-2,4,6-trihydroxy-phenyl)-propan-1-one	Mou-o	>1000	1	6	>166	---
1-(3-Allyl-2,4,6-trihydroxy-phenyl)-hexan-1-one	Mou-o	>1000	1	6	>166	---
1-(3-Allyl-2,4,6-trihydroxy-phenyl)-4-methyl-pentan-1-one	Mou-o	>1000	1	6	>166	---
1-(3-Benzyl-2,4,6-trihydroxy-phenyl)-4-methyl-pentan-1-one	Mou-o	1000	1	6	166	---
1-(5,7-Dihydroxy-2,2-dimethyl-chroman-8-yl)-butan-1-one	Mou-o	>1000	1	6	>166	---
Nystatin	Mou-o	8000	10	<0,1 - 50	80000 - 160	Drug
Lauric acid	Rat-o	12000	100	100	120	Food*
Undecylenic acid	Mou-o	8150	6	25 - 100	326 - 81	Drug*
Fluconazole	Mou-o	1408	>100	0,06 - >64	23467 - 33	Drug
Itraconazole	Mou-o	>320	>100	0,03 - 10	>10666 - 32	Drug
Citral	Mou-o	6000	3	20 - 190	288 - 31	Food***
Ketoconazole	Mou-o	618	>100	0,01 - 50	61800 - 12	Drug
Miconazole	Mou-iv	*****90	>100	0,01 - 16	9000 – 6	Drug

Notes: * may cause digestive tract irritation, ** tumorigen, *** mutagen, ******** active against 'dermatophytes', ********* intravenous LD_{50}

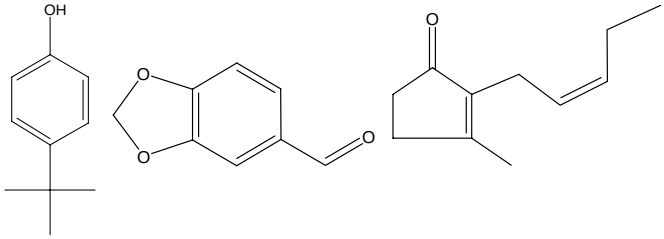
When the activity of essential oil and related compounds was examined on their effectiveness against *Trichophyton* species three sesquiterpene alcohols and two C-11 alcohols were selected (Table 13).

Table 13. Effective Inhibitors of *Trichophyton* Species, oral $TI_{max.}$ >= 100.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Undecylenic alcohol	Rat-o	>5000	1	10	500	Food*
Farnesol	Rat-o	6000	1	12,5	480	Food
Nerolidol	Rat-o	>5000	1	12,5	400	Food
4-(1,1-Dimethylethyl)phenol	Rat-o	3250 ul/kg	5	10	325	---***
n-Undecyl alcohol	Rat-o	3000	1	12,5	240	Food*
alpha-Bisabolol	Rat-o	14850	6	50 – 100	297 – 149	Food
Piperonal	Rat-o	2700	1	25	108	Food*, **
cis-Jasmone	Rat-o	5000	1	50	100	Food*
Cinnamic aldehyde	Rat-o	2220	18	5 – 400	444 – 1	Food*, **

Notes: * may cause skin irritation, ** mutagen, *** tumorigen

The structures of further effective inhibitors (4-(1,1-dimethylethyl)phenol **47**, piperonal **48**, cis-jasmone **49**) are shown next, which are all reported to have undesired side effects.



47 **48** **49**

To get information about the most successful compounds by the way of dermal application, the TIs of compounds against *Microsporum* species were calculated. As result, only a few compounds were selected as effective inhibitors and none of the investigated natural compounds were free of side effects. The highest calculations were obtained with the synthetic antifungal drugs haloprogine and triclosan (Table 14).

Table 14. Effective Inhibitors of *Microsporum* Species, dermal TI $_{max.}$ >= 100.

