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Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance ☆

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Abstract

There is cumulative resistance against antibiotics of many bacteria. Therefore, the development of new antiseptics and antimicrobial agents for the treatment of skin infections is of increasing interest. We have screened six plant extracts and isolated compounds for antimicrobial effects on bacteria and yeasts with dermatological relevance. The following plant extracts have been tested: *Gentiana lutea*, *Harpagophytum procumbens*, *Boswellia serrata* (dry extracts), *Usnea barbata*, *Rosmarinus officinalis* and *Salvia officinalis* (supercritical carbon dioxide [CO_2] extracts). Additionally, the following characteristic plant substances were tested: usnic acid, carnosol, carnosic acid, ursolic acid, oleanolic acid, harpagoside, boswellic acid and gentiopicroside. The extracts and compounds were tested against 29 aerobic and anaerobic bacteria and yeasts in the agar dilution test. *U. barbata*-extract and usnic acid were the most active compounds, especially in anaerobic bacteria. *Usnea* CO_2-extract effectively inhibited the growth of several Gram-positive bacteria like *Staphylococcus aureus* (including methicillin-resistant strains – MRSA), *Propionibacterium acnes* and *Corynebacterium* species. Growth of the dimorphic yeast *Malassezia furfur* was also inhibited by *Usnea*-extract. Besides the *Usnea*-extract, *Rosmarinus*-, *Salvia*-, *Boswellia*- and *Harpagophytum*-extracts proved to be effective against a panel of bacteria. It is concluded that due to their antimicrobial effects some of the plant extracts may be used for the topical treatment of skin disorders like acne vulgaris and seborrhoic eczema.

Keywords: *Usnea barbata*; Usnic acid; Agar dilution test; *Staphylococcus aureus*; *Propionibacterium acnes*; *Malassezia furfur*; Antimicrobial plant extracts

Introduction

Plants and plant extracts have been used for the treatment of skin disorders for centuries (Augustin and Hoch, 2004; Avalos and Maibach, 2000; Schempp et al., 1999). Because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004; Blaschek et al., 2004; Norton, 2000). We screened six plant extracts and

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8 isolated compounds for their antimicrobial effects on bacteria and yeasts. The most important selection criterion of the plants was the absence of allergy-inducing compounds. Therefore, pure essential oils have not been included in the screening. We utilized commercially available alcoholic fluid extracts, dry extracts and carbon dioxide (CO_2)-extracts. The tested fluid extracts contain polar secondary plant substances such as bitter agents (*Menyanthes trifoliata* L.), steroid saponines (*Smilax regelii* KILL. et C.V. MORTON), or cucurbitacines (*Bryonia cretica* L.). The dry extracts are rich in bitter agents (*Gentiana lutea* L.), harpagoside (*Harpagophytum procumbens* BURCH. DC.), or boswellic acids (*Boswellia serrata* ROXB. ex COLEBR.). The supercritical liquid CO_2-extraction method enriches lipophilic compounds such as usnic acid (*Usnea barbata* L.) or diterpene phenols (*Salvia officinalis* L., *Rosmarinus officinalis* L.). CO_2-extracts usually are viscous and are characterized by a high drug–extract-ratio (10:1–20:1 w/w). Additional extraction of polar compounds is achieved by using entrainers such as isopropylic alcohol. Detailed specification of the extracts used in this study is given in the "Materials and methods" section and in Table 1. If available, we also investigated characteristic compounds isolated from the tested plants. Details on these compounds are given in the "Materials and methods" section.

