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Abstract

The antimicrobial activity of essential oils is discussed in this review taking in account studies which were published in the period of time from 2008 until September 2010. Furthermore, the most important methods to examine the antimicrobial efficiency of essential oils are presented. The studies are divided into the following three groups depending on the activity of the applied essential oil against the test microorganisms: antimicrobial, antifungal active agents and substances which inhibit the growth of yeasts. Various interesting possible applications are revealed such as the use of essential oils instead of synthetic drugs to circumvent the increasing resistance of some pathogens. Moreover, they could not only be used for the therapy of infectious illnesses, but also as preservatives in the food industry. A further possibility is among others the application of essential oils in skin products in order to treat or avoid dermal infections. Additionally, the prevalent constituents of the individual antimicrobial active essential oils are elaborated.

Zusammenfassung

Die antimikrobielle Wirkung von ätherischen Ölen wird in diesem Review unter Berücksichtigung von Studien, die in der Zeitspanne von 2008 bis September 2010 veröffentlicht wurden, diskutiert. Außerdem werden die wichtigsten Methoden zur Bestimmung der antimikrobiellen Wirksamkeit von ätherischen Ölen präsentiert. Die Studien werden in die folgenden drei Gruppen unterteilt, abhängig von der Aktivität des verwendeten ätherischen Öls gegen die Testkeime: antimikrobielle, antifungale Wirkstoffe und Substanzen, die das Wachstum von Hefen hemmen. Verschiedene interessante Anwendungsmöglichkeiten werden aufgezeigt, wie zum Beispiel die Anwendung von ätherischen Ölen an Stelle von synthetischen Wirkstoffen, um die ansteigende Resistenz von einigen Pathogenen zu umgehen. Außerdem können sie nicht nur zur Therapie von infektiösen Erkrankungen eingesetzt werden, sondern auch als Konservierungsmittel in der Lebensmittelindustrie. Eine weitere Möglichkeit ist unter anderem die Anwendung von ätherischen Ölen in Hautprodukten, um dermale Infektionen zu behandeln oder zu vermeiden. Des Weiteren sind die vorherrschenden Bestandteile der einzelnen antimikrobiell wirksamen ätherischen Öle ausgearbeitet.

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INTRODUCTION

Essential oils (EOs) possess a wide spectrum of different impressive qualities including antiphlogistic, spasmolythic, antinociceptive and antioxidant activity. Moreover they exert immunomodulant, psychotrope, acaricide and expectorant effects.[1]Due to their multifunctionality, EOs find a huge application area in medicine and aromatherapy.

Also antiviral, antidiabetic and cancer suppressive activities are observed. In addition to further other effects, EOs show significant antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria. That is why they were already used for embalming in Ancient Egypt.[2]

In the course of history EOs were always applied for their antimicrobial effects in traditional medicine. Therefore, plants were used for the treatment of infectious illnesses since ancient times even though no knowledge about microorganisms existed by then.[3]

Medicinal plants are of course still in use nowadays, but now the investigation of the active agents is possible by modern means. The isolation of EOs and their characterization by using gas chromatography (GC) and mass spectrometry (MS) systems are common practice. Moreover their antimicrobial activity can be verified by *in-vitro* tests. EOs get even more popular regarding the fact that many synthetic drugs are connected with unpleasant side-effects. Volatile oils also represent an interesting alternative due to emerging resistance of microorganisms against synthetic agents.

EOs cannot only exert bacteriostatic and bactericidal effects, but also demonstrate activity against fungi and yeasts.

This paper focuses on the antimicrobial and antifungal activity of EOs concentrating on studies that have been published since 2008 until September 2010.

IN VITRO TESTS TO ASSESS ANTIMICROBIAL ACTIVITY

Several methods are used to investigate the antimicrobial activity of EOs. The three most important ones are: The agar diffusion test, the agar or broth dilution test and the vapour phase test.[4]

Agar Diffusion Test:

A petri dish filled with microorganisms containing agar is needed to perform this method. The EO is either directly applied to the surface – in this case small holes are punched into the agar surface - or put on a small paper disk which is afterwards placed onto the agar. The antimicrobial activity can be estimated from the size of the originating inhibition zone. Nevertheless it is important to point out that this test method is not completely free of any problems. This conclusion can be drawn from the fact that in some cases the results of the agar diffusion test showed small antimicrobial activity, but the same EO proved high activity in dilution tests. Especially components with low water solubility showed misleadingly low antimicrobial activity. Moreover the different volatility of single constituents, then often unknown diffusion coefficients and other side effects have to be considered.[4]

Dilution Test:

In the **broth dilution** test concentration series of the antimicrobial substance are established using a broth medium which is seeded with microorganisms. The minimal inhibitory concentration (MIC) is evaluated in order to determine the antimicrobial potency of the tested substance.

In the **agar dilution** test a concentration gradient of the tested substance is placed onto an agar plate. By evaluating the microbial growth the MIC can be stated likewise. This method is declared to be the gold standard but it is not that often used since it is connected to higher costs and laborious handling.[5]

When performing the dilution test method with EOs it is adjuvant to create a saturated moistened atmosphere to adjust volatility.[4]

Vapour Phase Test:

Up to now, there is no standardized method available for the vapour phase test. In general, a seeded agar plate is placed upside-down onto a reservoir which comprises

a certain amount of volatile oil. In this case the generated inhibition zone is considered as criterion for the antimicrobial activity.[4]

Aromatogram:

The procedure of developing an aromatogram resembles the agar diffusion test. That is why the test microorganisms which are cultivated on an agar plate are exposed to certain amounts of EOs which are spread on paper disks. The antimicrobial efficacy of the EO is likewise determined by inhibition zones.[6]

The crucial difference between these two techniques is not the course of action itself, but the substances which are investigated for their potentially antimicrobial activity. Therefore, aromatograms always indicate the use of exclusively EOs whereas antibiograms include also other active substances such as synthetic drugs.[7]

Air washer coupled with Air Sampler:

This test method allows the determination of the antimicrobial activity of EOs against air-borne microbes. A special machine called air washer is filled with diluted EO which is vaporized into the room. By the air sampler air-borne microorganisms are fixed on agar strips. After incubating these strips the number of microbes in the air can be counted. As a result, the comparison between the amount of bacteria before and after the application of EO vapours is facilitated.[8]

ANTIMICROBIALS

Antimicrobial agents inhibit the growth of microorganisms or lead to their death. In the following chapter studies are presented that deal with the effect of EOs on bacteria and yeasts.

Former studies indicate a higher antibacterial effect of EOs against Gram-positive than against Gram-negative bacteria. The outer cell membrane of Gram-negative bacteria obtains hydrophilic qualities that impede the contact of the hydrophobic constituents of the EO with the bacterial cell.[9]

Contrary to this, EOs can directly impair the cell membrane of Gram-positive bacteria leading to cell membrane rupture, blocking of enzyme systems and progressivity of ion permeability.[10]

EOs against drug-resistant bacteria strains

The increasing tolerance of several microorganisms against commonly used antibiotic drugs represents a challenge for scientists to find alternative ways for the treatment of such infections. One of the main causes that provokes the higher resistance of microorganisms is the loose application of drugs.[11] This includes that they are applied in too low concentrations, not specific enough or without serious indication. Especially methicillin-resistant *Staphylococcus aureus* (*MRSA)* strains are popular test microorganisms.

S. aureus – a Gram-positive bacterium which is common part of the human microbial skin flora - can cause minor infections, but nevertheless also severe diseases such as pneumonia, sepsis, endocarditis or meningitis particularly in hospitalized patients. The increasing resistance of these pathogens against current drugs tremendously complicates the therapy of these infections.[12]

effective against	EO	main constituents	test method	Ref.
MRSA, vancomycin-resistant *Enterococcus faecium (VRE),* multidrug-resistant strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*	*Cleistocalyx operculatus* (Roxb.) Merr and Perry (Myrtaceae)	γ-terpinene (5.8%) globulol (5.6%) cis-linalool oxide (5.2%)	MIC = 5 - 20 μl/ml	[13]
MRSA	*Eucalyptus globulus* Labill. (Myrtaceae)	1,8-cineole (47.2%)	MIC = 85.6 μg/ml	[14]
MRSA, VRE	*Kadsura longipedunculata* Finet & Gagnepain (Schisandraceae)	δ-cadinene (21.8%)	diffusion test, dilution test	[15]
MRSA	*Lavandula angustifolia* Mill. (Lamiaceae)	linalyl acetate (37.0%), linalool (29.5%)	disk diffusion	[16]
MRSA	*Lavandula latifolia* Medik (Lamiaceae)	linalool (38.8%), 1,8-cineole (28.5%)	disk diffusion	[16]
MRSA	*Lavandula luisieri* Rozeira Riv.-Mart. (Lamiaceae)	α-necrodyl acetate (34.5%), 1,8-cineole (17.6%)	disk diffusion	[16]

MRSA	*Lavandula stoechas* L. ssp. *stoechas* (Lamiaceae)	α-fenchone (39.2%), myrtenyl acetate (9.5%), α-pinene (6.1%), camphor (5.9%)	MIC = 31.2 μg/ml	[17]
MRSA	*Salvia rosifolia* Sm. (Lamiaceae)	α-pinene, 1,8-cineole	MIC = 125 μg/ml	[18]
MRSA	*Tanacetum parthenium* (L.) Schultz Bip. (Asteraceae)	camphor (49.0-60.8%)	MIC = 125 μg/ml	[19]
MRSA	*Thymus vulgaris* L. (Lamiaceae)	thymol (48.1%)	MIC = 18.5 μg/ml	[14]
MRSA	*Zataria multiflora* Boiss. (Lamiaceae)	thymol (38.7%), carvacrol (15.3%), rho-cymene (10.2%)	MIC = 0.25-1.0 μl/ml MBC = 0.5-2.0 μl/ml	[20]
MRSA	*Zanthoxylum tingoassuiba* St.-Hil. (Rutaceae)	α-bisabolol, methyl-N-methylanthranilate	disk diffusion	[21]

Table 1: EOs and MRSA

Helichrysum italicum (Roth) G.Don fil. (Asteraceae) EO which contained among other constituents geraniol showed an inhibitory activity against multidrug resistant strains of the Gram-negative bacteria *Acinetobacter baumannii, Enterobacter aerogenes, Escherichia coli* and *P. aeruginosa.* The susceptibility of these pathogens was considerably enhanced by combining commonly used drugs such as β-lactams, chloramphenicol and quinolones with geraniol. *H. italicum* EO was presumed to obtain substances which act as efflux pump inhibitors since the EO revealed to be

especially active against bacteria which over-expressed efflux pumps and therefore developed tolerance towards drugs.[22]

The volatile oil of *Melaleuca alternifolia* Cheel. (Myrtaceae) comprises among other constituents the antimicrobial active agent terpinen-4-ol. In *in-vitro* tests the bacteriostatic and bactericidal activity of both *M. alternifolia* EO and its isolated component terpinen-4-ol was ascertained against coagulase-negative staphylococci and *MRSA* showing much stronger activity when using terpinen-4-ol on its own. As a consequence terpinen-4-ol could constitute an interesting alternative in the therapy of *MRSA* infections of the skin.[23] *MRSA* and *MSSA* strains got adjusted to *M. alternifolia* EO when it was applied at sub-lethal concentrations. These strains developed higher resistance to the EO but also to antibiotics. After the same treatment coagulase-negative Staphylococci showed likewise lower vulnerability to antibiotics, but the effect of *M. alternifolia* EO was not decreased. Therefore, it is important to use EOs in high enough concentrations to avoid this adaptation.[24]

The above mentioned results support the idea of using EOs as an alternative to well-established drugs since they show high efficacy in inhibiting drug-resistant bacteria strains. The EOs could be used on their own, but also in combination with other EOs or synthetic active agents since synergy was observed by combining these substances. Therefore, synergistic effects were noticed regarding *Z. multiflora* EO in combination with the synthetic active agent vancomycin[20], but also when combining different EOs such as *L. luisieri* EO with *L. angustifolia* or *L. stoechas* EO.[16]

EOs against *Propionibacterium acnes* and *Staphylococcus epidermidis*

The following studies show that EOs are capable of inhibiting the growth of bacteria which are linked to the occurrence of skin infections, such as *P. acnes, Propionibacterium granulosum*[25] or *S. epidermidis.* Interestingly, no differences were noticed between the activity against drug-sensitive and drug-resistant bacteria strains.[26] Due to that, EOs could be used in acne therapy or in cosmetic products for the prevention and treatment of skin infections.[27]

effective against	EO	main constituents	test method	Ref.
P. acnes, *S. epidermidis*	*Abies koreana* E.H.Wilson (Pinaceae)	bornyl acetate (30.4%), limonene (19.0%)		[28]
S. epidermidis	*Acronychia pedunculata* (L.) Miq. (Rutaceae)	α-pinene (57.4%), *(E)*-β-caryophyllene (13.6%)		[29]
P. acnes, *S. epidermidis*	*Citrus natsudaidai* Hayata (Rutaceae)	limonene (81.6%)	MIC = 0.31 μl/ml MIC = 10.0 μl/ml	[27]
P. acnes, *S. epidermidis*	*Citrus obovoidea* Hort. ex Takahash (Rutaceae)	limonene (83.4%)	MIC = 0.31 μl/ml MIC = 2.5 μl/ml	[27]
P. acnes, *S. epidermidis*	*Citrus sunki* Hort. ex. Tan. (Rutaceae)	dl-limonene (68.2%)		[30]
P. acnes	several *Citrus* species (Rutaceae)	limonene (67.7 to 91.7%), myrcene (2.6 to 25.3%)	MIC = 1.25 to 20 μl/ml	[31]
P. acnes, *S. epidermidis*	*Cryptomeria japonica* (Thunb. ex L. f.) D.Don (Cupressaceae)	kaurene (17.2%), elemol (10.9%), γ-eudesmol (9.4%), sabinene (8.9%)	MIC = 0.156 to 10.00 μl/ml	[26]
P. acnes, *S. epidermidis*	*Fortunella japonica* (Thunb.) Swingle var. *margarita* (Swingle) Makino (Rutaceae)	dl-limonene (61.6%)		[30]

S. epidermidis	*Helichrysum pallasii* (Spreng.) Ledeb. (Asteraceae)	hexadecanoic acid (14.7%), *(Z,Z)*-9,12-octadecadienoic acid (14.2%)	MIC = 100 µg/ml	[32]
P. acnes	*Syzygium aromaticum* (L.) Merr. Et Perry (Myrtaceae) *S. aromaticum*		agar diffusion tests, MIC = 0.31 mg/ml	[33]
P. granulosum, *P. acnes*	*Thymus quinquecostatus* Celak. (Lamiaceae)	p-cymen-3-ol (50.4%), p-cymen-2-ol (24.1%), cymene (19.0%)	disk diffusion method, MIC = 0.5 mg/ml	[25]

Table 2: EOs against skin infections

Also linalool and α-terpineol revealed high efficiency against *P. acnes* and *S. epidermidis* with MICs ranging from 0.625 to 1.25 µl/ml.[27]

By regarding the chemical composition of the individual EOs which exert antibacterial activity against *P. acnes* and *S. epidermidis* the presence of limonene in most of these EOs stands out. In general, the EOs are predominated by non-phenolic monoterpenes.