Trivial name	Animal test	LD_{50}-dermal mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Haloprogine	Rabbit-dermal	1625	10	0,001 - 0,4	1625000 - 4062	Drug
Triclosan	Rabbit-dermal	9300	5	1 – 10	9300 - 930	Drug
o-Methoxycinnamaldehyde	Rabbit-dermal	>5000	2	3,13 – 6,25	>1597 – 625	Food*
4-(1,1-Dimethylethyl)phenol	Rabbit-dermal	2520	1	10	252	---**
Citral	Rabbit-dermal	2250	3	20,8 – 190	108 - 12	Food*
Hexachlorophene	Rat-dermal	>600	7	1 – 100	>600 - 6	Drug

Notes: *mutagen, ** tumorigen

It would be desirable to compare dermal TIs of natural compounds with drugs used to treat topical fungal infection. However, the dermal toxicity of most of topical antifungal drugs is not published [10]. A list of TIs calculated with oral toxicity data is shown below (Table 15).

Table 15. Effectiveness of Topical Antifungals towards *Microsporum* Species.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Tolciclate	Mou-o	4000	>20	0,001 - 0,13	1000000 - 64516	Drug
Sulconazole	Mou-o	nitrate: 2475	2	<0,04 – 0,08	>61875 – 30937	Drug
Siccanin	Mou-o	>6000	2	0,8 - 1,6	>7500 - 3750	Drug
Oxiconazole	Mou-o	nitrate: 2630	2	0,3 - 1	8766 - 2630	Drug
Tioconazole	Mou-o	1870	10	0,2 - 1,56	9350 - 1200	Drug
Fenticonazole	Mou-o	>3000	1	2,5	>1200	Drug
Amphotericin B	Mou-o	>8000	>100	0,125 - 8	>64000 - 1000	Drug
Amorolfin	Mou-o	400	50	0,001 - 0,2	400000 - 800	Drug
Bifonazole	Mou-o	2629	>100	0,025 - 5	105000 - 525	Drug
Triclosan	Mou-o	4530	4	0 - 10	4530 – 453	Drug
Flutrimazole	Mou-o	>1000	14	0,025 - 2,5	>40000 – 400	Drug
Econazole	Mou-o	nitrate: 463	10	0,03 - 12,5	15433 – 370	Drug
Clotrimazol	Mou-o	923	>100	0,01 - 2,69	92300 – 369	Drug
Ciclopirox	Mou-o	1740	>40	0,98 - 10	1775 – 174	Drug
Nystatin	Mou-o	8000	10	<0,1 - 50	80000 - 160	Drug
Natamycin	Mou-o	1500	1	12,5	120	Drug
Haloprogine	Mou-o	LD >3000	7	31,2	>2000000 - 96	Drug
Miconazole	Mou-o	519	>100	0,01 - 16	51900 - 16	Drug
Ketoconazole	Mou-o	618	>100	0,01 - 50	61800 - 12	Drug
Tolnaftate	Mou-o	10000	>60	0,0015 - 1000	6666666 - 10	Drug
Protiofate	Mou-o	423	8	0,78 - 50	542 - 8	Drug

3.7. Candida albicans

Yeasts live as normal microorganisms in and on the human body. Many species are without any clinical significance, however, some of them develop pathological changes in debilitated persons, e.g. following administration of anticancer drugs or immunsuppressive agents such as corticosteroids and by overuse of broad-spectrum antibiotics [17]. *Candida albicans* causes systemic and topical

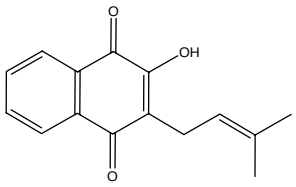
infections in children, and therefore, respective TIs are calculated for oral (Table 16) and topical (Table 17) administration routes.

Table 16. Effective Inhibitors of *Candida albicans*, oral TI $_{max.}$ >= 100.