A broad panel of microbial pathogens associated with various skin infections has been included in the screening: the Gram-positive *Staphylococcae* and *Streptococcae* are causing wound infections, furuncles, carbuncles, abscesses, impetigo and erysipelas (Köhler et al., 2001; Madigan et al., 2003). The Gram-negative *Enterobacteria* are part of the physiological intestinal flora. However, outside the intestine they may cause wound infections and sepsis (Köhler et al., 2001; Madigan et al., 2003). *Pseudomonas*, another Gram-negative rod, is a frequent pathogen of wound infections. Anaerobic Gram-negative rods may cause skin infections under certain circumstances, i.e. in immunocompromised subjects (Köhler et al., 2001; Madigan et al., 2003). The Gram-positive *Corynebacteria* and *Propionibacteria* are part of the physiological skin flora. However, *Corynebacteria* may cause opportunistic skin infections in immunosuppressed patients. *Propionibacterium acnes* plays an important role as causative agent in acne vulgaris (Köhler et al., 2001). The yeasts *Candida albicans* and *Candida krusei* may occur in low frequency on skin and mucous membranes without causing symptoms. As opportunistic pathogens they may overgrow the normal skin flora and cause skin diseases like intertrigo and candidiasis in diabetics, adipose and immunodeficient subjects. The dimorphic yeast *Malassezia furfur* that is growing in skin areas rich in sebaceous glands is associated with the pathogenesis of seborrhoic eczema and dundruff (Faergemann, 2004; Grigoriu et al., 1984). Details on the germs and their cultivation are given in the "Materials and methods" section and in Tables 2–5.

Materials and methods

Plant extracts and chemicals

The alcoholic fluid extracts of *S. regelii*, *M. trifoliata* and *Bryonia cretica* were purchased from Hetterich (Fürth, Germany). *G. lutea* and *H. procumbens* dry extracts were from Finzelberg (Andernach, Germany). *B. serrata* dry extract was provided by HWI (Rheinzabern, Germany). Supercritical CO_2-extracts from *U. barbata*, *R. officinalis* and *S. officinalis* were from Flavex (Rehlingen, Germany). Table 1 provides detailed information on drug material, solvent, drug–extract relation and preparation of stock solutions from the extracts. We tempted to obtain highly concentrated water-soluble stock solutions that could easily be incorporated into the aqueous liquid agar medium. On the other hand, it was necessary to dilute toxic solvents like ethanol below a concentration of 1% v/v. The stock solutions differ somewhat with respect to the concentration of the plant extracts. The stock solutions were incorporated into the agar plates at the following concentrations: 100, 20, 10, 2, 1, 0.4 and 0.2 µg/ml.

Most of the isolated plant substances were purchased from Roth (Karlsruhe, Germany) (usnic acid, carnosic acid, oleanolic acid, ursolic acid, harpagoside, gentiopicroside, cucurbitacin E, cucurbitacin I and aucubin). Carnosol was purchased from Alexis (Grünberg, Germany), and 11-keto-boswellic acid was from Phytoplan (Heidelberg, Germany). The substances were dissolved in ethanol 20% v/v and water 80% v/v. Cucurbitacin E was dissolved in dimethyl sulfoxide 20% v/v and water 80% v/v. Stock solutions were prepared at a concentration of 1.28 mg/ml and incorporated into the agar plates according to the two-fold dilution method of the German DIN-standard (Deutsches Institut für Normung, 2002). The final concentrations were 128, 64, 32, 16, 8, 4, 2 and 1 µg/ml.

Cultivation of bacteria and yeasts

In the present screening we have analyzed 18 aerobic and 9 anaerobic bacteria strains, 2 *Candida* strains and 1 *M. furfur* isolate. All strains derived from Type Culture Collections (ATCC and DSM) or patient isolates from the Institute of Medical Microbiology, University of Freiburg (for origin and strain number see Tables 2–5). The susceptibility of all germs to a panel of antibiotics has been described in the ATCC specification sheets or has been tested according to the German Network for Antimicrobial Resistance Surveillance (GENARS, 2004). The test germs were precultivated on appropriate

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Table 1. Specification of the tested extracts and characteristic compounds