EOs against *Helicobacter pylori*

H. pylori is a Gram-negative bacterium which colonizes the stomach of many people. On the one hand these infections can proceed without any symptoms, but on the other hand ulcers and gastritis can occur. These complications are treated with proton-pump-inhibitors in combination with antibiotics.[34] Also EOs possess antibacterial activity against *H. pylori*.

effective against	EO	main constituents	test method	Ref.
H. pylori	*Apium nodiflorum* (L.) Lag. (Apiaceae)	limonene (27.7%), p-cymene (23.1%), myristicine (18.5%)	MIC = 12.5 µg/ml	[35]
H. pylori	*Plinia cerrocampanensis* Barrie (Myrtaceae)	α-bisabolol (42.8%)	MIC = 62.5 µg/ml	[36]
H. pylori	*Thymus caramanicus* Jalas (Lamiaceae)	carvacrol (68.9%)	Disk diffusion test, MIC = 14.5 to 58.0 µg/ml	[37]

Table 3: EOs against *Helicobacter pylori*

The EO of *Dittrichia viscosa* subsp. *revoluta* (Hoffmanns. & Link) P.Silva & Tutin (Asteraceae) comprised 3-methoxy cuminyl isobutyrate (12%), α-cadinol (6.3%) and eudesm-6-en-4α-ol (4.8%). The number of *H. pylori* bacteria significantly decreased using a concentration of 0.33 µl/ml. Especially oxygenated compounds contributed to the antibacterial effect.[38]

EOs as food-preservatives/ EOs against food-related bacteria

The use of EOs as biopreservatives is a matter of great interest for the food industry since the consumers prefer natural additives instead of synthetic ones. That is why lot of studies were performed on this subject in the last years.[39]

effective against	EO	main constituents	test method	Ref.
Bacillus cereus, *Listeria monocytogenes*	*Artemisia echegarayi* Hieron.	camphor, thujone	disk diffusion test, dilution test	[40]

		(Asteraceae)			
L. monocytogenes, S. aureus; B. cereus, Enterobacter cloacea	*Artemisia incana* (L.) Druce (Asteraceae)	camphor (19.0%), borneol (18.9%), 1,8-cineole (14.5%)	MIC = 31.3 μg/ml; MIC = 125 μg/ml	[41]
Salmonella typhi, E. coli	*Chaerophyllum macropodum* Boiss. (Apiaceae)	trans-β-ocimene, myristicin	microdilution broth test	[42]
S. typhi, E. coli	*Chrysanthemum parthenium* (L.) Bernh. (Asteraceae)	α-pinene, camphor	microdilution broth test	[43]
E. aerogenes, E. coli, L. monocytogenes, S. aureus, Salmonella enteritidis, Salmonella typhimurium	*C. operculatus*	γ-terpinene (5.8%), globulol (5.6%), cis-linalool oxide (5.2%)	disk diffusion test, MIC = 1 to 4 μl/ml	[13]
Salmonella species	*Citrus* species	(+)-limonene, terpenes	MIC = 1%	[44]
E. coli	*Jasminum sambac* (L.) Aiton (Oleaceae)	methyl salicylate, benzyl acetate, methyl anthranilate	MIC = 31.25 μl/ml	[45]
Enterococcus faecalis, L. monocytogenes, S. aureus; B. cereus; Yersinia	*Laurus nobilis* L. (Lauraceae)	1,8-cineole (60%)	MIC = 0.02% (v/v); MIC = 0.2%; MIC = 1.0%	[46]

enterocolitica				
S. aureus, Vibrio cholerae; B. cereus, E. coli, L. monocytogenes, S. typhimurium	*Mentha pulegium* L. (Lamiacae)	piperitone (38.0%), piperitenone (33.0%)	MIC = 0.5 µl/ml MIC = 1.0 to 4.0 µl/ml	[47]
S. typhi, Bacillus subtilis	*Minthostachys mollis* (Kunth) Griseb Vaught var. *mollis* (Lamiaceae)	pulegone (55.2%), trans-menthone (31.5%)	MIC = 4 µg/ml	[48]
S. aureus; B. subtilis, P. aeruginosa, L. monocytogenes; S. typhimurium	*Nandina domestica* Thunb. (Berberidaceae)	1-indolizino-carbazole (19.7%), 2-pentanone (16.4%)	agar diffusion assays, MIC = 62.5 µg/ml; MIC =125 µg/ml; MIC= 500 µg/ml	[49]
S. enteritidis	*Ocimum basilicum* L. (Lamiaceae)	linalool (64.4%), 1,8-cineole (12.3%)	MIC = 20.0 to 80.0 µg/ml	[50]
E. coli, Salmonella enterica enterica	*Phoebe lanceolata* (Nees) Nees (Lauraceae)	β-caryophyllene (27.4%), 1,8-cineole (18.2%)	disk diffusion test, dilution test	[51]
E. faecalis	*Retama raetam* (Forssk.) Webb (Fabaceae)	nonanal (35.8%), α-humulene (29.3%)	MIC = 0.625 mg/ml	[52]
E. coli; P. aeruginosa, E. faecalis	*Salvia officinalis* L. (Lamiaceae)	1,8-cineole (33.3%), β-thujone (18.4%)	MIC = 4.5 mg/ml; MICs = 9 mg/ml	[53]

E. coli, E. faecalis	*Schinus molle* L. (Anacardiaceae)	α-phellandrene (35.9%), β-phellandrene (29.3%)	MICs = 9 mg/ml	[53]
B. cereus	*Tanacetum argenteum* (Lam.) Willd. ssp. *argenteum* (Asteraceae)	α-pinene (36.7%), β-pinene (27.5%)	MIC = 125 µg/ml	[54]
B. cereus	*Tanacetum argyrophyllum* (C. Koch) Tvzel var. *argyrophyllum* (Asteraceae)	camphor, borneol and 1,8-cineole	MIC = 125 µg/ml	[55]
L. monocytogenes	*Zizyphus jujuba* Mill. (Rhamnaceae)	eugenol (48.3%), isoeugenol (11.8%)	agar disk diffusion test, dilution test	[56]
B. cereus, E. coli, L. monocytogenes, S. enteritidis, Proteus mirabilis	*T. vulgaris, Origanum vulgare* L. (Lamiaceae), *S. aromaticum, Citrus sinensis* (L.) Osbeck (Rutaceae)		disk diffusion test	[57]

Table 4: EOs as biopreservatives

Not only classic *in-vitro* tests were conducted to investigate the antimicrobial activity. Therefore, the EOs were also applied on different media (e.g. meat). Subsequently, the effect on the microbial growth was observed over a period of time. Such studies are mentioned here:

Govaris et al. investigated the usage of *Origanum vulgare* subsp. *hirtum* Link. (Lamiaceae) EO as food preservative. Therefore it was either applied alone at a percentage of 0.6 or 0.9% or in combination with nisin in minced sheep meat. When the EO which primarily consisted of carvacrol (80.2%) was used singularly at a percentage of 0.9%, it exerted quite high activity against *S. enteritis*, whereas the use of nisin alone did not harm these pathogens. Even bactericidal activity was observed when the EO was combined with nisin.[58] Another study about *O. vulgare* EO verifies the antimicrobial effect against *S. aureus*. The germ's growth and its enterotoxin synthesis were inhibited by the volatile oil. Since this EO is especially powerful against foodborne bacteria, it might be used as biopreservative in food-industry.[59]

A research was performed in which edible tomato puree films were produced which were containing allspice, oregano and garlic EO in order to impair microbial growth. This method might be used in food industry for the extension of shelf life. The antibacterial effect against *E. coli, L. monocytogenes* and *S. enterica* was evaluated by vapor phase and overlay diffusion tests. Oregano EO – rich in carvacrol (63.4%) - obtained the strongest antibacterial effect, but also allspice EO which contained 68.6% eugenol and garlic EO (dominated by diallyl disulfide) showed antibacterial effects. *L. monocytogenes* revealed to be the most vulnerable pathogen. All three bacteria were inhibited by direct contact as well as by the vapors.[60]

Aim of a further study was to investigate the consequence of adding *Origanum onites* L. (Lamiaceae) EO containing pads to wrapped chicken drumsticks concerning the food's shelf-life. The storability was prolonged from three to five days using 5 ml of the diluted EO (1.5%) due to the fact that the number of enterobacteriaceae, lactic acid bacteria, pseudomonads, psychrotrophs and yeasts was kept down. Unfortunately, the chemical composition of this EO was not investigated in this research.[61]

T. vulgaris EO was incorporated at a concentration of 0.6% in minced beef meat. Higher concentrations could not be applied since they proved disadvantageous for the food flavor. The growth of *L. monocytogenes* bacteria was effectively inhibited especially at storage at 10 degrees. Moreover synergy was observed in combination

with nisin. Therefore the number of these pathogens revealed to be lower than the official boundary value determined by the EU when nisin (1000 IU/g) and *T. vulgaris* EO (0.6%) were applied and when the meat was subsequently refrigerated at 4 degrees.[62]

Cinnamaldehyde was capable of inhibiting the growth of *B. cereus* in nutrient and carrot broth at a concentration of 2µl/100ml stored at a temperature of 12 degrees, whereas the application of eugenol and carvacrol was ineffective. That is why cinnamaldehyde could be used for the preservation of food based on carrots.[63]

The inhibitory potency of carvacrol and cinnamaldehyde was also evaluated against the food-poisoning causing pathogen *Campylobacter jejuni*. Both EO components were effective at concentrations from 0.1% upwards independent on the potential resistance of the individual strains against drugs. Cinnamaldehyde was noticed to exert even stronger antibacterial agency in comparison to carvacrol.[64]

Various EO compounds were tested for their antibacterial activity against *Clostridium perfringens*. Trans-cinnamaldehyde, 2-tert-butyl-6-methylphenol, carvacrol and geraniol showed the strongest activity with MICs of 167 µg/ml, 175 µg/ml, 300 µg/ml and 450 µg/ml, respectively. Contrary to this, *Lactobacillus* strains which are part of the natural intestinal flora were not harmed.[65]

The following study shows that there exists a certain framework of the concentration in which the tested EO is efficient against pathogenic bacteria, but does not yet exert any influence on the salutary bacteria of the gastrointestinal tract: The EO of *Foeniculum vulgare* var. *azoricum* (Mill.) Thell. (Apiaceae) appeared to be rich in the antimicrobial active agent *(E)*-anethole (59.3-71.7%). The EO exhibited antimicrobial effect against a large number of foodborne pathogenic bacteria, but also against probiotic bacteria such as *Lactobacillus* strains and *Streptococcus thermophilus*. The lowest MIC value of 15.62 µg/ml was measured against *Acinetobacter lwoffi*, followed by a MIC of 31.25 µg/ml against *S. aureus* and *P. aeruginosa*. The inhibiting effect on probiotic bacteria was reported at MIC values superior than 250 µg/ml. Due to its antimicrobial effect against food related pathogens the EO could be used as food preservative, but one has to keep in mind

that the exaggerated ingestion of fennel products could influence the bacterial flora in the gastrointestinal tract by inhibiting the growth of probiotic bacteria.[66]

A study was conducted about the usage of specific EOs for the therapy of gastrointestinal dysbiosis, an imbalance of the intestinal microflora. Therefore, the effect of eight EOs which are traditionally used for the treatment of gastrointestinal ailments and diseases was examined by MIC evaluation against *Bacteroides fragilis, Clostridium difficile, C. perfringens, E. faecalis, E. coli, Eubacterium limosum, Bifidobacterium bifidu, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus plantarum, Peptostreptococcus anaerobius* and *Candida albicans*. The volatile oil of *Trachyspermum copticum* (L.) Link (Apiaceae) exhibited the strongest antibacterial effect since it stopped bacterial growth of all tested germs at concentrations lower than 2.2%. Moreover, it revealed high selectivity against pathogenic bacteria. The same is true for *Carum carvi* L. (Apiaceae) and *L. angustifolia* EO. *Citrus aurantium var. amara* L. (Rutaceae) revealed lower antibacterial potency but showed likewise selectivity. Therefore, these EOs could be used for the treatment of dysbiosis without impairing the growth of salutary bacteria.[67]