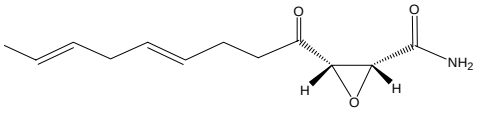
Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Lapachol	Mou-o	487	1	0,03	16233	---
Dodecanal	Rat-o	23000	1	25	920	Food*
Linoleic acid	Mou-o	>50000	1	125	>400	Food*
Swartziadione	Mou-o	LDL0 >300	1	0,78	>384	---
Cerulenin	Mou-o	547	1	<1,5	>364	---
Amphotericin B	Mou-o	>8000	>100	0,02 - 25	>400000 - 320	Drug
Ketoconazole	Mou-o	618	>100	0,039 - 2	19776 - 309	Drug
Nystatin	Mou-o	8000	>100	0,78 - 33	10256 - 242	Drug
Oenanthic acid	Mou-o	6400	1	32,5	197	Food
Fluconazole	Mou-o	1408	>100	<=0,0313 - 8	>45000 - 176	Drug
Flucytosine	Mou-o	>15000	>100	0,005 - >128	>3000000 - <117	Drug
4-(1,1-Dimethylethyl)phenol	Rat-o	3250	1	31,25	104	---**
Butylparaben	Mou-o	13200	6	75 - 130	176 - 101	Food
o-Methoxycinnamaldehyde	Rat-o	>5000	3	50	>100	Food***
Decylenic alcohol	Rat-o	>10000	1	100	>100	---
Octanoic acid	Rat-o	10080	2	18 – 145	560 – 69	Food*
alpha-Bisabolol	Mou-o	11350	4	100 – 500	113 - 22	---
Miconazole	Mou-iv	90	82	0,02 - >25	4500 - <3,6	Drug
Citral	Mou-o	6000	16	10 – 10000	600 – 0	Food
n-Dodecanol	Rat-o	>12800	2	25 – 100000	>512 - 0	Food*
Undecylenic acid	Mou-o	8150	1	70 – 1000	116 - 8	Drug*
Sorbic acid	Mou-o	3200	1	25 – 10000	128 - 0	Food

Notes: * may cause digestive tract irritation, ** tumorigen, *** mutagen

The most successful compound against *Candida albicans* was lapachol **50** according to the oral TI calculations. Lapachol is a green-yellow substance found in the inner bark of Brazilian Taheboo tree (*Tabebuia avellanedae*), which is traditionally used in South America to treat *Candida* and bacterial infections. Lapachol inhibited growth of *Candida albicans* at a very low concentration, but the germicidal concentration was found to be much higher (32 ppm). Beside long-chains aliphatics having a terminal polar group and 8 to 12 carbon atoms, swartziadione **16** was selected, a structurally related compound to lapachol, and in addition, cerulenin **51** from microbial origin (e.g. *Cephalosporium caerulens*). Cerulenin was found to inhibit growth of yeast-type fungi by inhibiting the biosynthesis of fatty acids [26]. All before mentioned compounds superimpose the minimum TIs of orally administered antibiotics usually taken to treat *Candida* infections. Among terpenoids only alpha-bisabolol resulted in promising a TI, while acute toxicity data of other interesting compounds have not been determined.



50



51

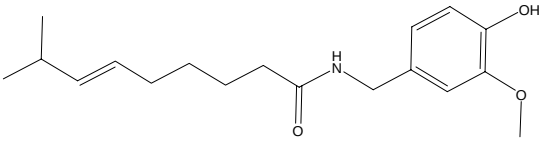
When calculating dermal TIs of anti-candidal drugs no results were calculable with standard therapeutics amphothericin B, nystatin, clotrimazole and miconazol due to missing acute dermal toxicity data [10]. The residual calculations of the most effective compounds are shown in Table 17.

Table 17. Effective Inhibitors of *Candida albicans*, dermal $TI_{max.} >= 100$.