	Drug material	Solvent	Drug–extract relation	Extract form	Characteristic compounds	Preparation of stock solutions	Tested single compounds
Usnea barbata L.	Usneae barbatae lichen	Carbon dioxide	3–4:1 (w/w)	Suspension	Usnic acid (4% w/w)	250 mg/ml in H_2O	(+)-usnic acid
Rosmarinus officinalis L.	Rosmarini officinalis folium	Carbon dioxide/ isopropyl alcohol	14–17:1 (w/w)	Viscous paste	Diterpene phenols (14% w/w)	250 mg/ml in H_2O	Carnosol, carnosic acid, ursolic acid, oleanolic acid
Salvia officinalis L.	Salviae officinalis folium	Carbon dioxide/ isopropyl alcohol	14–20:1 (w/w)	Viscous paste	Diterpene phenols (35% w/w)	10 mg/ml in 20% ethanol	Carnosol, carnosic acid, ursolic acid, oleanolic acid
Boswellia serrata ROXB. ex COLEBR.	Boswellia serrata resin, Indian origin	Ethanol (70% v/v)	3.8–4.2:1 (w/w)	Powder	Boswellic acids (80% w/w)	10 mg/ml in 20% DMSO/10% H_2O	11-keto-β-boswellic acid
Harpagophytum procumbens (BURCH.) DC.	Harpagophyti radix	H_2O	1.5–2.5:1 (w/w)	Powder	Harpagoside (2.6% w/w)	200 mg/ml in PBS	Harpagoside
Gentiana lutea L.	Gentianae radix	H_2O	2–4:1 (w/w)	Powder	Secoiridoide bitter agents	400 mg/ml in PBS	Gentiopicroside
Menyanthes trifoliata L.	Menyanthidis folium	Ethanol (55% v/v)	1:1 (w/v)	Fluid extract (dry substance 23%)	Secoiridoide bitter agents	1:7 (v/v) in H_2O	Aucubin
Bryonia cretica L.	Bryoniae radix	Ethanol (70% v/v)	1:1 (w/v)	Fluid extract (dry substance 10%)	Curcurbitacines	1:7 (v/v) in H_2O	Curcurbitacin E cucurbitacin I
Smilax regelii KILL. et C.V. MORTON	Sarsaparillae radix	Ethanol (70% v/v)	1:1 (w/v)	Fluid extract (dry substance 10%)	Saponines	1:5.5 (v/v) in H_2O	

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Table 2. Effects of plant extracts on aerobic bacteria and *Candida* species in the agar dilution test

	Usnea		*Salvia*		*Rosmarinus*		*Boswellia*		*Harpagophytum*		*Gentiana*		*Bryonia*		*Sarsaparilla*		*Menyanthes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus (ATCC 25923)	2	100	100	100	10	10	100	—	10	20	—	—	—	—	—	—	100	100
Staphylococcus aureus, Pen. res. (E 12431/98)	2	100	100	100	10	10	100	—	10	10	—	—	—	—	—	—	100	100
Staphylococcus aureus, MRSA (RV5/98)	10	100	100	100	10	10	—	—	10	20	—	—	—	—	—	—	100	100
Staphylococcus epidermidis (DSM 1798)	1	100	20	100	10	10	100	100	10	100	—	—	—	—	—	—	100	100
Staphylococcus lugdunensis (NG 3406/94)	1	100	20	100	10	10	—	—	10	100	—	—	—	—	—	—	100	100
Streptococcus pyogenes gr. A (E 12449/98)	1	1	10	100	10	10	1	100	100	100	100	n. e.	100	100	20	100	20	100
Streptococcus agalactiae gr. B (DSM 2134)	1	1	20	100	10	10	2	20	100	100	—	—	—	—	—	—	100	—
Enterococcus faecalis (ATCC 19212)	1	100	100	100	20	20	2	100	100	100	—	—	—	—	—	—	—	—
Escherichia coli (ATCC 25922)	—	—	—	—	100	100	—	—	100	100	—	—	—	—	—	—	—	—
Morganella morganii (E 10679/93)	—	—	100	100	10	20	—	—	20	20	—	—	—	—	—	—	100	100
Klebsiella pneumoniae (ATCC 27736)	—	—	—	—	—	—	—	—	100	100	—	—	—	—	—	—	—	—
Pseudomonas aeruginosa (ATCC 27853)	—	—	100	100	20	20	—	—	100	100	—	—	—	—	—	—	—	—
Serratia marcescens (E 8382/92)	—	—	—	—	100	100	—	—	100	100	—	—	—	—	—	—	—	—
Pseudomonas maltophilia (D 141/92)	—	—	100	100	10	10	—	—	20	100	—	—	—	—	—	—	100	100
Bacillus subtilis (ATCC 6633)	1	20	20	20	10	10	2	2	100	100	—	—	—	—	—	—	100	—
Micrococcus luteus (DSM 348)	1	1	100	100	10	10	100	n.a.	100	100	—	—	—	—	—	—	—	—
Corynebacterium amycolatum (ATCC 494368)	1	10	20	100	2	10	1	20	10	20	10	n.e.	—	—	—	—	20	100
Corynebact. pseudodiphtericum (RV 2/95)	1	1	10	20	2	2	10	20	20	100	100	n. e.	—	—	—	—	20	100
Candida albicans (ATCC 90028)	—	—	—	—	20	100	—	—	100	—	—	—	—	—	—	—	—	—
Candida krusei (ATCC 6258)	—	—	—	—	20	100	—	—	100	100	—	—	—	—	—	—	—	—