In the two following mentioned studies it becomes aware that the extent of the antimicrobial activity is among others dependant on the pH-level and the composition of the food[68, 69]:

The qualification of several EOs as food preservatives was evaluated using four food-borne bacteria strains. Especially useful seemed to be the combination of *O. vulgare* (carvacrol 68.5%) with *Origanum majorana* L. (Lamiaceae) (4-thujanol 36.2%), *T. vulgaris* (thymol 52.9%, p-cymene 34.0%) or with *O. basilicum* (linalool 42.3%, estragole 26.9%) exhibiting an additive effect against *B. cereus, P. aeruginosa* and *E. coli*. The growth of *L. monocytogenes* was additively impaired by using blends of *O. majorana* or *T. vulgaris* with *Rosmarinus officinalis* L. (Lamiaceae) (eucalyptol 39.6%, camphor 19.0%), *Salvia triloba* L. (Lamiaceae) (eucalyptol 42.0%, camphor 12.0%) or *O. basilicum*. The strength of activity was influenced by the pH level and the food ingredients. Therefore, the conclusion could be drawn that a low pH level of about 5 and high protein content in the food supports

the inhibitory properties of the used EOs whereas carbohydrates and fat diminish it.[68]

The antimicrobial activity against food-related bacteria was observed using the EOs of *Melissa officinalis* L. (Lamiaceae), *O. majorana*, *O. vulgare* and *T. vulgaris*. Three different media were established which were based on meat, milk and salad. The *Listeria* strains were found to be more susceptible than *Lactobacillus*, *Enterobacter* and *Pseudomonas* strains. *O. vulgare* and *T. vulgaris* – the two most efficient EOs - showed additive effects when used in combination. The EOs obtained the strongest antimicrobial activity in food with high pH level and protein content.[69]

The organoleptic changes which are associated with the application of EOs as food-preservatives in a high enough concentration to avoid the bacterial growth can represent a problem which could be solved by using aromas[70] or additional measures to extent the shelf life of food products, such as refrigeration[53]:

The antimicrobial effect of several substances which were found in EOs was investigated using the Gram-positive bacteria *B. cereus, E. faecalis, L. monocytogenes* and *S. aureus*. Moreover, the inhibitory activity against Gram-negative bacteria (*E. coli, Salmonella choleraesuis, Y. enterocolitica*), yeasts (*C. albicans, Zygosaccharomyces rouxii, Debaryomyces hansenii*) and fungi was observed. Carvacrol, cinnamaldehyde and thymol displayed the strongest antimicrobial activity. EOs can influence the taste of packaged food in an unfavourable way. That is why the combination of these substances with aromas (banana, vanilla, strawberry) was examined. Organoleptic tests revealed that all of them could be used in combination with vanilla, but not with banana. Only the combination of strawberry aroma with thymol resulted in an organoleptic acceptable taste.[70]

In an experiment with minced meat the bacteriostatic activity of the EOs of *S. officinalis* and *S. molle* was noticed against *Salmonella anatum* and *S. enteritidis* at concentrations of 1.5% using *S. officinalis* EO and 2.0% using *S. molle*. Unfortunately, the taste was impaired at these concentrations. That is why the

combination of these EOs in lower concentrations with NaCl and storage at low temperatures was detected to be more useful.[53]

Sinapis alba L. (Brassicaceae) EO which was isolated from the seeds contained phenethyl isothiocyanate as active agent. This lead molecule was obtained by high performance liquid chromatography (HPLC) and silica gel column chromatography and subsequently subjected to chemical modifications. Paper disk diffusion assays were performed in order to investigate the effect on the following intestinal bacteria: *E. coli*, *C. difficile*, *C. perfringens*, *Bifidobacterium breve*, *B. bifidum*, *B. longum*, *L. acidophilus* and *L. casei*. The EO inhibited the growth of *C. difficile*, *C. perfringens* and *E. coli* at 5 mg/disk. The same *Clostridium* strains were effectively inhibited at a dose of 1 mg/disk when phenethyl isothiocyanate was singularly used. The semi-synthetic derivates of this molecule which contained aromatic functional groups, such as benzyl-, benzoyl- and phenethyl-groups revealed higher selectivity and higher antibacterial agency against pathogenic intestinal bacteria, such as *E. coli* and *Clostridium* strains.[71]

The majority of investigated EOs was rich in non-phenolic monoterpenic compounds. Nevertheless, also phenolic monoterpenes, such as carvacrol[58] and phenylpropanoid constituents (e.g. cinnamaldehyde[64]) contributed to the antimicrobial activity against food-borne pathogens.

EOs as bio-preservatives in cosmetic industry

Due to the preserving activity of EOs, these substances could also be applied for the preservation of cosmetic products. Since some EOs show synergistic effects in combination with commercially used preservatives the application of EOs makes a diminution of these synthetic substances possible as the two below-mentioned studies revealed.[72, 73]

Patrone et al. investigated the combination of several EOs with synthetic preservatives which are used in cosmetic industry. *Eucalyptus globosus* Labill. (Myrtaceae) and *Mentha piperita* L. (Lamiaceae) EO showed synergistic activity

against *P. aeruginosa* when they were applied in combination with methylparabene. Moreover, synergy was noticed against *S. aureus* using *S. officinalis*, *O. vulgare* and *M. piperita* in combination with imidazolidinyl urea and propylparabene. These findings constitute a further proof of the advantages of combining EOs with common preservatives in cosmetic products.[72]

The application of commercial lavender, lemon and tea tree EO in body milks was investigated observing the inhibition of microbial growth. The main constituents of the lavender oil were linalool (34.1%) and linalyl acetate (33.3%). The tee trea oil mainly consisted of terpinen-4-ol (41.3%) and γ-terpinene (19.1%). The most abundant substance in lemon oil was limonene (79.8%). The growth of the involved microorganisms *S. aureus, P. aeruginosa, Aspergillus niger* and *Candida* species was sufficiently inhibited using these EOs in combination with 0.2% of a synthetic preservative. Since synergy was noticed when the EOs were combined with the synthetic agent, the applied quantity of the synthetical component could be cut down about 8.5 times.[73]

EOs against dental bacteria

This chapter deals with the antimicrobial activity of EOs against dental bacteria - especially against the tooth-decay causing bacteria *Streptococcus pyogenes* and *Streptococcus mutans*. EOs are capable of inhibiting the growth of these bacteria as well as the formation of biofilms. In various cases the potency of chlorohexidine was found to be even lower than the efficacy of the EOs.[74] Therefore, the application of EOs is recommended in products which prevent caries.[75]

effective against	EO	main constituents	test method	Ref.
S. mutans	*Achillea ligustica* All. (Asteraceae)	viridiflorol (14.5%), terpinen-4-ol (13.0%)	MIC = 39 µg/ml	[75]
S. mutans, S. pyogenes	*Mentha longifolia* L. (Lamiaceae)	(-)-menthol	disk diffusion test,	[76]

			microdilution test	
S. mutans	*Hyptis pectinata* L. Poit. (Lamiaceae)	β-caryophyllene (28.3%), caryophyllene oxide (28.0%)	MIC = 200 μg/ml	[77]
Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Parvimonas micra, Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia	*Satureja hortensis* L. (Lamiaceae)	carvacrol (86.6%)	MICs < 0.125 μl/ml	[78]

Table 5: EOs against dental bacteria

The antimicrobial activities of *R. officinalis* EO, *M. piperita* EO and chlorohexidine were compared to each other using the tooth-decay causing bacteria *S. pyogenes* and *S. mutans*. *R. officinalis* EO whose main constituents were piperitone (23.7%), α-pinene (14.9%) and linalool (14.9%) obtained MBC of 2000 ppm against *S. mutans* and 4000 ppm against *S. pyogenes*. Chlorohexidine showed MICs of 8000 and 1000 ppm. *M. piperita* EO which mainly comprised α-terpinene (19.7%) and piperitenone oxide (19.3%), but also trans-carveol (14.5%) and isomenthone (10.3%) showed MBCs of 6000 ppm against *S. mutans* and 1000 ppm against *S. pyogenes*. The decimal reduction times (D-values) of the EOs were lower than that of chlorohexidine with 2.8 min against *S. mutans*. The lowest D-value against *S.*

pyogenes and the highest anti-biofilm activity was achieved by application of *M. piperita* EO. Hence, the EOs displayed even higher activity than chlorohexidine.[74] Further *in-vitro* as well as *in-vivo* experiments verified the high antibacterial activity of *M. piperita* EO against the plaque-causing bacteria *S. pyogenes* and *S. mutans*. Also thereby the EO showed stronger effects in preventing the formation of biofilms and keeping the number of bacteria in the mouth low in comparison to chlorohexidine.[79]

A similar study was conducted comparing the anti-biofilm activity of *Eucalyptus camaldulensis* Dehnh. var. *obtuse* (Myrtaceae) EO and *Mentha spicata* L. (Lamiaceae) EO. The MBC values of both oils turned out to be 2 mg/ml against *S. pyogenes* and 4 mg/ml against *S. mutans*. An *in-vivo* experiment proved the ability of preventing biofilm formation. The principal constituents of *M. spicata* EO were detected to be limonene (48.0%) and piperitone (20.3%). *E. camaldulensis* EO comprised 1,8-cineole (64.0%) and α-pinene (9.6%). *E. camaldulensis* EO reached a D-value of 2.8 min against *S. mutans* using the MBC, so did *M. spicata* EO. For comparison only, the D-value of chlorohexidine (2%) was 12.8 min. Only 3.6 min were measured against *S. pyogenes* using *E. camaldulensis* EO, whereas the D-value was 4.3 min using *M. spicata* EO.[80]

It becomes quite obvious that *Mentha* species play an important role in inhibiting the growth of tooth-decay causing bacteria. Although the composition of the individual species differ from each other all of them achieved remarkable results in impairing the microbial growth of periodontal pathogens.

EOs against diverse human pathogens

effective against	EO	main constituents	test method	Ref.
Cryptococcus neoformans; K. pneumoniae	*Abies holophylla* Maxim. (Pinaceae)	bicyclo[2.2.1] heptan-2-ol (28.1%)	MIC = 0.5 mg/ml; MIC = 10.9 mg/ml	[81]
Candida	*Abies koreana*	bornyl ester	MIC = 0.5	[81]

glabrata; K. pneumoniae; B. subtilis, E. coli	E.H.Wilson (Pinaceae)	(41.8%)	mg/ml; MIC = 5.5 mg/ml; MIC = 10.9 mg/ml	
B. subtilis, S. aureus	*Ageratum conyzoides* L. (Asteraceae)	precocene I (52.2%), caryophyllene (26.2%)	disk diffusion tests	[82]
E. coli, K. pneumoniae	*Anaphalis nubigena* DC. var. *monocephala* (DC.) C. B. Clarke (Asteraceae)	α-guaiene (12.3%), γ-muurolene (10.4%)	MIC = 125 μg/ml, MIC = 500 μg/ml	[83]
S. aureus, S. epidermidis, C. albicans, C. neoformans	*Artemisia absinthium* L. (Asteraceae)	trans-sabinyl acetate (26.4%), myrcene (10.8%), trans-thujone (10.1%)	agar diffusion tests	[84]
B. subtilis, K. pneumoniae, Bacillus mycoides	*Ballota nigra* L. (Lamiaceae)	p-vinylguiacol (9.2%), borneol (7.5%)	disk diffusion test, dilution test	[85]
Streptococcus agalactiae, S. pyogenes	*Bupleurum marginatum* Wall. ex DC. (Apiaceae)	tridecane (13.2%), undecane (10.4%)	MIC = 0.125 to 4.00 mg/ml	[86]
S. aureus, Streptococcus faecalis	*Callistemon citrinus* (Curtis) Skeels (Myrtaceae)	1,8-cineole (61.2%)	disk diffusion test, broth microdilution test	[87]
S. aureus, S. faecalis, B. cereus, Serratia	*Callistemon viminalis* (Gaertn.) G.Don	1,8-cineole (83.2%)	disk diffusion test, broth microdilution	[87]

marcescens	(Myrtaceae)		test	
Clostridium bifermentas, Enterococcus faecium, Enterococcus hirae, Streptococcus salivarius subsp. *thermophilus*	*Cannabis sativa* L. (Cannabaceae)	myrcene, α-pinene, β-caryophyllene	broth dilution test	[88]
Staphylococcus simulans, Staphylococcus lugdunensis, S. *aureus,* S. *epidermitis, Candida tropicalis*	*Carum montanum* (Coss. et Dur.) Benth. et Hook. (Apiaceae)	nothoapiole (62.8%)	diffusion test	[89]
B. subtilis, C. albicans	*Chamaecyparis nootkatensis* (D. Don) Spach. (Cupressaceae)	limonene (53.2%)	diffusion test	[90]
E. faecalis, S. aureus	*Cordia verbenacea* D.C. (Boraginaceae)	tricyclene (23.9%), bicyclogermacrene (11.7%)	MIC = 200 μg/ml, MIC = 170 μg/ml	[91]
S. aureus, Pasteurella multocida	*Dodecadenia grandiflora* Nees (Lauraceae)	germacrene D (26.0%), furanodiene (13.7%)	disk diffusion test, dilution test	[51]
B. cereus, B. subtilis, Micrococcus luteus, S. aureus	*Enterolobium contortisiliquum* (Vell.) Morong	carvone		[92]