Trivial name	Animal test	LD_{50}-oral mg/kg bw	n-strains	MIC range in ppm	TI max. - TI min.	Status
Capsaicin	Mou-dermal	>512	1	<1,5	>340	Food**, ***
Triclosan	Rabbit-dermal	9300	12	10 - 33	930 - 282	Drug
Haloprogine	Rabbit-dermal	1625	>100	0,05 - 6,25	32500 - 260	Drug
Sclareol	Rabbit-dermal	>5000	1	32	>156	---
Undecanal	Rabbit-dermal	>5000	1	50	>100	Food*
o-Methoxycinnamaldehyde	Rabbit-dermal	>5000	2	50	>100	Food**
Cloconazole	Rabbit-dermal	HCl: >310	>100	0,12 - 8	>160 - 39	Drug
Caprylic acid	Rabbit-dermal	>5000	4	18 - 1000	>277 - 5	Food*
Camphor	Rabbit-dermal	>5000	5	50 - 2000	>100 - 2,5	Food*
Citral	Rabbit-dermal	2250	17	10 - 10000	225 - 0	Food*, **

Notes: * may cause skin irritation, **mutagen, *** tumorigen

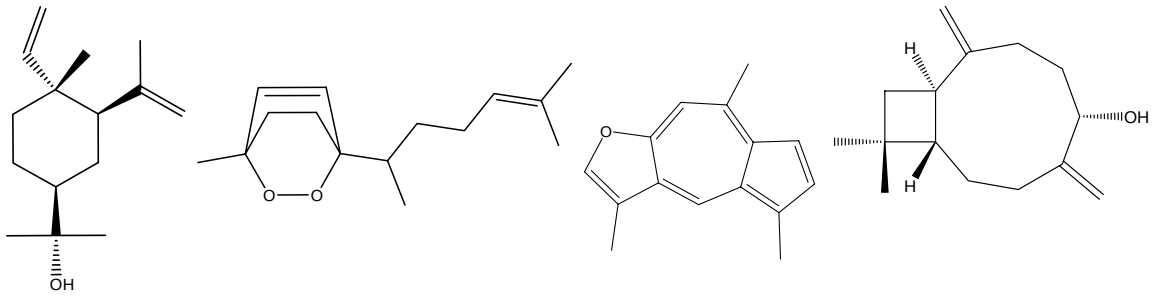
Only a few effective compounds were found, of which the most successful compound is sclareol **31** from Clary Sage (*Salvia sclarea*). This compound was patented to treat human fungal infections and its recommended to be used internally at daily doses of 1 to 1000 mg/kg [27]. Other compounds may have undesired side effects, such as capsaicin **52,** the main compound in Chilly (*Capsicum frutescens*) or Red Pepper (*Capsicum annuum*).



52

Alpha-bisabolol (MIC = 100 ppm) **6** is widely used in topical cosmetic preparations and it is regarded as safe on human skin, although its numeric dermal LD_{50} value has not been published [19]. A few other compounds show also promising activity against *Candida albicans*, however, their acute toxicity is unknown. These compounds are commercially available elemol (MIC = 70 ppm) **53**, while

others (3,6-epoxydioxy-bisabola-1,10-diene: MIC: 6,25 ppm **54**, linderazulene: 12,5 ppm **55** and caryophyllodienol: 12,5 ppm **56**) are isolated from plants only in a few cases.



53 **54** **55** **56**

3.8. Other Important Microorganisms Causing Children's Infections

About causative microorganism of other important diseases typical for children, such like pertussis, epiglottis, tetanus and diphtheria, not much information is obtained about effective inhibitors in TI calculations.

Bordetella pertussis (responsible for pertussis) was found to be susceptible towards aliphatic acids. No effective compound was found in the cases of *Haemophilus influenzae* (epiglottis) and *Clostridium tetani* (tetanus). Alpha-bisabolol (oral TI > 200) **6** and to a lesser extend camphor (oral TI = 90) **2** were effective against the diphtheria causing microorganism *Corynebacterium diphtheriae*.

4. Discussion

The effectiveness of antibiotics used to treat children's diseases is superior to that of compounds produced by higher plants according to the before mentioned criteria and data analysis.