MIC and MBC of stock solutions are indicated in μg/ml.
—, no inhibition; n.e., not evaluable.

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Table 3. Effects of (+)-usnic acid and carnosic acid on aerobic bacteria and *Candida* species in the agar dilution test

	(+)-Usnic acid		Carnosic acid	
	MIC	MBC	MIC	MBC
Staphylococcus aureus (ATCC 25923)	16	64	64	64
Staphylococcus aureus, Pen. res. (E 12431/98)	16	64	128	128
Staphylococcus aureus, MRSA (RV5/98)	16	64	—	—
Staphylococcus epidermidis (DSM 1798)	4	32	64	128
Staphylococcus lugdunensis (NG 3406/94)	16	64	128	128
Streptococcus pyogenes group A (E 12449/98)	8	64	64	128
Streptococcus agalactiae group B (DSM 2134)	16	64	64	n.e.
Enterococcus faecalis (ATCC 19212)	4	64	—	—
E. coli (ATCC 25922)	—	—	—	—
Morganella morganii (E 10679/93)	—	—	—	—
Klebsiella pneumoniae (ATCC 27736)	—	—	—	—
Pseudomonas aeruginosa (ATCC 27853)	—	—	—	—
Serratia marcescens (E 8382/92)	—	—	—	—
Pseudomonas maltophilia (D 141/92)	64	128	—	—
Bacillus subtilis (ATCC 6633)	8	8	64	128
Micrococcus luteus (DSM 348)	8	32	—	—
Corynebacterium amycolatum (ATCC 494368)	8	32	64	128
Corynebacterium pseudodiphtericum (RV 2/95)	4	8	32	128
Candida albicans (ATCC 90028)	—	—	—	—
Candida krusei (ATCC 6258)	—	—	—	—

MIC and MBC of compounds are indicated in μg/ml.
—, no inhibition; n.e., not evaluable.

agar plates. Fresh colonies were suspended in Müller–Hinton-Bouillon and the bacterial count was assessed according to the McFarland standard 0.5–1 (Madigan et al., 2003). Yeasts were adjusted to McFarland standard 2 (6×10^6 germs/ml). The lipophilic yeast *M. furfur* was grown on Sabouraud agar containing glucose and olive oil (Grigoriu et al., 1984).

Agar dilution test

Stock solutions of plant extracts or single compounds and their serial dilutions were mixed thoroughly with the liquid agar before solidification to obtain the final concentrations. Subsequently aerobic bacteria were inoculated with a multipoint-inoculator on Müller–Hinton-agar, and anaerobic bacteria on Wilkins–Chalgren-agar. The inoculum of 1 μl of the germ suspensions contained 10^{4-5} colony forming units (CFU) for aerobic bacteria and 10^{5-6} CFU for anaerobic bacteria. Aerobic bacteria and *Candida* were incubated at 36 °C for 24 h, and anaerobic bacteria were incubated at 36 °C for 48 h in an anaerobic jar using Anaerocult^R (90% N_2, 10% CO_2). *Malassezia* was incubated at 36 °C for 48 h with 5–10% CO_2. The plates were then evaluated visually for the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). The MIC was defined as the concentration that resulted in no visible growth or less than 10 CFU, corresponding to an inhibition of 99.9% of the inoculum. For the determination of the MBC we used a variant on the agar dilution method. The inoculation spots with no visible growth were cut and top down streaked on Columbia blood agar (aerobes) or yeast-cysteine blood agar (anaerobes) without inhibitory substances The MBC was determined according to the MIC. The lowest concentration without visible growth corresponded with the MBC.