	(Fabaceae)			
E. coli, *S. aureus*	*Erigeron mucronatus* DC (Asteraceae)	caryophyllene (11.4%), limonene (10.3%)	Disk diffusion test	[93]
P. aeruginosa	*Eugenia beaurepaireana* (Kiaersk.) D. Legrand (Myrtaceae)	β-caryophyllene (8.0%), bicyclogermacrene (7.2%)	MIC = 278.3 μg/ml	[94]
S. aureus	*Eugenia brasiliensis* Lam. (Myrtaceae)	spathulenol (12.6%), τ-cadinol (8.7%)	MIC = 156.2 μg/ml	[94]
S. aureus	*Eugenia umbelliflora* Berg. (Myrtaceae)	viridiflorol (17.7%), β-pinene (13.2%)	MIC = 119.2 μg/ml	[94]
B. subtilis, *S. aureus*, *S. mutans*, *E. coli*, *E. faecalis*, *C. albicans*	*Ferula glauca* L. (Apiaceae)	leaf EO: (E)-caryophyllene (24.9%), fruit EO: α-pinene (24.2%), root EO: *(E)*-β-farnesene (10.0%), elemicin (9.0%), flower EO: germacrene D (14.2%), myrcene (13.6%)	MIC = 38 to 1250 μg/ml	[95]
S. aureus, *E. coli*, *S. enterica enterica*, *Shigella flexneri*, *P. multocida*	*Hedychium aurantiacum* Wall. ex Roscoe (Zingiberaceae)	terpinen-4-ol	disk diffusion test, MIC = 2.0 – 15.6 μl/ml	[96]
S. aureus, *E. coli*,	*Hedychium*	trans-meta-mentha-	disk diffusion	[96]

S. enterica enterica, Shigella flexneri, P. multocida	*coronarium* J.König (Zingiberaceae)	2,8-diene, linalool	test, MIC = 7.8 – 31.3 μl/ml	
S. aureus, E. coli, S. enterica enterica, Shigella flexneri, P. multocida	*Hedychium ellipticum* Sm. (Zingiberaceae)	1,8-cineole, sabinene	disk diffusion test, MIC = 7.8 – 31.3 μl/ml	[96]
S. aureus	*Hymenocrater longiflorus* Benth. (Lamiaceae)	δ-cadinol (18.5%), α-pinene (10.2%), p-menth-1-en-8-ol (9.8%)	MIC = 120 μg/ml	[39]
B. subtilis, S. aureus	*Hypericum hirsutum* L. (Guttiferae)	*(E,E)*-α-farnesene (7.0–13.8%) and *(E)*-β-farnesene (7.2–9.4%)	broth microdilution test	[97]
B. subtilis, S. aureus	*Hypericum richeri* Vill. *subsp. richeri* (Guttiferae)	germacrene D (26.9%)	broth microdilution test	[97]
B. subtilis, S. aureus	*Hypericum tetrapterum* Fr. (Guttiferae)	α-copaene (12.7%), α-longipinene (8.1%)	broth microdilution test	[97]
E. faecium, B. cereus, S. aureus, C. albicans, Candida tropicalis, C. glabrata, Candida parapsilosis	*Inula helenium* L. (Asteraceae)	alantolactone, isoalantolactone	MIC = 0.009 to 0.6 mg/ml	[98]
B. subtilis, B. cereus, S. aureus,	*Juniperus phoenicea* L.	α-pinene	agar diffusion test	[99]

L. monocytogenes, P. aeruginosa	(Cupressaceae)			[100]
S. agalactiae, S. pyogenes	*K. longipedunculata*	δ-cadinene (21.8%)	MIC = 60 μg/ml	[15]
S. aureus, S. epidermidis, M. luteus	*Laserpitium zernyi* Hayek (Apiaceae)	α-pinene (31.6%), α-bisabolol (30.9%)	microdilution test	[101]
S. aureus, S. enterica enterica, P. multocida	*Lindera pulcherrima* (Nees) Hook. f. (Lauraceae)	furanodienone (46.6%)	disk diffusion test, dilution test	[51]
S. aureus; E. faecalis; Citrobacter freundii, Staphylococcus saprophyticus	*Lindera strychnifolia* (Sieb. & Zucc.) Fern. (Lauraceae)	root EO: zerumbone (26.7%), leaf EO: sesquithuriferol (35.9%)	MIC = 0.01 mg/ml; MIC = 0.02 mg/ml; MIC = 0.78 mg/ml	[102]
B. subtilis, E. faecalis, E. coli, P. aeruginosa, S. aureus, Monilia albicans	*Litsea cubeba* (Lour.) Pers. (Lauraceae)	neral, β-terpinene, β-phellandrene	disk diffusion test, microbroth dilution test, MIC = 100 to 1000 μg/ml	[103]
K. pneumoniae, L. monocytogenes, C. albicans	*Mentha longifolia* L. (Lamiaceae)	pulegone (54.4%)	Diffusion test	[104]
K. pneumoniae, L. monocytogenes, C. albicans	*Mentha viridis* L. (Lamiaceae)	carvone (50.5%)	Diffusion test	[104]
B. subtilis; S.	*Metasequioa*	2-butaneone	MIC = 125	[105]

aureus, *P.* *aeruginosa;* *E.* *coli*	*glyptostroboides* Miki ex Hu. (Taxodiaceae)	(30.6%)	µg/ml; MIC = 250 µg/ml; MIC = 500 µg/ml	
S. aureus	*Momordica charantia* L. (Cucurbitaceae)	trans-nerolidol (61.6%)	MIC = 125 µg/ml	[106]
E. faecalis, *S. aureus, E. coli, P. aeruginosa,* *K. pneumoniae*	*Monticalia andicola* Turcz. (Asteraceae)	α-pinene (19.6%), β-pinene (10.5%)	MIC = 10 to 150 µg/ml	[107]
P. multocida	*Neolitsea pallens* (D. Don) Momiyama & Hara (Lauraceae)	furanogermenone (59.5%)	disk diffusion test, dilution test	[51]
K. pneumoniae, S. aureus, *B. macerans;* *S. epidermidis,* *S. pyogenes,* *B. subtilis;* *Burkholderia cepacia, Brucella abortus, E. coli, C. albicans*	*Nepeta cataria* L. (Lamiaceae)	4aα,7α,7aβ-nepetalactone (70.4%)	MIC = 15.62 µg/ml; MIC = 62.5 µg/ml; MICs = 125 µg/ml	[108]
B. subtilis, *S. aureus,* *C. albicans*	*Ocimum forskolei* Benth (Lamiaceae)	estragole	diffusion test	[109]
P. mirabilis, *P. aeruginosa,* *S. aureus, E. coli*	*Ocimum gratissimum* L. (Lamiaceae)	eugenol (68.8% - 74.1%)	disk diffusion test	[110]
Proteus vulgaris,	*Origanum*	carvacrol	disk diffusion	[111]

S. typhimurium, E. cloacae, S. aureus, E. coli, K. pneumoniae, Corynebacterium diphtheriae, C. albicans	*acutidens* (Hand.-Mazz.) Ietswaart (Lamiaceae)		test	[112]
S. aureus; P. aeruginosa	*Origanum compactum* Benth. (Lamiaceae)	carvacrol (37.8%), thymol (19.8%)	MIC = 1% (v/v); MIC = 0.031% (v/v)	[113]
B. subtilis; S. flexneri, S. aureus; E. coli, K. pneumoniae, Salmonella choleraensius	*O. majorana*	terpinen-4-ol (30.4%)	MIC = 0.069 mg/ml; MIC = 0.782 mg/ml; MIC = 0.920 mg/ml	[114]
B. subtilis, E. coli	*Pamburus missionis* (Wight) Swingle (Rutaceae)	1-tridecanol (38.3%), 1-hexadecanoic acid (16.1%)	MIC = 10 mg/ml	[115]
E. coli, P. multocida	*Persea duthiei* King ex. Hook f. (Lauraceae)	*(E)*-nerolidol (13.2%), limonene, α-pinene, β-pinene (10.0% each)	disk diffusion test, dilution test	[51]
S. aureus, S. enterica enterica	*Persea gamblei* (King ex Hook.f.) Kosterm. (Lauraceae)	β-caryophyllene (22.1%), γ-gurjunene (16.8%)	disk diffusion test, dilution test	[51]
S. aureus, E. coli, S. enterica enterica	*Persea odoratissima* (Nees) Kost.	α-pinene (16.6%), sabinene (13.1%), β-caryophyllene	disk diffusion test, dilution test	[51]

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	(Lauraceae)	(10.4%)		
B. cereus, B. subtilis, S. aureus, E. coli, P. aeruginosa, S. typhi, C. albicans, C. tropicalis	*Phyllanthus emblica* L. (Phyllanthaceae)	β-caryophyllene, β-bourbonene	MIC = 100 to 1000 µg/ml	[116]
S. aureus, Enterococcus hirae; P. aeruginosa, E. coli; C. albicans	*Pituranthos chloranthus* Benth. and Hook. (Apiaceae)	terpinen-4-ol (30.3%)	MIC = 1.875 mg/l; MICs = 3.75 mg/l; MIC = 7.5 mg/l	[117]
S. aureus, M. luteus, S. typhimurium; S. epidermidis	*Rhaponticum acaule* DC (Asteraceae)	methyl eugenol, epi-13-manool	disk diffusion test, MIC = 500 µg/ml; MIC = 800 µg/ml	[118]
S. aureus; L. monocytogenes, C. albicans; E. faecalis, S. pyogenes	*Rhaponticum carthamoides* (Willd.) Iljin (Asteraceae)	13-norcypera-1(5),11(12)-diene (22.6%), aplotaxene (21.2%)	MIC = 32 µg/ml; MIC = 128 µg/ml; MIC = 256 µg/ml	[119]
S. aureus, Streptococcus pneumoniae, Shigella spp, *E. faecalis*	*Ridolfia segetum* (L.) Moris (Apiaceae)	dillapiole (47.4%)	MIC = 1.25 mg/ml	[120]
B. subtilis, Chromobacterium violaceum, E. coli; S. aureus, Erwinia carotovora	*Rosa damascena* Mill. (Rosaceae)	citronellol (35.2%), geraniol (22.2%)	MIC = 0.25% (v/v); MIC = 0.5% (v/v)	[121]
E. coli, K.	*R. officinalis*	1,8-cineole,	MIC = 1.25 to	[122]

pneumoniae, S. aureus, B. subtilis, B. cereus, S. epidermidis, S. faecalis	*var. typicus* and *var. troglodytorum*	camphor	10 µl/ml	
A. lwoffii; C. perfringens, S. pneumoniae	*Salvia aramiensis* Rech. fil. (Lamiaceae)	1,8-cineole (46.0%)	disk diffusion test, MIC = 4.5 mg/ml; MIC = 18 mg/ml	[123]
A. lwoffii	*Salvia aucheri* var. *aucheri* Boiss. (Lamiaceae)	1,8-cineole (30.5%), camphor (21.3%)	disk diffusion test, broth microdilution test	[123]
B. cereus, B. subtilis, S. aureus, S. epidermidis, S. faecalis; P. vulgaris, S. typhi	*Salvia bracteata* Banks et Sol. (Lamiaceae)	caryophyllene oxide (16.6%)	MIC = 50 µg/ml; MIC = 100 µg/ml	[124]
B. subtilis, E. faecalis, S. aureus, S. epidermidis E. coli, P. aeruginosa, K. pneumoniae	*Salvia chloroleuca* Rech. F. & Aell. (Lamiaceae)	β-pinene (10.6%), 1,8-cineole, β-caryophyllene, α-pinene (9.0% each) and carvacrol (7.9%)	disk diffusion test, dilution test	[125]
S. aureus, S. epidermidis	*Salvia eremophila* Boiss. (Lamiaceae)	borneol (21.8%), α-pinene (18.8%), bornyl acetate (18.7%)	MIC = 7.8 µg/ml, MIC = 125 µg/ml	[126]
A. lwoffii	*Salvia pilifera* Benth.	α-thujene (36.1%)	disk diffusion test, broth	[123]

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			microdilution test	
B. cereus, B. subtilis, S. aureus, S. epidermidis, S. faecalis; *P. vulgaris, P. aeruginosa*	*Salvia rubifolia* Boiss. (Lamiaceae)	γ-muurolene (11.8%)	MIC = 50 μg/ml; MIC = 100 μg/ml	[124]
B. cereus, E. faecalis, bark EO: also *P. mirabilis*	*Santiria trimera* (Oliv.) Aubrév. (Burseraceae)	leaf EO: α-humulene (34.6%), bark EO: α-pinene (51.5%)	agar disc diffusion test, broth microdilution test	[127]
MRSA, P. vulgaris, S. typhimurium, C. albicans, C. tropicalis	*Satureja cuneifolia* Ten. (Lamiaceae)	thyme	MIC = 62.5 to 500 μg/ml	[128]
B. subtilis, S. aureus, E. faecalis, K. pneumoniae, E. coli, P. aeruginosa	*Satureja spicigera* (C. Koch) Boiss. (Lamiaceae)	carvacrol (53.7%), thymol (36.0%)	disk diffusion test	[129]
S. aureus, P. aeruginosa; C. albicans	*Schinus terebinthifolius* Raddi. (Anacardiaceae)	cis-β-terpineol (17.9%), *(E)*-caryophyllene (17.6%)	MIC = 0.80 mg/ml; MIC = 0.85 mg/ml	[130]
B. subtilis, P. aeruginosa	*Stachys cretica* L. subsp. *smyrnaea* Rech. fil. (Lamiaceae)	trans-β-caryophyllene (51.0%), germacrene D	disk diffusion test	[131]