The antimicrobial plant constituents differ in their spectrum of activity from antibiotic drugs and show only poor effectiveness against gram-negative pathogens, such like *Escherichia coli*, *Salmonella species*, *Bordetella pertussis* and *Haemophilus influenzae*. Several effective inhibitors of low mammalian toxicity ($TI_{min.}$ >100) were selected in the analysis with gram-positive bacteria: *Streptococcus pneumoniae* (m-menthene-phenol **5**, alpha-bisabolol **6**, lupulone **7**), *Staphylococcus aureus* (m-menthene-phenol **5**, alpha-bisabolol **6**, lupulone **7**, bakuchiol **15**, swartziadione **16**, shikonin **17**, hinokitiol **19**) and *Mycobacterium tuberculosis* (bakuchiol **15**, farnesol **21**, sclareol **31**, cis- **32** and trans-phytol **33**, vitamin A acid **34**, methyl- **36** and propylparaben **37**, phenylsalicylate **38**, asym.-m-xylenol **39**, hexyresorcinol **40**, alpha- **41** and beta-naphthol **42**). As effective inhibitors of dermatophytic fungi were found: alpha-bisabolol **6**, bakuchiol **15**, nerolidol **20**, farnesol **21**, a series of phenylbutanone derivatives **43**, alpha-hederin **44**, o-methoxycinnamaldehyde **46**, 4-(1,1-dimethylethyl)phenol **47**, piperonal **48**, and cis-jasmone **49**. As the most successful compound against

Candida albicans turned out to be: swartziadione **15**, sclareol **31**, lapachol **50**, capsaicin **52**. In addition, alpha-bisabolol **6** was effective against *Corynebacterium diphtheriae*.

Among the typical components of essential oils especially sesquiterpene and diterpene alcohols were selected (alpha-bisabolol **6**, nerolidol **20**, farnesol **21**, cis- **32** and trans-phytol **33**, sclareol **31**). Monoterpenes, such as thymol **8** or carvacrol **9**, and phenylpropanes, such as eugenol **3**, being prominent for their antimicrobial activity failed in this analysis due to their comparatively high mammalian toxicity.

Not many of the isolated natural inhibitors of children's pathogens have been tested against a greater number of microbial strains of one species as it the case with standard therapeutics. Thus, the ranges of antimicrobial activity are generally unknown, which are important for the judgement about the usefulness of such compounds as therapeutic agent. Another important topic aspect is the use of non-standardized test methods in microbiology, which also causes variation of data.

The calculation of oral TIs aimed to analyze the effectiveness of compounds by oral application. Other than in laboratory *in vitro* experiments an orally administered compound undergoes metabolic changes inside the body of living organism. For instance, the metabolites of trans-anethole **57**, the main constituent of the essential oil of anise *(Pimpinella anisum*), were studied in two human volunteers [28]. Following ingestion of 1 mg methoxy ^{14}C-labelled trans-anethole approx. 21% of the administered dose was demethylated within 8 hours as it could be determined by the amount of exhaled $^{14}CO_2$. Within 24 hours approx. 65% of the totally administered radioactivity was found in the urine, while about 10% of the radioactivity was not recovered (Figure 2).

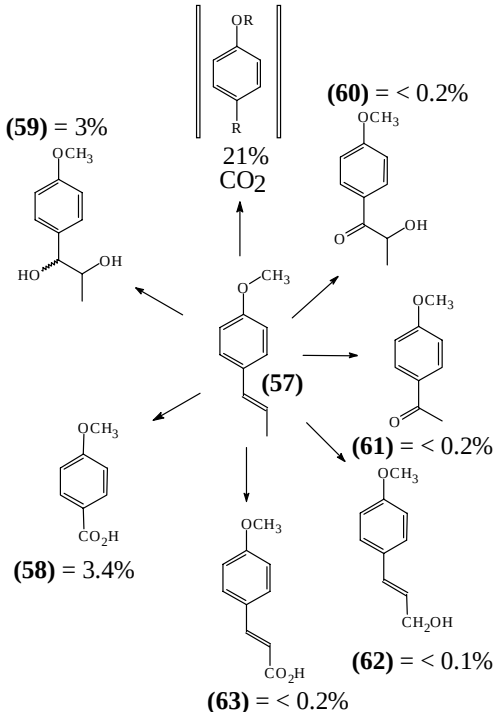


Figure 2. Metabolization of Orally Administered trans-Anethole in Humans.