Results

Aerobic bacteria and *Candida*

Table 2 summarizes the results obtained with plant extracts in aerobic bacteria and *Candida*. The CO_2-extracts and *Harpagophytum* were the most active plant extracts. *Usnea* CO_2-extract inhibited the growth of all tested Gram-positive bacteria including penicillin-resistant *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA), and *Enterococcus faecalis*. *Rosmarinus*-extract inhibited the growth of all tested aerobic bacteria, with the exception of *Klebsiella pneumoniae*. *Salvia*-extract showed a similar inhibition spectrum compared to *Rosmarinus*-extract, but with higher MIC and MBC and some gaps in the Gram-negative spectrum. *Harpagophytum*-extract was effective in all tested germs, but generally at higher concentrations compared to *Rosmarinus*-extract. The *Boswellia*-extract

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Table 4. Effects of plant extracts on anaerobic bacteria and *Malassezia* in the agar dilution test

	Usnea		*Salvia*		*Rosmarinus*		*Boswellia*		*Harpagophytum*		*Gentiana*		*Bryonia*		*Sarsaparilla*		*Menyanthes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Clostridium perfringens (ATCC 13124)	0.4	0.4	10	10	1	1	1	10	—	—	—	—	100	100	—	—	20	100
Propionibactrium acnes (FR 024/12-10)	0.4	1	10	100	10	100	1	10	100	—	—	—	—	—	100	100	100	100
Fusobacterium nucleatum (ATCC 25586)	0.4	1	—	—	10	10	—	—	100	100	100	n.a.	100	100	—	—	100	100
Bacteroides fragilis (ATCC 25285)	0.4	0.4	10	20	10	10	—	—	—	—	—	—	—	—	—	—	—	—
Bacteroides vulgatus (ATCC 29327)	0.4	0.4	10	10	2	10	20	—	—	—	—	—	—	—	—	—	—	—
Prevotella intermedia (NCTC 9336)	0.4	1	20	20	10	10	—	—	—	—	—	—	—	—	—	—	—	—
Veillonella parvula (A 11-9)	—	—	100	—	10	20	—	—	—	—	—	—	—	—	—	—	—	—
Porphyromonas gingivalis (W 83)	0.2	0.2	2	10	1	1	1	20	100	100	100	n.a.	20	100	10	20	20	20
Peptococcus magnus (D385-8/94)	0.4	0.4	100	100	10	20	20	100	—	—	—	—	—	—	100	100	20	20
Malassezia furfur (HK 001)	10	10	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.	n.e.

MIC and MBC of stock solutions are indicated in µg/ml.
—, no inhibition; n.e., not evaluable.

was effective against *Streptococcus pyogenes*, *Corynebacterium amycolatum* and *E. faecalis*. *Menyanthes*-extract inhibited the growth of some Gram-positive and Gram-negative bacteria at a higher concentration (mostly at 100 µg/ml). *G. lutea*, *Bryonia cretica* and *S. regelii* did not display any activity, except against *S. pyogenes*. *Candida* was only inhibited by *Rosmarinus*- and *Harpagophytum*-extracts. Testing of the single compounds revealed that only (+)-usnic acid and carnosic acid display significant activity in aerobic bacteria (negative data not shown). Both substances inhibited the growth of Gram-positive bacteria and of *E. faecalis* and *Pseudomonas maltophilia*, (+)-usnic acid being much more active than carnosic acid. The compounds were not active against *C. albicans* and *C. krusei*. The MIC and MBC data of (+)-usnic acid and carnosic acid are summarized in Table 3.