		(32.8%)		
B. subtilis, Bacillus pumulis, E. coli, E. faecalis, K. pneumoniae, S. aureus, S. epidermidis, P. aeruginosa, C. albicans	*Tanacetum balsamita* L. subsp. *balsamita* (Asteraceae)	carvone (51.0%), β-thujone (20.8%)	disk diffusion test, dilution test	[132]
B. subtilis, S. epidermidis, S. aureus, S. faecalis	*Teucrium divaricatum* Sieb. ssp. *villosum* (Celak.) Rech. fil. (Lamiacae)	*(E)*-caryophyllene (30.1%)	MIC = 25 to 100 μg/ml	[133]
A. lwoffii, S. pyogenes, E. coli, Listeria species, *C. albicans, C. parapsilosis, Candida krusei*	*Thymbra spicata* L. (Lamiaceae)	carvacrol (60.4%)	disk diffusion test, dilution test	[134]

Table 6: EOs against human pathogens

Since the chemical composition of EOs can change according to the growing place and the point of time at which the plants are collected, the antimicrobial activity can be influenced by these parameters. That is why the EO of *S. cuneifolia* which was isolated of plants in the post-flowering stage presented lower MIC values than the EOs of pre-flowering and flowering stage.[128] In another study the chemical composition of *H. spicatum* Buch.-Ham. (Zingiberaceae) was noticed to be dependent on the collection area. Therefore, some samples contained primary sabinene and terpinen-4-ol whereas others mainly obtained 10-epi-γ-eudesmol and

1,8-cineole. Both samples showed activity against *S. aureus, P. multocida* and *E. coli*.[96]

Beside of exerting bacteriostatic and bactericidal effects EOs are also capable of impairing the development of capsules[135] and spores[136].

Cuminum cyminum L. (Apiaceae) is on the one hand a popular spice on the other hand it is traditionally applied for its astringent and carminative effects. The EO of this plant was investigated presenting a high content of α-pinene (29.1%), limonene (21.5%) and 1,8-cineole (17.9%). During *in-vitro* tests *S. aureus, Streptococcus faecalis* and *E. coli* appeared to be the most susceptible pathogens whereas *K. pneumoniae* was tolerant to the EO. Diverse chemotypes of this plant exist.[137] This explains why the seed EO of *C. cyminum* mainly comprised cumin aldehyde (25.2%) and γ-terpinene (19%) in another study. This oil exerted antibacterial activity against *K. pneumoniae* demonstrated by MIC and MBC results in the range from 0.8 to 3.5 μg/ml. At concentrations lower than the MIC the formation of capsules was prevented and the function of urease was impaired.[135]

The development of bacterial spores of *B. subtilis* was impaired by various EOs of which *Elettaria cardamomum* (L.) Maton (Zingiberaceae) and *M. alternifolia* showed the strongest inhibitory impact. The main compounds of *M. alternifolia EO* (terpinen-4-ol, 38.0% of the EO) and those of *E. cardamomum* (α-terpinyl acetate 46.0% and 1,8-cineole 34.0%) possessed sporicidal activity, but not in such extent as the whole EO. This indicated the potential existence of synergistic interactions among the individual constituents and the importance of substances which were represented in lower levels.[136] Despite of the fact that *M. alternifolia* EO exerts strong inhibitory activity against microbes, some bacteria are nevertheless capable of developing protection measures against it. A study proved that some *P. aeruginosa* strains obtain special pumps (MexAB-OprM pumps) which induce resistance towards monoterpenes which occur in *M. alternifolia* EO such as terpinen-4-ol, α-terpineol and 1,8-cineole by ejecting them.[138]

Various studies show that the extent of antimicrobial activity and the mode of action are dependent on the additive and synergistic or even antagonistic effects of the individual constituents.[136, 139, 140]

The additive interactions of two *T. vulgaris* chemotypes were observed involving the cravacrol and the linalool cemotype. The most abundant substances in the EO of these plants were carvacrol, linalool and thymol. Additive antimicrobial activity was noticed when these two oils were combined, when their isolated monosubstances linalool and carvacrol were used in combination or linalool with thymol. When using the monosubstances in combinations as previously described they exhibited a partial synergistic effect against *K. pneumoniae*. The conclusion can be drawn that the antimicrobial effect of *T. vulgaris* EO correlates with the additive effects between the single components.[139]

When combining farnesol with geraniol or geranylgeraniol the mechanism of action against *S. aureus* was affected in comparison to using farnesol singularly. Therefore, the damaging effect of farnesol to the bacterial cell membrane was reduced in combinations with geraniol, but nevertheless cell proliferation was more strongly impaired. Geranylgeraniol impeded both modes of action. That is why it is not sufficient to investigate the mode of action of the major component of an EO, since the mechanism of the EO is a result of the single constituents interactions.[140]

The following study verifies that the single compounds of EOs could be used as starting material for the development of semi-synthetic substances which are characterized by stronger antimicrobial efficacy: In a study published by Pintore et al. the EO of *R. officinalis* was divided into oxygenated fractions whose main components were 1,8-cineole (37.6%) and bornyl acetate (21.4%) and hydrocarbon fractions consisting of α-pinene (44.2%), camphene (24.5%) and limonene (11.7%). Moreover, the hydrocarbon fraction was transformed into a hydroformulated fraction. These three fractions and the original EO were tested using different microbes to determine their antibacterial activity. The highest antimicrobial effect was achieved against *Aeromonas sobria* and *Candida* strains. The hydroformulated fraction even displayed a fungicidal effect on *Candida* strains that were robust against the natural EO and the other two fractions.[141]

The antimicrobial activity of EOs can absolutely keep up with the bacteriostatic activity of synthetic active agents. Therefore, equal or even better results were achieved in tests involving EOs and amphotericin B[142], chloroamphenicol or streptomycin.[143]

The EO of *Perovskia abrotanoides* Karel (Lamiaceae) – a plant which is traditionally applied in the therapy of leishmaniasis – contained a high quantity of camphor (23%) and 1,8-cineole (22%) and α-pinene (12%). The most susceptible germs revealed to be *S. aureus* determined by a MIC and MBC of 8 μl/ml and *B. cereus* with MIC and MBC values of 2 μl/ml. The EO showed no activity against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). The activity against *C. albicans* was equal to the potency of amphotericin B with MIC and minimal fungicidal concentration (MFC) values of 8 μl/ml. Since the EO showed antimicrobial activity, it could inhibit the manifestation of secondary microbial infections in leishmaniasis patients. When using camphor, 1,8-cineole and α-pinene against the above-mentioned microorganisms singularly camphor achieved the lowest MIC results of 1 or 2 μl/ml in microbroth dilution assays, whereas 1,8-cineole showed the lowest effect.[142]

The volatile oil obtained from the rhizomes of *Zingiber officinale* Rosc. (Zingiberaceae) primarily comprised geranial (25.9%) and α-zingiberene (9.5%). Antimicrobial efficacy was observed against *S. aureus, P. vulgaris, P. aeruginosa, K. pneumoniae*, whereas *E. coli* revealed to be insensitive to the EO. The activity was higher than that of chloramphenicol and similar to streptomycin.[143]

By flow cytometry the damaging effect of thymol and carvacrol to the *E. coli* cell membrane was proved. Both substances inhibited the growth of this microorganism using a concentration of 200 mg/l.[144]

EOs with aldehydic or phenolic compounds exerted the strongest antimicrobial efficiency with MIC values lower than 2% (v/v) in a study involving thirteen different EOs and 65 bacteria strains. *Cymbopogon citratus* (DC.) Stapf (Poaceae) and *Cinnamomum verum* J.Presl (Lauraceae) bark revealed EOs with high aldehyde content, such as geranial, neral and cinnamaldehyde, respectively. Components of

the EOs rich in phenolic compounds were thymol and carvacrol in *O. compactum*, thymol in *Trachyspermum ammi* (L.) Sprague (Apiaceae), eugenol in *Eugenia caryophyllus* (Sprengel) Bullock & Harr. (Myrtaceae) and *C. verum* leaf EO. The growth of *P. aeruginosa* was most effectively inhibited by *O. compactum* and *C. verum* bark EO with MICs lower than 2%. *M. alternifolia* (terpinene-4-ol), *Cymbopogon martinii* (Roxb.) Wats. (Poaceae) (geraniol) and *L. angustifolia* (linalyl actetate, linalool) EOs obtained a high amount of alcohols and therefore fluctuating antibacterial efficacy. Hydrocarbons (such as limonene) and the bicyclic ether 1,8-cineole which were present in *C. sinensis*, *E. globulus* and *Melaleuca cajeputii* Powell (Myrtaceae) showed weaker antibacterial activity with MICs higher than 10% (v/v).[145]

First and foremost plants of the *Lamiaceae* family exhibited high antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria. Especially different *Origanum, Salvia* and *Mentha* species which are representatives of this family achieved significant results in antimicrobial tests. In general, the most frequently occurring substances were identified as the sesquiterpenes caryophyllene and germacrene D as well as the phenolic monocyclic monoterpenes carvacrol and thymol. Moreover, the monocyclic monoterpenes 1,8-cineole, terpinen-4-ol and the bicyclic monoterpene α-pinene were often detected in the EOs.

EOs against *Borrelia burgdorferi*

B. burgdorferi is a bacterium belonging to the class of spirochetes which is spread by ticks and causes the lyme disease in humans.[146]

The EO of *Cistus creticus* L. (Cistaceae) was subjected to GC/MS analysis and to *in-vitro* tests to investigate its impact on the growth of *B. burgdorferi* as a consequence to the fact that borreliosis patients observed reduced pain after intake of *C. creticus* leaf products. It turned out that the EO decreased the quantity of these germs to 2% used at a concentration of 0.02%. GC/MS screenings revealed the presence of carvacrol and various diterpenes of the labdane-type including manoyl oxide. These substances are proved to have antimicrobial properties.[147]

EOs against nocardiform actinomycetes

a) EOs against *Mycobacteria*

Mycobacterium tuberculosis is a Gram-positive pathogen which is responsible for the emergence of tuberculosis. Also in this case drug-resistant strains were identified which impede an effective cure and indicate alternative active agents.[148]

effective against	EO	main constituents	test method	Ref.
M. tuberculosis	*Achyrocline alata* (Kunth) DC. (Asteraceae)	thymol (24.0%)	MIC = 62.5 µg/ml	[149]
M. tuberculosis	*Anemia tomentosa* (Sav.) var. *anthriscifolia* (Schrad). (Anemiaceae)	(-)-epi-presilphiperfolan-1-ol (30.6%), silphiperfol-6-ene (14.7%)	MIC = 100 µg/ml	[150]
M. tuberculosis	*Lantana fucata* Lindl. (Verbenaceae)	β-elemene (27.1%), germacrene D (11.6%), *(E)-*caryophyllene (7.7%)	MIC = 100 µg/ml	[151]
M. tuberculosis	*Lantana trifolia* L. (Verbenaceae)	germacrene D (45.1%), *(E)-*caryophyllene (12.8%), bicyclogermacrene (12.7%)	MIC = 80 µg/ml	[151]
M. tuberculosis	*Swinglea glutinosa* Merr. (Rutaceae)	β-pinene (49.6%)	MIC = 100 µg/ml	[149]
Mycobacterium		trans-	MIC = 25.9	[152]

avium subsp. *paratuberculosis*		cinnamaldehyde	µg/ml	
M. avium subsp. *paratuberculosis*		carvacrol	MIC = 72.2 µg/ml	[152]
M. avium subsp. *paratuberculosis*		2,5-dihydroxybenz-aldehyde	MIC = 74 µg/ml	[152]
M. avium subsp. *paratuberculosis*		2-hydroxy-5-methoxybenz-aldehyde	MIC = 90.4 µg/ml	[152]

Table 7: EOs against mycobacteria

The growth of *M. tuberculosis* was most effectively inhibited by the application of an EO characterized by a high amount of the phenolic monoterpene thymol[149], but also EOs with non-phenolic monoterpenes (such as β-pinene[149]) and sesquiterpenes (e.g. germacrene D[151]) obtained low MIC results.

b) EOs against *Nocardia asteroides*

Especially immunosuppressed patients are susceptible to *N. asteroides* infections which are usually generated by inhalation of the germs. In most cases these bacteria lead to pulmonary diseases.[153] In the two below-mentioned studies a strong antimicrobial activity of the EOs was assessed against *N. asteroides*.