Among urinary metabolites main conversion products were *O*-demethylated compounds and compounds of known structure **57** to **62**. This rapid biotransformation of a trans-anethole may help to interpret the findings obtained in a comparative *in vivo* - *in vitro* study with mycobacteria and trans-anethole [29]. Anethole was most effective in tuberculosis protection of guinea pigs *in vivo*, but failed to have considerable activity *in vitro* (Table 18).

Table 18. Comparison of the *in vitro* Inhibition of *Mycobacterium tuberculosis* by Essential Oils and some of their Components with the *in vivo* Disease Protection by such Materials of Guinea Pigs Infected with Tubercle bacilli.

Test materials	***in vivo***	***in vitro***
Anethole	++++	++
Lemon oil	+++	+
Nutmeg oil	++	+
Terpineol	++	++++
d-Limonene	+	+++
l-Limonene	+	+++
Geraniol	+	++
Eugenol	+	++++
d-Pinene	+	+++
l-Pinene	-	++

Notes: ++++ very active, +++ moderate active, ++ active, poor effect, - no effect

By means of these results, it is obvious that the effects of test compounds can not be explained with *in vitro* testing alone, although this method-strategy was successful many times in antibiotic research.

If a metabolizing system, however, is absent or plays a minor role, the active compound is available without major losses. Treatment of localized infections of outer and inner surfaces of the body with antimicrobials from higher plants is imaginable, e.g. topical application onto skin, inhalation (respiratory tract) and ingestion (gastrointestinal tract).

The compounds selected on the basis of dermal toxicity data are may be useful in the therapy or support of therapy of localized skin infections. Especially, several alcohols occurring in essential oils were effective against hair and skin pathogens, which are n-undecanol, n-undecenol, alpha-bisabolol **6**, nerolidol **20** and farnesol **21**.

Inhalation of volatile antimicrobials causes direct contact to undesired microorganisms present on mucous membranes of the respiratory tract. Similar to entire essential oils, a treatment of respiratory tract diseases with selected compounds from essential oils is imaginable.

In the gastrointestinal tract structural changes of essential oil compounds are known and influence in part markedly the chemical constitution. For instance, reports exist on rapid rearrangement of linalool to geraniol **14** through influence of gastric juice in the stomach [30], or the formation 3,4-dihydroxy-propylbenzene from methyl isoeugenol (3,4-dimethoxy-1-propenylbenzene) by action of the caecal microbial flora [31].

Appearance of essential oil compounds in the bloodstream following administration to skin and percutaneous resorption [32] or following inhalation [33] was demonstrated in humans and animals, respectively, which implies further pharmacological effects.

Among compounds found in essential oils, alpha-bisabolol **6** from Chamomile turned out to be safe following inhalation and simultaneously it is inhibitory to several microbial species pathogenic for children.

5. Conclusion

A comparison of the effectiveness of natural compounds with such of antibiotics on the basis of oral 'Therapeutic Indices' calculations revealed that natural compounds are inferior to antibiotics in general. The natural compounds are mild oral antimicrobials and they are unfit to treat severe infections of children.

Only in the case of tuberculosis several natural compounds of low mammalian toxicity and strong *in vitro* activity exist, however, these findings need further verification in living organisms.

Despite the limited value of essential oil compounds as oral antibiotics, the dermal 'Therapeutic Index' calculations may lead to findings, which are helpful in the therapy or in the supportive therapy of skin infections in children. Inhalation of isolated compounds from essential oil may be also useful in the support of therapy of respiratory tract infections.

This data analysis shows that the natural resources of antimicrobials are not fully explored. The effectiveness of most of the natural compounds as antimicrobials cannot be fully evaluated due to many missing toxicity data. The existing data material on volatile sesquiterpenes indicates their low mammalian toxicity and relatively strong antimicrobial activity, and therefore, this group of compounds turned out to be the most interesting one.