Anaerobic bacteria and *Malassezia furfur*

Usnea-extract was effective at low concentrations against all anaerobic bacteria except *Veillonella parvula*. *Usnea* was also tested in *M. furfur* that was inhibited at a concentration of 10 µg/ml (Table 4). *Salvia*- and *Rosmarinus*-extracts were effective in most of the anaerobic bacteria, but at higher concentrations compared to *Usnea*-extract. *Boswellia* inhibited the growth of *Clostridium perfringens*, *P. acnes* and *Porphyromonas gingivalis* at low concentrations. *Harpagophytum*, in contrast to its activity in aerobic bacteria, was not effective in anaerobic bacteria. The other plant extracts did not display marked effectivity in anaerobic bacteria (Table 4). (+)-Usnic acid and carnosic acid were effective in all anaerobic bacteria except *V. parvula*, (+)-usnic acid being superior to carnosic acid (Table 5). The other purified compounds were not active against anaerobic bacteria (not shown).

Discussion

We have tested nine plant extracts for antimicrobial activity differing in their production method and drug-extract relation. The CO_2-extracts from *U. barbata*, *R. officinalis* and *S. officinalis* were the most active compounds with respect to the inhibition panel and the MIC and MBC concentrations. Antibacterial effects of lichen species have been described recently (Gulluce et al., 2006; Rowe et al., 1999), but antimicrobial effects of *U. barbata* have poorly been investigated. In contrast, broad antimicrobial activity of usnic acid has been reported by several authors (Cocchietto et al., 2002; De Battisti et al., 1991; Dobrescu et al., 1993; Ingolfsdottir, 2002; Lauterwein et al., 1995). In addition to these effects, we have described here for the first time antimicrobial activity of (+)-usnic acid against *P. acnes*, *Corynebacterium pseudodiphtericum* and *M. furfur*. *P.*

Table 5. Effects of (+)-usnic acid and carnosic acid on anaerobic bacteria and *Malassezia* in the agar dilution test

	(+)-Usnic acid		Carnosic acid	
	MIC	MBC	MIC	MBC
Clostridium perfringens (ATCC 13124)	2	8	16	16
Propionibacterium acnes (FR 024/12-10)	1	4	16	32
Fusobacterium nucleatum (ATCC 25586)	2	4	128	128
Bacteroides fragilis (ATCC 25285)	1	8	64	64
Bacteroides vulgatus (ATCC 29327)	2	4	32	32
Prevotella intermedia (NCTC 9336)	2	2	64	64
Veillonella parvula (A 11-9)	64	—	—	—
Porphyromonas gingivalis (W 83)	1	2	16	32
Peptococcus magnus (D 385-8/94)	4	16	32	64
Malassezia furfur (HK 001)	64	64	n.e.	n.e.

MIC and MBC of compounds are indicated in μg/ml.
—, no inhibition; n.e., not evaluated.

acnes and *M. furfur* are associated with the pathogenesis of acne, seborrhoic eczema and dundruff (Bolognia et al., 2003; Faergemann, 2004). Therefore, *Usnea*-extract may be useful in the topical treatment of these skin disorders. Both *Usnea*-extract and (+)-usnic acid additionally inhibited the growth of aerobic Gram-positive bacteria including *Streptooccae*, *Corynebacteria* and MRSA. The inhibition spectrum and MIC/MBC of *U. barbata*-extract and (+)-usnic acid are similar, suggesting that the antimicrobial activity of *Usnea*-extract is mainly mediated by usnic acid. *Candida*-yeasts were inhibited neither by *Usnea*-extract nor by (+)-usnic acid.

