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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