The most prevalent substance in *Daucus crinitus* Desf. (Apiaceae) EO revealed to be a rare phenylpropanoid, namely isochavicol isobutyrate (39.0%). Also isochavicol propionate – a molecule which has never been before found in nature - was detected in a low quantity. The antimicrobial activity against several bacteria and fungi was examined presenting the highest activity against *N. asteroides* with a MIC value of 310 µg/ml. Moreover, moderate activity was noticed against Gram-positive bacteria such as *S. aureus* and against *C. albicans*. Gram-negative bacteria strains (*K. pneumoniae* and *S. enteriditis*) were found to be tolerant to the EO. Isochavicol isobutyrate showed no significant inhibiting effect in the disk diffusion test. This leads to the conclusion that other components of the EO might be responsible for its

agency, such as α-pinene (9.9%), β-caryophyllene (5.4%) or myrcene (3.4%). Nevertheless, isochavicol derivatives showed noteworthy MIC results in the range from 16 to 61 µg/ml against *N. asteroides* in the microdilution test.[154]

The EO of *Bupleurum plantagineum* Desf. (Apiaceae) and *Bupleurum montanum* Coss & Dur. (Apiaceae) was isolated from the aerial plant parts and afterwards submitted to GC/MS analysis. The oil of *B. plantagineum* was characterized by a high amount of α-pinene (31.9%), myrcene (24.8%) and cis-chrysanthenyl acetate (28.2%). The main compound of *B. montanum* EO was megastigma-4,6-(E),8(2)-triene (25.3%). *N. asteroides* as well as *E. faecalis* and *S. aureus* were assessed to be the most vulnerable pathogens.[155]

c) EOs against *Rhodococcus equi*

R. equi was primarily detected as pneumonia-causing bacterium in foals, but it turned out that these pathogens can likewise infect humans. Also in this case especially immunocompromised persons are infected.[156]

Costa et al. investigated the chemical composition and the antibacterial activity of the leaf EOs isolated from three *Guatteriopsis* species collected in Brazil. Caryophyllene oxide (69.3%) was identified as the predominant substance in *G. blepharophylla* Mart. (Annonaceae) EO whereas β-pinene (38.2%), α-pinene (30.8%) and *(E)*-caryophyllene (20.6%) were prevalent in *G. hispida* R.E.Fr. (Annonaceae) EO. The strongest antibacterial agency was shown by *G. friesiana* W. A. Rodrigues (Annonaceae) EO whose major constituents were β-eudesmol (51.6%) and γ-eudesmol (23.7%). All involved bacteria were inhibited by this EO, among others *B. subtilis* (MIC of 60 µg/ml), *S. epidermidis* and *E. hirae* with a MIC result of 100 µg/ml. *R. equi* was ascertained to be the most vulnerable pathogen with MICs of 50 µg/ml no matter which oil was applied. In tests using the individual components of the EOs the eudesmol molecules showed the highest antimicrobial effect, but did not achieve as high activity as the EO.[157]

EOs as water disinfectants

Legionella pneumophila is a pathogen which can lead to Legionnaire's disease, a life-threatening infection of the respiratory system. The presence of these bacteria was noticed in air conditioning systems and water pipes.[158] The following studies demonstrate that EOs could be used to impair the growth of these germs, e.g. in pharmaceutical aerosols or for the maintenance of water quality.[159]

One of these bacteriostatic EOs was isolated from *M. alternifolia* which comprised a high content of terpinen-4-ol (42.4%) and led to a remarkable growth inhibition by using MICs between 0.125 and 0.5% (v/v). [159]

In a further study the potential use of *Cinnamomum osmophloeum* Kaneh (Lauraceae) EO whose lead molecule was by far cinnamaldehyde (91.3%) as water disinfectant was investigated. The EO turned out to have significant antimicrobial activity against *L. pneumophila.* Its activity was found to be even higher at basic pH levels. Thus, it could be used in spas for disinfection of water which obtains a basic pH level, especially because the effectiveness of chlorine is diminished in alkaline surroundings.[160] Moreover, it could be applied for the prevention of *L. pneumophila* growth in the water of hot water pipes.[161]

Thymus capitatus (L.) Hoffmanns. & Link (Lamiaceae) EO which is rich in carvacrol and thymol presented high potential as water disinfectant just as its single constituent carvacrol did. Therefore, the employment of this EO impaired the growth of coliform bacteria when using 94 mg of EO per litre of spoiled water. At concentrations of 468 mg/l the number of coliforms decreased so massively that the non-existence of these pathogens could be verified. Moreover this condition continued for two weeks.[162]

EOs as air disinfectants

The EOs of *Pelargonium graveolens* L'Hér. (Geraniaceae) and *Cymbopogon flexuosus* (Nees ex Steud.) Will.Watson (Poaceae) were used in a mixture which contained geranial (22.3%) – the major component of *C. flexuosus* - and β-citronellol

(18.4%) which equates the major constituent of *P. graveolens* EO. The antimicrobial agency of this mixture used as vapor was evaluated in different tests using a special vapor machine. Therefore the number of air-borne bacteria was reduced to 11% in an office room within 15 hours. This EO blend could be applied as air disinfectant. Moreover, it demonstrated inhibitory activity against *A. baumanii, C. difficile, MRSA* and *VRE* strains in *in-vitro* tests.[163]

S. officinalis contained an EO whose lead molecules were β-thujone (17.8%), 1,8-cineole (16.3%) and camphor (14.2%). By microdilution tests a high antimicrobial activity was determined represented by MIC results between 0.015 and 0.125 μl/ml. The lowest MIC results were reached in tests involving *Bacillus* strains (*B. cereus, B. liqueniformis, B. subtilus*) and *E. hirae*. *S. aureus* showed a MIC value of 0.031 μl/ml. *E. coli, P. aeruginosa, Pseudomonas cepacia, Pseudomonas fluorescens, S. enterica* and *S. typhimurium* were also susceptible to the EO referring to MICs of 0.062 μl/ml. The yeasts *C. albicans, Pichia subpeliucolosa* and *Trichosporum fermentans* exhibited the strongest resistance with MICs of 0.125 μl/ml. Due to the observed high vapor agency of the volatile oil, it might find application as disinfectant against airborne microorganisms.[164]

Anti-biofilm activity of EOs

Certain bacteria and yeasts can develop biofilms on medicinal devices, such as catheters or dialysis access. Since these biofilms are often drug-resistant it is important to develop active agents against them.[165]

That is why the ability of *Boswellia rivae* Engl. (Burseraceae) and *Boswellia papyrifera* (Delile ex Caill.) Hochst. (Burseraceae) EO to prevent the development of *S. aureus*, *S. epidermidis* and *Candida*-biofilms was evaluated. GC/MS analysis revealed limonene (28.0%), α-pinene (13.3%) and 3-carene (15.7%) as the major constituents in *B. rivae* EO. *B. papyrifera* comprised n-octyl acetate (63.5%) and n-octanol (17.8%). On the one hand the EOs turned out to be very effective against already existing biofilms, on the other hand the development of biofilms was inhibited. The generation of *C. albicans* biofilms was prevented at sub-MIC

concentrations of 0.88 µg/ml by application of *B. rivae* EO, whereas the formation of *S. epidermidis* biofilms was inhibited at 0.27 µg/ml by *B. papyrifera* EO.[165]

Nostro et al. investigated the activity of carvacrol on *S. aureus* and *S. epidermidis* biofilms. The direct application of carvacrol in liquid form revealed to be more efficient than the use of vapor. Therefore, the number of colony forming units significantly decreased after exposure to the carvacrol liquid. It can be assumed that carvacrol is capable of destroying biofilms formed by *Staphylococcus* strains.[166]

M. alternifolia EO exerted remarkable activity against *S. aureus* biofilms used in concentrations lower than 1% (v/v). These concentrations were twice as high as the measured MBC values. The EO diminished the number of biofilm-forming cells especially during the first 15 minutes after application. The grade of extinction did not change when concentrations higher than 1% (v/v) were applied.[167]

EOs in combination with synthetic active agents

Combinations of EOs with well-established antibiotics can lead on the one hand to additive and synergistic but on the other hand also to antagonistic effects. Synergy was observed when *R. officinalis* EO was combined with ciprofloxacin to inhibit the growth of *K. pneumoniae*. Apart from that, antagonistic interactions were noticed using combinations of ciprofloxacin with the EOs of *M. alternifolia, M. piperita, R. officinalis* and *T. vulgaris* against *S. aureus*.[168] Otherwise, the combined use of *M. alternifolia* EO with tobramycin exerted synergistic interactions against *E. coli* and *S. aureus*.[169]

The EO of *Foeniculum vulgare* Mill. (Apiaceae) showed its strongest inhibitory effect against *S. aureus, E. coli* and *C. albicans*. Synergy was observed in combinations with tetracycline and amoxicillin concerning a number of pathogens such as *E. coli*. Also bactericidal activity was noticed starting at concentrations twice as high as the MIC results. The EO comprised trans-anethole, fenchone, estragole, but also α-pinene, γ-terpinene and limonene.[170]

O. vulgare EO exhibited significant antimicrobial activity against multi-drug resistant *E. coli* strains demonstrated by a MIC value of 0.5 µl/ml. Synergistic and additive activities were noticed against extended-spectrum β-lactamase-producing *E. coli* when the EO was combined with various antibiotics. That is why the combination of *O. vulgare* EO with synthetic drugs such as doxycycline, fluoroquinolones and lincomycin was recommended allowing a reduction of the drug dosage and therefore reducing the risk of side effects.[171]

EOs against phytopathogenic bacteria

Kotan et al. studied the inhibitory effect of *Thymus fallax* Fisch. & CA Mey (Lamiaceae) and *S. spicigera* on several phytopathogenic bacteria, including some *Erwinia, Pseudomonas* and *Xanthomonas* strains. The composition of the EOs was examined by GC/MS presenting a high content of thymol, carvacrol, p-cymene and γ-terpinene in *T. fallax* and *S. spicigera*. These two plants exhibited a strong antibacterial effect against a wide range of agricultural pathogens leading to the idea of using their EOs for plant and seed disinfection.[172]

Eugenol is capable of reducing the cell number of *Xanthomonas campestris pv. phaseoli var. fuscans* as an experiment with bean seeds revealed. Eugenol was applied at concentrations of 2, 4 and 8 mg/ml on infected seeds. Within 72 hours the growth significantly decreased about 3, 7 and 16%. Therefore, eugenol could be used as seed disinfectant for the prevention of infections caused by this pathogen.[173]

YEASTS

Yeasts are unicellular, eukaryotic organisms that belong to the kingdom of fungi. They are divided into basidiomycetes and ascomycetes. By developing true hyphae or pseudohyphae multicellular cell structures can originate. In order to proliferate they are forming buds. The two most well-known yeasts are *Saccharomyces cerevisiae* and *Candida albicans* which have been studied frequently in order to gain knowledge about the eukaryotic cell.[174]

In the following chapter recently published studies are summarized which exclusively deal with the effect of EOs on yeasts. In most cases *C. albicans* was utilized.

Candida

Candida species are a natural part of the human flora in the gastrointestinal tract, genitor-urinary system and on the skin. Nevertheless, they can cause infections in these body regions, since they are opportunistic pathogens. In worse cases even systematic infections can emerge. The most common pathogen of Candidiasis is *C. albicans*, followed by *Candida glabrata* and *Candida tropicalis*. In newborns also *Candida parapsilosis* is a prevalent pathogen that can lead to candidiasis, including candiduria.[175]

Nowadays the majority of nosocomial blood stream infections are linked to *Candida* all over the world. Severe infections usually occur in immunosuppressed patients or in persons who are likely to develop infections due to an already existing serious disease.[176]

Mahboubi et al. investigated the possible synergistic effect of Amphotericin B combined with *Myrtus communis* L. (Myrtaceae) EO whose most abundant substance was 1,8-cineole (36.1%), followed by α-pinene (22.5%). The MICs of *C. albicans, Aspergillus niger, A. parasiticus* and *A. flavus* were evaluated. *Candida* and *Aspergillus* achieved similar MICs - and minimum fungicidal concentrations (MFCs) - results with 8-16 μl/ml and 16-32 μl/ml, respectively. *M. communis* EO in combination with Amphotericin B showed a remarkable synergistic effect. In this case the MIC was significantly lower with 0.06 μg/ml against *C. albicans* compared

to the MIC when Amphotericin was used alone (2.00 µg/ml). This study recommends the combination therapy of Amphotericin B with EOs of *M. communis* against *Aspergillus* and *Candida* infections.[177]

The main part of *Zataria multiflora* Boiss (Lamiaceae) EO consisted of thymol with 27.1% to 64.9% depending on the collection area. Further components were p-cymene and carvacrol. Whereas the MIC values of the observed bacteria (*B. subtilis, S. epidermidis, P. aeruginosa, E. coli*) were quite high, *C. albicans* and *C. tropicalis* showed significant susceptibility to the EO with MIC values of 0.25 mg/ml and 0.062 mg/ml.[9]

The use of *Ocimum sanctum* L. (Lamiaceae) is common practise in ayurvedic medicine for its antimicrobial potency. In a recently published study the anticandidal activity of this plant was investigated. The main component of the EO which comprises 53 compounds was methyl chavicol (44.6%), followed by linalool (21.8%). These two constituents turned out to be the most effective ones. The antimicrobial study was performed with different C. *albicans, C. tropicalis, C. glabrata, C. parapsilosis* and *C. krusei* strains of which some were fluconazole/ketoconazole-resistant and others fluconazole/ketoconazole-sensitive. All of these strains were found to be susceptible to *O. sanctum* EO with MIC results between 0.1 µl/ml and 0.5 µl/ml. Since fluconazole is very often used to prevent or cure *Candida* infections, drug resistant strains have emerged. The result of this study confirms the idea of using *O. sanctum* EO in combination with established synthetic antifungal agents to obtain synergistic effects.[178] In another study about *O. sanctum* eugenol, linalool (which is the most effective constituent), methyl eugenol and 1,8-cineole were identified as the main components of the EO. The oil showed higher anticandidal activity against *C. albicans* and *C. tropicalis* compared to peppermint EO. Moreover the mode of action was investigated: The main components of *O. sanctum* EO exert a synergistic effect in inhibiting essential proton pumps. That is why the release of hydrogen protons was blocked by the EO.[179]