Another CO_2-extract with a broad antimicrobial spectrum was the *Rosmarinus*-extract. The growth of 28 out of 29 germs was inhibited by *Rosmarinus*-extract, but usually at higher concentrations compared to the *Usnea*-extract. Remarkably, *Rosmarinus*-extract was the only extract that also inhibited the growth of both *Candida* strains. *Rosmarinus*-extract was most effective against *C. perfringens* and *P. gingivalis* (MIC and MBC < 1 μg/ml). In the literature, only sparse information on antimicrobial activities of *Rosmarinus* is available. Most of the published work refers to essential oil from *Rosmarinus* (Blaschek et al., 2004; Mangena and Muyima, 1999). Here we have used a CO_2-extract from *Rosmarinus* leaves that contains other secondary metabolites compared to the essential oil (Del Bano et al., 2003). Major components of the essential oil are campher, α-pinene and 1,8-cineol (Blaschek et al., 2004). In contrast, major compounds of the leaf-extract are diterpene phenols (carnosol and carnosic acid), and the triterpenoids oleanolic acid and ursolic acid (Del Bano et al., 2003). Additionally, the CO_2 leaf-extract contains 1–2% (v/w) essential oil according to manufacturers specification (Flavex Naturextrakte, Rehlingen, Germany). To our knowledge, there are no comparable studies that investigated *Rosmarinus* CO_2 leaf-extract in the germs tested here. Solely an inhibitory effect of an ethanolic *Rosmarinus* leaf-extract on the growth of *Clostridium botulinum* has been reported (Blaschek et al., 2004), providing a rationale for the preservation of sausage with *Rosmarinus*. Here we have found inhibitory activity of *Rosmarinus*-extract on *C. perfringens* (MIC and MBC < 1 μg/ml).

S. officinalis CO_2-extract showed a similar inhibition spectrum as *Rosmarinus*-extract, but generally with higher MIC/MBC concentrations. It has been shown in the past that essential oil from *S. officinalis* inhibits the growth of bacteria, yeasts and dermatophytes (Jalsenjak et al., 1978; Janssen et al., 1989). An ethanolic extract from *S. officinalis* leaves was highly effective against *P. gingivalis* (Osawa et al., 1991).

B. serrata gum resin and boswellic acids have recently gained new interest because of the rediscovery of its antiinflammatory properties (Ammon et al., 1991). Antimicrobial effects of *B. serrata* have not been reported so far. *Boswellia* has been used in traditional medicine for the treatment of thrush that is caused by *Candida* species (Blaschek et al., 2004). We could not find antimicrobial activity of *Boswellia* dry extract or 11-keto-β-boswellic acid against *C. albicans* or *C. krusei*. However, the extract was highly effective against selected aerobic and anaerobic bacteria such as *Streptococcae*, *Corynebacteria*, *C. perfringens* and *P. acnes*. Because 11-keto-β-boswellic acid was not effective in these germs, we suggest that the effective components are other boswellic acids or essential oils contained in the extract.

H. procumbens-extract is known for its antiinflammatory properties (Fiebich et al., 2001). In the present screening, *Harpagophytum*-extract inhibited the growth of all aerobic bacteria, *C. krusei* and two anaerobic bacteria strains. The characteristic compound of *Harpagophytum*, harpagoside, was not effective in the screening, suggesting that other compounds in the extract represent

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the active principle. Bitter agents of *G. lutea* have been described to possess antifungal activity (Guerin and Reveillere, 1985). In the screening presented here, *Gentiana* dry extract did not display significant antimicrobial activity against bacteria and yeasts.

Bryonia, Smilax and Menyanthes did not display pronounced antimicrobial effects at the concentrations tested.

The most interesting extract is the supercritical CO_2-extract from *U. barbata* with a defined content of (+)-usnic acid. Although usnic acid is a lichen acid with a weak sensitizing potential, this should not be a problem in clinical practice because the MIC/MBC concentrations of *Usnea*-extract are clearly below the sensitizing concentrations (Hausen et al., 1993). Because of its high efficacy against *P. acnes*, *Corynebacteria, Fusobacterium* and *M. furfur*, *Usnea*-extract may be effective in the treatment of acne, rosacea, seborrhoic eczema and dundruff (Bolognia et al., 2003). These skin conditions are associated with superinfection of the skin with the above-mentioned germs. Topical standard treatment modalities for these skin disorders are problematic in some aspects (Leyden, 2004a). Benzoyl peroxide and retinoids are skin irritating. Frequently used topical antibiotics such as macrolides or tetracyclines may cause resistance of bacteria that prevents the use of these substances in severe infections (Leyden, 2004b). Therefore, it would be interesting to perform clinical studies with *Usnea*-extract in these skin disorders.

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