References

1. Schilcher, H. Pharmakologie und Toxikologie ätherischer Öle. *Therapiewoche* **1986**, *36*, 1100-1112.
2. Österreichisches Arzneibuch (1990) + supplements 1-6 (1990-1999), European Pharmacopoeia 3 (1997) + supplements (1998 – 2000), Pharmacopée Française X + supplements 1-13 (1983 – 1996), Deutsches Arzneibuch (2000) and Deutscher Arzneimittel-Codex (1997-2000), Farmacopea Ufficiale della Repubblica Italiana IX + supplements I-IV (1985 – 1996), The Japanese Pharmacopoeia XIII (1996) + supplements I + II (1999), Pharmacopoeia Helvetica 8 (1997) + supplements (1998-1999), Britisch Pharmacopoeia (1999) and US National Formulary 19 (2000) + supplements (2000).
3. Craig, J.O. Poisoning by the Volatile Oils in Childhood. *Archives of Disease in Childhood* **1953**, *28*, 475-483.
4. Schilcher, H. Sind pflanzliche Arzneimittel bzw. ist die "NATURMEDIZIN" eine Gefahr für den Anwender. *Ärztezeitschrift für Naturheilverfahren* **2002**, *43*, 253-254.

5. Dorsch, W.; Loew, D.; Meyer-Buchtela, E.; Schilcher, H. *Kinderdosierungen von Phytopharmaka. Teil I. Empfehlungen zur Anwendung und Dosierungen von Phytopharmaka, monographierte Arzneidrogen und ihren Zubereitungen in der Pädiatrie*. Kooperation Phytopharmaka GbR: 2nd edition ; ISBN 3-929964-14-7; Bonn; **1998**, p. 9-185.
6. Giacosa, P. Studj sulla azione fistologica di alcune sostanze aromatiche messa in rapporto collo loro struttura atomica. *Ann. Chim. Medicofarm. Farm. Ser*. **1886**, *3*, 273-293.
7. Schilcher, H. *Phytotherapie in der Kinderheilkunde – Handbuch für Ärzte und Apotheker*; Wissenschaftliche Verlagsgesellschaft mbH Stuttgart: 3. Auflage, ISBN 3-8047-1644-X; Stuttgart; **1999.**
8. Kuschinski, G.; Lüllmann, H.; Peters, T. *Kurzes Lehrbuch der Pharmakologie und Toxikologie*; Georg Thieme Verlag: 10. Auflage, ISBN 3-13-368510-4, Stuttgart – New York; **1984.**
9. Pauli, A. *AMICBASE-EssOil: Database on Natural Antimicrobials and Officinal Antiinfectives*; ReviewScience: http://www.reviewscience.com; Zirndorf; **2003.**
10. Woodard, D.; Stuart, R. *Toxicology Reports*; Vermont Safety Information Resources, Inc.: http://www.hazard.com/msds; Vermont; **2003.**
11. Merck Sharp and Dohme Research Laboratories <Rahway, N.Y.>. *Merck Manual of Diagnosis and Therapy*; Urban & Schwarzenberg: 16th ed.; ISBN 3-541-01855-0; München – Wien – Baltimore; **1993.**
12. World Healh Organization. *World Health Report: Global Burden of Disease Estimates;* Geneva; **2002.**
13. Kahlmeter, G.; Olsson-Liljequist, B. *Species related MIC-breakpoints*; Landstinget Kronoberg: http://www.ltkronoberg.se; Kronoberg; **2003.**
14. World Health Organization: Department of Communicable Disease Surveillance and Response. *Whonet 5: Microbiology Laboratory, Database Software*; World Health Organization (Geneva) and WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, (Microbiology Department, Brigham and Women's Hospital, Boston); **1999.**
15. United States Food and Drug Administration: *Code of Federal Regulations. Title 21. Food and Drugs*; Rockville; **2003.**
16. Daschner, F. *Antibiotika am Krankenbett*; Springer Verlag: 11th ed.; Berlin – Heidelberg – New York – Barcelona – Hongkong – London – Mailand – Paris – Singapur – Tokio; **2002.**
17. Murray, P.R.; Baron, E.J.; Pfaller, M.A.; Tenover, F.C.; Yolken, R.H. (eds.). *Manual of Clinical Microbiology*; ASM Press: ISBN 1-55581-086-1; Washington, D.C.; **1995.**
18. Lamar, R.V. Some Further Observations Upon the Action of Certain Soaps on the Pneumococcus and its Experimental Infection. *Journal of Experimental Medicine* (New York) **1911**, 14, 256-64.
19. Anonymous. Final report on the Safety Assessment of Bisabolol. *International Journal of Toxicology* **1999**, *18* (Supplement 3), 33-40.
20. Sieradzki, K.; Roberts, R.B.; Haber, S.W.; Tomasz, A. The Development of Vancomycin Resistance in a Patient with Methicillin-Resistant Staphylococcus aureus Infection. *The New England Journal of Medicine* **1999**, *340*, 517-523.