The EO of *Origanum vulgare* L. (Lamiaceae) whose main components were o-cymene, thyme and γ-terpinene showed antimicrobial activity against *C. albicans* and *C. dubliniensis* with MIC values in the range of 200 to 800 µg/ml.[180] *C.*

dubliniensis strains can lead to oral candidiasis in HIV patients just as *C. albicans* can. Moreover, this *Candida* species is capable of getting adjusted to fluconazole administration. The germ's susceptibility to volatile oils could be utilized for the treatment of *C. dubliniensis* infections.[181]

The most prevalent substance in *S. aromaticum* EO was detected to be eugenol (85.3%). The antimicrobial effect against several *Candida* strains and fungi was evaluated revealing high agency against all tested microorganisms. Therefore *C. parapsiliosis* was inhibited at a concentration of 0.32 µl/ml and the other *Candida* strains including *C. albicans, C. glabrata, C. tropicalis and C. krusei* at 0.64 µl/ml. On the one hand the EO impaired ergosterol synthesis and induced cell membrane rupture and on the other hand germ tube development was impaired. Eugenol was supposed to be responsible for the antimicrobial agency since equal MIC results were achieved when this constituent was applied singularly.[182]

Nystatin is an effective agent in the cure of fungal diseases. Nevertheless, its use can lead to several side effects including for example kidney damage. Minimizing the Nystatin dose through combination with EOs might be a solution to reduce adverse reactions. The EO of *O. vulgare* showed synergistic effect when combined with Nystatin against *C. albicans, C. crusei* and *C. tropicalis*. The Fractional Inhibitory Concentration (FIC) levels ranged from 0.11 to 0.17 mg/ml. The GC-MS analysis revealed the presence of cymenol (58.6%) and cymene (25.0%). Nystatin associated with the EO of *Pelargonium graveolens* L'Hérit. (Geraniaceae) exhibited lower synergistic effect against fewer *Candida* strains. The main components of *P. graveolens* EO were found to be citronellol (47.3%), geraniol (9.1%) and linalool (8.8%). Although no synergistic effect was detected in combination with *Melaleuca alternifolia* Cheel. (Myrtaceae) EO, an additive effect was noticed. The EO of *M. alternifolia* mainly consisted of terpinen–4-ol (30.3%) and γ-terpinene (16.3%).[183]A similar study was carried out based on the same EOs. Aim of this study was to investigate the potential synergistic effect of these oils in combination with Amphotericin B. The antimicrobial potency of the EOs against *Candida* was tested revealing that *Pelargonium* EO had the highest activity. The synergistic effect of *P. graveolens* EO in combination with Amphotericin B was confirmed in further tests.[184]

Nevertheless, EOs must be used carefully combined with antibiotics since their combination might also imply antagonistic effects. Thus, *Mentha piperita* L. (Lamiaceae), *M. alternifolia, Thymus vulgaris* L. (Lamiaceae) and *R. officinalis* EOs showed antagonistic effects in combination with Amphotericin B against *C. albicans*. The additive, synergistic and antagonistic effect was often linked to the percentage in which the EO and the antibiotic were applied.[168]

The volatile oil of *Piper ovatum* Vahl (Piperaceae) was isolated and examined by GC/MS analysis. The detected lead molecules were δ-amorphene (16.5%), γ-muurolene (13.3%) and cis-muurola-4(14),5-diene (14.3%). Antifungal tests proved the inhibition of *C. tropicalis*.[185]

The efficiency of geraniol against *C. albicans* was investigated in an *in-vivo* experiment with mice. Mice were infected with vaginal candidiasis and afterwards treated with geraniol. As a consequence, the development of mycelia was inhibited but not candidal cell proliferation. When vaginal washing was additionally performed to the geraniol administration, also the cell growth was impaired.[186]

Dalleau et al. compared the anti-biofilm activity of molecules which are often prevalent in EOs. The effect of thymol, carvacrol, α-terpinene, 1,8-cineole, menthol, citral, linalool, eugenol, farnesol and geraniol was measured by 24-hour treatment of *Candida*-biofilms which had been developed for 1 to 5 days. Thymol, carvacrol and geraniol exhibited the most significant antibiofilm activity against all three tested strains, including *C. albicans, C. parapsilosis* and *C. glabrata*. Above all, carvacrol was capable of reducing *Candida*-biofilm development regardless of the maturation state and attained more than 75% inhibition used in concentrations of 0.03% against *C. albicans* and 0.125% against the other two *Candida* species. Thymol and geraniol showed similar potency against *C. parapsilosis* biofilms used at 0.125% independent on their age.[187]

Giordani et al. compared the anticandidal effect of several *Thymus* types, *Origanum majorana* L. (Lamiaceae) and *R. officinalis* L. collected in Algeria. It is the first study including *Thymus numidicus* Poiret. (Lamiaceae) which presented the highest

activity against *Candida* reaching a MIC of 0.000479 µg/ml. This equates 1357 fold higher potency compared to Amphotericin B whose measured MIC was found to be 0.65 µg/ml. The main component in the EO of *T. numidicus* was identified as thymol. *O. majorana* volatile oil presented a MIC of 1.564 µg/ml. The *O. majorana* EO comprised 25.4% of thymol, 21.4% of carvacrol and 20.8% of γ-terpinene as main constituents. The EO of *T. vulgaris* L. (Lamiaceae) whose main constituents were p-cymene (26.4%) and thymol (25.6%) showed likewise a low MIC value of 3.71 µg/ml. *R. officinalis* EO whose MIC was determined to be 2.208 µg/ml consisted mainly of α-pinene (19.7%), camphor (12.6%) and borneol (11.2%). *Thymus algeriensis* Boiss. et Reut (Lamiaceae) showed the highest MIC value of 11.38 µg/ml. This EO was characterized to contain 25.5% α-pinene, 7.7% 1,8-cineole and 8.5% camphor. The constituents of the singular EOs were almost the same but their ratio differed significantly. The dimension of the antifungal activity was assumed to be dependent on the quantity of carvacrol, thymol, γ-terpinene and p-cymene found in the EO.[188]

Cymbopogon citratus (DC.) Stapf (Poaceae) EO exhibited antifungal potency against various *Candida* species, including *C. albicans, C. parapsilosis, C. glabrata, C. tropicalis* and *C. krusei* of which *C. albicans* appeared to be the most susceptible one. The GC analysis identified citral as major component with a percentage of 76%. Since equal anticandidal results were obtained when citral was singularly used, it is obviously the most powerful constituent.[189]

The essential volatiles of *Pinus koraiensis* Siebold et Zucc (Pinaceae) cones was investigated by GC-MS analysis revealing the presence of limonene (27.9%), α-pinene (23.9%) and β-pinene (12.0%). The antimicrobial effect was examined by both broth microdilution and agar disk diffusion tests. In contrast to the relatively weak antibacterial activity with MICs equally or higher than 21.8 mg/ml much better efficacy was noticed against the involved yeast strains, such as *C. neoformans* (MIC of 0.136 mg/ml) or *C. glabrata* (MIC >0.545 mg/ml).[190]

Cryptococcus

Cryptococcus neoformans enters the human body through inhalation. Whereas it is not threatening for healthy persons, it can lead to critical infections in immuno-compromised patients, cancer or HIV-patients. The germs are able to disseminate to the brain causing meningoencephalitis.[191]

The EO of *Thymus x viciosoi* (Pau) R. Morales (Lamiaceae) whose main components were thymol, carvacrol and p-cymene proved antifungal activity using broth microdilution assays. The quite low MIC values ranged from 0.08 to 0.32 µl/ml against all utilized yeasts and fungis. The lowest MIC of 0.08 emerged at tests against *C. neoformans* and *Trichophyton mentagrophytes*. Since similar low MIC values were measured during tests using the isolated components carvacrol and thymol instead of the whole EO, they are assumed to be responsible for the impressive antifungal efficiency. The effect of the EO on the plasma membrane was observed by flow cytometry showing damage of the cell membrane, inhibition of the cell metabolism and as a result cell death.[192]

The EO of *Pinus densiflora* Siebold et Zucc. (Pinaceae) exhibited antifungal activity. The evaluated MIC results for *C. neoformans* were determined to be 0.545 mg/ml whereas the activity against *C. glabrata* was not that high demonstrated by a MIC value of 2.18 mg/ml. By means of GC/MS the main components of the EO were found to be β-phellandrene (16.7%) and α-pinene (14.9%).[193]

ANTIFUNGALS

This chapter deals with new findings concerning the antifungal activity of EOs. Various molds, dermatophytes and phytopathogenic fungi were included in these studies.

EOs against dermatophytes

The designation 'dermatophytic fungi' comprises different kinds of *Epidermophyton, Microsporum* and *Trichophyton* species. These pathogens are responsible for the generation of fungal infections concerning the human skin, nails and hair.[194]

effective against	EO	main constituents	test method	Ref.
Microsporum canis, Microsporum gypseum, Trichophyton rubrum, Fonsecaea pedrosoi	*Artemisia absinthium* L. (Asteraceae)	trans-sabinyl acetate (26.4%)	agar diffusion test	[84]
M. canis, M. gypseum, T. rubrum, F. pedrosoi	*Artemisia biennis* Willd. (Asteraceae)	*(E)*-β-farnesene (40.0%), *(Z)*-β-ocimene (34.7%)	agar diffusion test	[84]
M. canis, M. gypseum, T. rubrum, F. pedrosoi	*Artemisia ludoviciana* Nutt. (Asteraceae)	1,8-cineole (22.0%), camphor (15.9%)	agar diffusion test	[84]
Trichophyton mentagrophytes var. interdigitale	*Citrus macroptera* Lour. (Rutaceae)	β-pinene (33.3%), α-pinene (25.3%), p-cymene (17.6%)	MIC = 12.5 µg/ml	[195]

M. canis	*Croton argyrophylloides* Muell. Arg. (Euphorbiaceae)	spathulenol (20.3%), bicyclogermacrene (11.7%)	MIC = 9 to 19 µg/ml	[196]
M. canis	*Croton zenhtneri* Pax & Hoffman (Euphorbiaceae)	estragole (72.9%)	MIC = 620 to 1250 µg/ml	[196]
M. canis, T. mentagrophytes, T. rubrum	*Magnolia liliflora* Desr. (Magnoliaceae)		disk diffusion test, MIC = 62.5 to 500 µg/ml	[197]
M. canis, T. rubrum, T. mentagrophytes	*Nandina domestica* Thunb. (Berberidaceae)		MIC = 62.5 to 500 µg/ml	[198]
M. gypseum, M. canis, T. mentagrophytes, T. rubrum	*Ocimum forskolei* Benth (Lamiaceae)	estragole	diffusion test	[109]
T. mentagrophytes; T. rubrum; M. gypseum	*Plinia cerrocampanensis* Barrie (Myrtaceae)	α-bisabolol (42.8%)	MIC = 32 µg/ml; MIC = 62.5 µg/ml; MIC = 125 µg/ml	[36]
Epidermophyton floccosum, T. rubrum, T. mentagrophytes, M. canis, M. gypseum	*Syzygium aromaticum* (L.) Merr. Et Perry (Myrtaceae)	eugenol (85.3%)	MIC = 0.08 to 0.16 µl/ml	[182]
T.	*Zanthoxylum*	α-bisabolol,	disk	[21]

mentagrophyes, E. floccosum, M. gypseum	*tingoassuiba* St.-Hil. (Rutaceae) EO	methyl-N-methylanthranilate	diffusion test	

Table 8: EOs against dermatophytes

The impact of the cultivation place on the chemical composition and therefore on the antimicrobial activity is revealed in the below-mentioned study.

The chemical composition of the EO obtained from *D. carota* subsp. *carota* differed depending on the growing location. That is why the plants from Portugal mainly comprised geranyl acetate and α-pinene whereas β-bisabolene and 11-α-(H)-himachal-4-en-1-β-ol were the primary constituents in the Sardinian plants. Both EOs exhibited antifungal effects especially against the yeast *C. neoformans* and dermatophytic fungi, such as *E. floccosum, M. canis, M. gypseum, T. mentagrophytes* and *T. rubrum*. The plants from Sardinia showed the strongest activity with MICs ranging from 0.16 to 0.64 μl/ml.[199] Of all tested fungi the dermatophytic strains showed the most distinctive vulnerability to the EO of *Daucus carota* subsp. *halophilus* (Brot.) A. Pujadas (Apiaceae) which mainly comprised sabinene, α-pinene, limonene and elemicin. Therefore, the MIC values ranged from to 0.16 to 0.64 μl/ml in tests including *Epidermophyton floccosum, M. canis, M. gypseum, T. mentagrophytes* and *T. rubrum*. Moreover, increased elemicin levels correlated with stronger fungistatic effects.[200]

Lavandula pedunculata (Miller) Cav. (Lamiaceae) EO was divided into different chemotypes depending on the major constituent which was either 1,8-cineole or fenchone. Dermatophytes such as *M. canis, M. gypseum, T. mentagrophytes* and *T. rubrum* showed higher susceptibility to the EOs in comparison to *Aspergillus* species and yeasts. Especially strong activity was assessed in tests with sub-chemotypes which additionally comprised high camphor levels achieving MIC results between 0.32 and 0.64 μl/ml.[201]

Besides of inhibiting the growth of dermatophytic fungi some EOs, such as *M. liliflora* EO, additionally succeeded in impairing the development of spores.[197]

In an *in-vivo* experiment involving horses which suffered from a *Trichophyton equinum* infection the antifungal potential of *Melaleuca alternifolia* Cheel. (Myrtaceae) EO against these pathogens was proved. The application of the volatile oil revealed to be as successful as the treatment with enilconazole. As a result, the horses recovered from the fungal infections within one month.[202]

The prevalent substances in the EOs which were investigated for their activity against dermatophytic fungi cannot be assigned to one particular chemical group, but it seems that especially sesquiterpenes, phenylpropanoids and bicyclic non-phenolic monoterpenes are connected with strong antifungal effects against skin-infection causing fungi.

