

In vitro* Activity of *Artemisia abrotanum* Extracts Against *Malassezia* Spp., *Candida albicans* and *Staphylococcus aureus

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Sir,

In order to combat diseases, research in modern medicine is constantly revising traditional medicinal flora. The genus *Artemisia*, of the family *Asteraceae*, comprises a wide array of species indigenous to southern Europe and western Asia. *Artemisia abrotanum* (*Aa*) is a perennial composite that is nowadays used mainly for culinary or cosmetic purposes (1, 2). Extracts of *Aa* contain mostly terpenes and flavonols. The terpenes 1,8-cineole and davanone represent the bulk of these. Their structures are shown in Fig. 1. Both terpenes have records of anti-inflammatory or antimicrobial activity and were therefore included as separate substances in this study (3–6). They are, however, probably best known as essential oils, which often have characteristic scents, e.g. mint, induced by menthol.

The aim of this study was to assess the antimicrobial properties of *Aa* extracts *in vitro*. The susceptibility of *Malassezia* spp., *Candida albicans* and *Staphylococcus aureus* to these substances was evaluated by estimating minimal inhibitory concentrations (MICs).

The *Malassezia* spp. (formerly *Pityrosporum ovale*) are members of the normal human cutaneous flora in adults (7, 8). The genus comprises 7 species, whereof *M. obtusa* and *M. restricta* are not included here. *M. pachydermatis* is the only non-lipophilic species. The *Malassezia* yeasts are opportunists requiring predisposing circumstances in order to manifest as disease. They are pathogens in skin diseases such as pityriasis versicolor and *Malassezia* folliculitis and are associated with seborrhoeic dermatitis and atopic dermatitis.

S. aureus was included on the basis of causing or aggravating several dermatological disorders and with respect to the increase in multi-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) (9).

C. albicans is a member of the normal flora of mucous membranes. Predisposing factors may give rise to

infections. The yeast causes problematic infections in immuno-suppressed individuals (7).

MATERIALS AND METHODS

Extracts of *Aa* were acquired from the Department of Organic and Bioorganic Chemistry, Lund University, Sweden (10). They were produced by Professor Olov Sterner using a patented vaporizing process. One oil extract and 2 aqueous ethanol extracts were produced. Both contained 25% of active substance. The essential oils 1,8-cineole and davanone in these were found to be 100% pure. When cultured in Scandinavia *Aa* will not bloom. The method of production of the extracts reduces the risk of allergic reactions (4).

The *Malassezia* strains CBS7019 *M. furfur*, CBS1871 *M. pachydermatis*, CBS7956 *M. slooffiae*, CBS7966 *M. globosa* and 42132 *M. sympodialis* were included in the study. Isolates of *S. aureus* and *C. albicans* H29 were obtained from the culture collection of the Department of Microbiology, Sahlgrenska University Hospital, Gothenburg, Sweden. *S. aureus* is coagulase-positive, non-MRSA and was from a patient with impetigo. *S. aureus* was grown on horse blood agar at 37°C. Cultures of *C. albicans* were grown on mycobiotic plates at 32°C. The *Malassezia* strains were grown on modified Leeming Notman agar plates at 37°C. Preceding the initial test the yeasts were subcultured and kept for 5 days at 32°C. Due to lack of growth in this first experiment they were thereafter continually re-inoculated every week to ensure viable strains. *S. aureus* was kept refrigerated and subcultured on horse blood plate and incubated for 1 day at 37°C before inclusion in the test.

Diagnostic Sensitivity Test (DST) agar (Oxoid, Basingstoke, UK) with glycerol monostearate (2.5 g/l) and Tween80 (2 mg/l) content was used to meet the growth requirements of *Malassezia*. The DST agar was obtained from the Department of Microbiology, Sweden. On receipt, the DST agar was in liquid form, requiring storage at 55°C to keep it from setting.

The oil extracts were diluted in dimethyl formamide (DMF), producing stock solutions able to mix with the agar. Secondary stock solutions were made by serial diluting a decided volume of the first stock solutions in phosphate-buffered saline (PBS). This was done to increase the accuracy of lower concentrations. Since the ethanol extract dissolved readily with the agar no organic solvent was needed. Each stock solution and the ethanol extract were then further diluted with agar to obtain the following concentrations in the agar: 200, 100, 50, 25, 12.5, 6.25, and 3.125 mg/ml for DMF; 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/ml for ethanol extract and control; and 5, 2.5, 1.25, 0.625, 0.3125, 0.2, 0.1, and 0.05 mg/ml for oil extract, davanone and cineole. No precipitations were observed.