21. Endtz, H.P.; Mouton, J.W.; Den Hollander, J.G.; Van den Braak, N.; Verbrugh, H.A. Comparative in vitro Activities of Trovafloxacin (CP-99,219) against 445 Gram-positive Isolates from Patients with Endocarditis and those with other Bloodstream Infections. *Antimicrobial Agents and Chemotherapy* **1997**, *41*, 1146-1149.
22. Katsura, H.; Tsukiyama, R.-I.; Suzuki, A.; Kobayashi, M. In Vitro Antimicrobial Activities of Bakuchiol against Oral Microorganisms. *Antimicrobial Agents and Chemotherapy* **2001**, *45*, 3009-3013.
23. Lin, Y. Compositions for Treatment of Antibiotic-Resistant Gram-Positive Bacterial Infections and Methods for Using and Preparing the Same. *United States Patent*, **2000**, 6025400.
24. Villegas, L.F.; Marcalo, A.; Martin, J.; Fernandez, I.D.; Maldonado, H.; Vaisberg, A.J.; Hammond, G.B. (+)-epi-Alpha-bisabolol [correction of bisbolol] is the Wound-Healing Principle of Peperomia galioides: Investigation of the in Vivo Wound-Healing Activity of Related Terpenoids. *Journal of Natural Products* **2001**, *64*, 1357-1359; **2002**, *65*, 248 (erratum).
25. Chin, Y.; Anderson, H.H.; Alderton, G.; Lewis, J.C. Antituberculous Activity and Toxicity of Lupulon for the Mouse. *Proceedings of the Society for Experimental Biology and Medicine* **1949**, *70*, 158-162.
26. Royer, G.P.; Townsend, C.A. Cerulenin Compounds for Fatty Acid Synthesis Inhibition. *United States Patent*, **1996**, 5539132.
27. Nozoe, S.; Takahashi, A.; Masuda, J.; Segawa, T. Antifungal Agent. *Japanese Patent*, **1998**, 10298070.
28. Sangster, S.S.; Caldwell, J.; Hutt, A.J.; Anthony, A.; Smith, R.L. The Metabolic Disposition of [Methoxy-14C]-Labelled trans-Anethole, Estragole and p-Propylanisole in Human Volunteers. *Xenobiotica* **1987**, *17*, 1223-1232.
29. Imura, K. On the Influence of the Ethereal Oils Upon the Culture of Tubercle Bacilli and Upon Development of Experimental Tuberculosis in Animals. *Journal of the Shanghai Science Institute (Section 4)* **1935**, *1*, 235-270.
30. Franchomme, P. Analytical and Pharmacological Approach of Aforementioned Species; Lecture; 4th International Scientific Aromatherapy Symposium, March 2 – 4; Grasse; **2001.**
31. Solheim, E.; Scheline, R.R. Metabolism of Alkenebenzenes in the Rat. II. Eugenol and Isoeugenol Methyl Ethers. *Xenobiotica* **1976**, *6*, 137-150.
32. Schalter, R. *Perkutane Resorption ätherischer Öle mit und ohne Zusatz von Nicotinsäuerestern*; Dissertation, München; **1985.**
33. Buchbauer, G.; Jirovetz, L.; Jäger, W.; Plank, C.; Dietrich, H. Fragrance Compounds and Essential Oils with Sedative Effects Upon Inhalation. *Journal of Pharmaceutical Sciences* **1993**, *82*, 660-664.