EOs against molds

Molds – in most cases *Aspergillus* species - can lead to invasive infections especially in patients with weakened immune system. Not all species are susceptible to the established active agents. Moreover, the emergence of resistance to applied drugs was observed.[203] In addition, the exposure to molds and their spores is assumed to be connected to asthmatic and allergic reactions.[204] Many molds are able to produce toxic molecules, so-called mycotoxins, which represent a health-damaging threat to human beings since some of them (e.g. aflatoxins) act as carcinogens. These harmful substances are taken up by the ingestion of contaminated food. Especially cereals and nuts are susceptible to fungal infestation.[205]

effective against	EO	main constituents	test method	Ref.
Aspergillus flavus	*Aegle marmelos* L. Correa (Rutaceae)	dl-limonene (39.2%)	MIC = 750 µl/l	[206]
Aspergillus parasiticus, *A. flavus*	*Ageratum conyzoides* L. (Asteraceae)	precocene I, precocene II	disk diffusion test	[82] [207]
Geotrichum candidum;	*Artemisia incana* (L.) Druce	camphor (19.0%), borneol (18.9%),	MIC = 31.3	[41]

Aspergillus and *Penicillium* species, *Cladosporium herbarum, Absidia repens, Trichothecium roseum*	(Asteraceae)	1,8-cineole (14.5%)	μg/ml; MIC = 125 to 500 μg/ml	
A. flavus, Aspergillus niger, Aspergillus glaucus, Aspergillus ochraceus, Fusarium and *Colletotrichum* species	*Chenopodium ambrosioides* L. (Chenopodiaceae)	*(Z)*-ascaridole (61.4%)		[208]
Alternaria alternata, A. niger, Penicillium roquefortii, Fusarium oxysporum	*Cymbopogon citratus* (DC.) Stapf (Poaceae)		MIC = 0.062 to 0.31 μl/ml	[209]
Mucor ramamnianus, Aspergillus westerdijkiae	*Juniperus phoenicea* L. (Cupressaceae)	α-pinene	agar diffusion test	[100]
A. niger, Rhizopus oryzae	*Laurus nobilis* L. (Lauraceae)	1,8-cineole (60%)	MIC = 0.02% (v/v)	[46]
A. niger	*Matricaria chamomilla* L.(Asteraceae)	α-bisabolol (56.9%)		[210]
A. ochraceus, M. ramamnianus	*Mentha longifolia* L. (Lamiaceae)	pulegone (54.4%)	diffusion test	[104]

A. ochraceus, M. ramamnianus	*Mentha viridis* L. (Lamiaceae)	carvone (50.5%)	diffusion test	[104]
Cladosporium cladosporioides	*Myrica gale* L. (Myricaceae)	α-pinene, germacrone	dilution test	[211]
A. flavus, Fusarium tabacinum, Fusarium solani	*Nepeta cataria* L. (Lamiaceae)	4aα,7α,7aβ-nepetalactone (70.4%)	disk diffusion test, MIC = 15.62 µg/ml	[108]
Aspergillus species, *A. alternata, Penicillium* species, *Fusarium nivale*	*Ocimum sanctum* L. (Lamiaceae)	eugenol (61.3%)	MIC = 0.3 µl/ml	[212]
C. cladosporioides, Cladosporium sphareospermum	*Piper divaricatum* G.F.W.Meyer. (Piperaceae)	methyleugenol (63.8%), eugenol (23.6%)	dilution test	[213]
A. niger	*Pituranthos chloranthus* Benth. and Hook. (Apiaceae)	terpinen-4-ol (30.3%)	MIC = 7.5 mg/l	[117]
A. parasiticus	*Rosmarinus officinalis* L. (Lamiaceae)	piperitone (23.7%), α-pinene (14.9%), limonene (14.9%)	MIC = 1750 ppm	[214]
Ashbiya gossypii, A. niger, R. oryzae, Trichoderma reesei	*Salvia officinalis* L. (Lamiaceae)	β-thujone (17.8%), 1,8-cineole (16.3%), camphor (14.2%)	MIC = 0.031 to 0.250 µl/ml	[164]
A. flavus, A. parasiticus	*Satureja hortensis* L. (Lamiaceae)	thymol, carvacrol	diffusion test, MIC = 6.25 µl/ml	[215] [216]
A. flavus, A. niger, Aspergillus	*S. aromaticum*	eugenol (85.3%)	MIC = 0.32 to	[182]

			0.64 µl/ml	
fumigatus				
A. alternata	*Thuja orientalis* L. (Cupressaceae)	α-pinene (29.2%), δ-3-carene (20.1%)	diffusion test	[217]
A. parasiticus	*Trachyspermum copticum* (L.) Link (Apiaceae)	thymol (37.2%), p-cymene (32.3%)	MIC = 600 ppm	[214]
A. flavus	*Zataria multiflora* Boiss (Lamiaceae)	carvacrol (71.12%)	MIC = 400 ppm	[218]
A. flavus, A. niger, Fusarium moniliforme	*Zingiber officinale* Rosc. (Zingiberaceae)	geranial (25.9%)	disk diffusion test	[143]
A. niger		camphor	MIC = 2 µl/ml	[142]
A. niger		α-pinene	MIC = 4 µl/ml	[142]

Table 9: EOs against molds

The prevalent substances of *Lippia alba* (Mill.) N.E. Brown (Verbenaceae) EO neral (14.2%) and geranial (22.2%) as well as the entire EO inhibited on the one hand the aflatoxin B1 production and on the other hand the growth of *A. flavus*. Moreover, the growth of other *Aspergillus* species and *Fusarium* strains was significantly impaired. That is why this EO seemed to be suitable for the preservation of food.[219] A further study involving two different chemotypes of *L. alba* was published emphasizing the different antimicrobial activity of each chemotyp. The citral chemotype of *L. alba* EO which consisted to 30.5% of geranial and to 23.6% of neral inhibited the growth of *A. fumigatus* at a concentration of 78.7 µg/ml. The carvone chemotype which comprised carvone (25.3%), limonene (22.4%), geranial and neral (10.4% each) was found to be not as successful since the measured MIC values revealed to be always higher than 500 µg/ml against all microorganisms. This circumstance is probably linked to the particular citral content since citral exhibited high efficiency in inhibiting *A. fumigatus* (MIC of 62.5 µg/ml) and *C. krusei* (39.7 µg/ml). Also

geraniol (6.3% of the citral chemotype) and citronellal were tested singularly revealing strong antifungal potency.[220]

The growth as well as the mycotoxin production of molds was significantly inhibited by the use of EOs. As a consequence, their application as bio-preservatives seems to be possible. The EOs of the following plants led to a noteworthy reduction or to an entire inhibition of the aflatoxin production: *A. conyzoides*[207], *A. marmelos*[206], *L. alba*[219], *O. sanctum*[212], *R. officinalis*[214], *S. hortensis*[216], *T. copticum*[214] and *Z. multiflora*[218].

The growth of *A. flavus* was entirely impeded by applying the EO of *S. hortensis* EO at the MIC on lemons one week before they were exposed to the pathogens.[215] In addition, this EO and its individual components thymol and carvacrol effectively suppressed the growth as well as the aflatoxin B1 and G1 synthesis of *A. parasiticus*.[216]

Besides of inhibiting the production of aflatoxines also other mycotoxins - such as deoxynivalenol and its derivates - were impaired by the use of EOs.
Piperitone were separated from the EO of *Eucalyptus dives* Schauer (Myrtaceae). Precocenes I and II were likewise purified from the other constituents of *Matricaria recutita* L. (Asteraceae) EO. All these substances were found to be capable of suppressing the synthesis of deoxynivalenol – a mycotoxin of *Fusarium* strains. In the present study the isolated substances effectively inhibited the biosynthesis of these harmful molecules in *Fusarium graminearum*.[221] Also the aflatoxin G1 synthesis of *A. parasiticus* was effectively suppressed by the application of *M. recutica* EO whereas the aflatoxin B1 levels were not reduced. Moreover the synthesis of the mycotoxin 3-acetyldeoxynivalenol was diminished in *F. graminearum*. The active agents were identified as *(Z)-* and *(E)*-spiroethers of which the latter ones displayed higher efficacy.[222]

One of the modes of action seems to be the destruction of existing mycelia as well as the inhibition of the development of new mycelia as some studies proved.
Therefore, the volatile oil of *Citrus sinensis* (L.) Osbeck (Rutaceae) which was noticed to be rich in limonene (84.2%) exerted antifungal activity against *A. niger* by

destroying its mycelial cell walls as microscopy techniques revealed. The fungal growth completely stopped by using a concentration of 3.0 μg/ml which was at the same time fungicidal.[223] The same effect was observed against the hyphae of *A. niger* by applying the EO of *M. chamomilla*.[210] Furthermore, the EO of *A. conyzoides* inhibited the formation of mycelia[82], just as *Z. multiflora* EO did.[218]

Moreover, EOs were found to be capable of inhibiting the formation of spores. Chamazulene was the lead molecule in *Achillea millefolium* L. (Asteraceae) EO constituting 42.2% of the whole oil. At the investigated concentration of 0.25 μl/ml the growth of *Aspergillus nidulans* was significantly reduced. This EO exerted genotoxic effects against the fungal cells and suppressed the development of spores.[224]

As the below-mentioned study shows the fungistatic activity of EOs can be influenced by the present pH-level. *Penicillium verrucosum, Penicillium expansum* and *A. ochraceus* were more sensitive to *Ocimum gratissimum* L. (Lamiaceae) EO compared to the EOs of *Thymus vulgaris* L. (Lamiaceae) and *C. citratus*. All EOs showed stronger effects against the *Penicillium* strains at a high pH-value of 9 whereas *A. ochraceus* was more vulnerable at a low pH-value of 3.[225]

In the present studies EOs effectively inhibited the growth of molds, but also the production of mycotoxins, mycelia and spores. Due to these properties they could be used among others for the preservation of food, e.g. for active-packaging as a study with *Cinnamomum zeylanicum* Breyne (Lauraceae) EO revealed.
Hence, *C. zeylanicum* EO which was predominated by the antimicrobially active substance trans-cinnamaldehyde was found to be capable of inhibiting the growth of *Rhizopus stolonifer* in bread when the wrapping included this EO at a percentage of 6% (w/w).[226]

By regarding the chemical composition of EOs which exhibit strong antifungal activity against molds no obvious pattern becomes apparent. Therefore, some EOs were predominated by non-phenolic terpenes while others exhibited a high percentage of sesquiterpenes, phenolic monoterpenes or phenylpropanoids.

EOs against phytopathogenic fungi

effective against	EO	main constituents	test method	Ref.
Botrytis fabae, F. oxysporum, Pythium debaryanum, Rhizocotonia solani	*Artemisia judaica* L. (Asteraceae)	Trans-ethyl cinnamate, piperitone		[227]
Colletotrichum gloeosporioides, F. oxysporum, F. solani, Ganoderma australe, Pestalotiopsis funereal, R. solani	*Calocedrus macrolepis* var. *formosana* Florin (Cupressaceae)	α-pinene (44.2%), limonene (21.6%)		[228]
Botrytis cinerea	*Foeniculum vulgare* (L.) Mill. (Apiaceae)			[229]
Botrytis, Fusarium and *Alternaria* species	Origanum acutidens (Hand.-Mazz.) Ietswaart (Lamiaceae)	carvacrol (87.0%)		[230]
A. alternata, B. cinerea, F. oxysporum	*R. officinalis*	p-cymene (44.0%), linalool (20.5%)	disk-diffusion test	[231]
Colletotrichum acutatum, Colletotrichum fragariae, C. gloeosporioides	*Salvia rosifolia* Sm. (Lamiaceae)	α-pinene, 1,8-cineole	dilution test	[18]

Table 10: EOs against phytopathogenic fungi

Eugenol was isolated from *Syzygium aromaticum* (L.) Merr. Et Perry (Myrtaceae) EO and subsequently used for the inhibition of phytopathogens. At a concentration of 150 μl/l the production of mycelia was totally suppressed in *B. cinerea, Monilinia fructigena, P. expansum* and *Phlyctema vagabunda*. Using a combination of eugenol and lecithin which protected the fruits from the phytotoxic effects of eugenol the occurrence of these fungal infections in stored apples was effectively diminished indicating its potential use as bio-fungicide.[232]

In addition, eugenol was detected to obtain fungistatic effects against fungi which infect plant seeds such as *Fusarium moniliforme, Alternaria solani, R. solani* and *Colletotrichum* species. Therefore, this substance inhibited the growth of the pathogens, but also led to the inhibition of mycelia formation and to the destruction of spores.[233]

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TABLES

Table 1: EOs and MRSA

Table 2: EOs against skin infections

Table 3: EOs against *Helicobacter pylori*

Table 4: EOs as biopreservatives

Table 5: EOs against dental bacteria

Table 6: EOs against human pathogens

Table 7: EOs against mycobacteria

Table 8: EOs against dermatophytes

Table 9: EOs against molds

Table 10: EOs against phytopathogenic fungi

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