Micro-organisms were harvested and suspended in PBS and counted manually in Bürker chambers to reach concentrations of 10⁷ cells/ml for the *Malassezia* spp. and 10⁵ cells/ml for the *S. aureus* and *C. albicans*. Due to poor growth of *M. globosa* and *M. slooffiae*, the suspensions were modified to contain 10⁸ cells/ml in the third experiment.

Using a Pasteur micropipette, plates were inoculated with 20 µl of emulsion. Either 3 or 4 different suspensions of

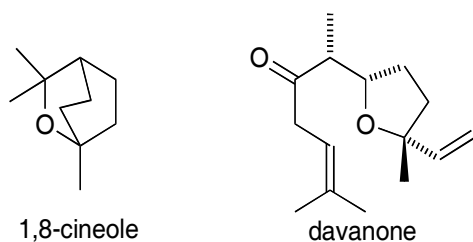


Fig. 1. Structures of 1,8-cineole and davanone found in *Artemisia abrotanum*.

micro-organisms were inoculated onto each plate. Control plates containing only agar and plates with concentration series of DMF and ethanol were made. All plates had duplicates.

Plates were incubated at 37°C and read after 1, 2 and 3 days.

RESULTS

The MICs are presented in Table I. The substances studied inhibited the growth of all micro-organisms. There were some variations in isolate-substance responsiveness. All plates showed consistency with the parallel control plates. Davanone was the most potent antimicrobial agent of the oil substances. The oil extract was more inhibitory than the ethanol extract. Contrary to our initial experience, *M. globosa*, *M. slooffiae* and *M. pachydermatis* obtained MICs equivalent to the other micro-organisms in our subsequent experiments. Note that the inoculum of *M. globosa* and *M. slooffiae* was increased ten-fold, which might account for the higher MICs.

Neither the ethanol controls, nor the DMF had lower MICs than the terpenes or *Aa* extracts. The most resistant isolate overall was *S. aureus*. The yeasts *M. globosa* and *M. slooffiae* seemed to be the least persistent micro-organisms. *C. albicans* was slightly less sensitive than *M. furfur*. The highest MIC obtained for the substances studied was 5 mg/ml (cineole) and the lowest 0.1 mg/ml (davanone).

DISCUSSION

Extracts of *Aa* contain ample secondary metabolites of interest; terpenes, flavonols, coumarins and cinnamic acid derivatives. Adding to this complexity, there are seasonal, geographical and genotype variations of *Aa* extract constituents that may explain its diverse historical and cultural usage. Contrary to the *Aa* genotype "tycho" used in this experiment, certain genotypes have been reported to harbour the toxic terpene thujone. Flowering *Aa* plants are more prone to contain toxins (2). Since "tycho" is cultivated in a Nordic climate on the island of Ven because the temperature is not high enough it does not come into flower. This will reduce the risk of contact allergy which may be a problem with many terpenes. Furthermore, oxidized terpenes are

inclined to be more allergenic. The chemical nature of terpenes and their listing in Pharmacopoeias suggest an easy transformation into topical solutions to be tested on human subjects.

The activity of *Aa* extracts against *Malassezia* spp, *C. albicans* and *S. aureus* is evident. Regarding davanone and 1,8-cineole, our results support previous findings (3, 5). The mechanism behind the inhibition is unknown.

Traditional therapies for *Malassezia* and *Candida* infections of the skin include bifonazole, ketoconazole and itraconazole. Published MICs for these substances (0.02–0.05 µg/ml for ketoconazole, 0.1–0.2 µg/ml for itraconazole and 0.5 µg/ml for bifonazole) are a thousand-fold as potent as the MICs measured in this study (11–13). This might appear to be a significant discrepancy, but considering the substances used here are natural and unmodified, the results are clearly interesting.

Further investigation is needed to clarify whether *Aa* extracts or terpenes have a role to play in future dermatological care.

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Table I. *In vitro* activity (mg/ml) of *Artemisia abrotanum* extracts against *Malassezia* spp, *C. albicans* and *S. aureus*

Substance (mg/ml)	<i>M. furfur</i>	<i>M. sympodialis</i>	<i>M. pachydermatis</i>	<i>M. slooffiae</i>	<i>M. globosa</i>	<i>C. albicans</i>	<i>S. aureus</i>
Ethanol extract	2	2	2	2	2	2	2
Oil extract	0.6	0.6	0.3	0.3	1.3	1.3	1.3
Cineole	5	5	5	5	5	5	5
Davanone	0.6	0.1	0.6	0.1	0.3	0.6	1.3
Dimethyl formamide	50	25	50	25	25	50	100
Ethanol control	— ^a	1	1	1	— ^a	— ^a	— ^a

^aMinimal inhibitory concentrations exceeded the highest concentration (2 mg/ml).

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