

Apple scab control with grapefruit seed extract: no alternative to chemical fungicides

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Summary

The growth inhibiting effect of four commercially available grapefruit seed extracts on the causal organism of apple scab *Venturia inaequalis* was tested. Germination of the conidia of *Venturia inaequalis* was pronouncedly inhibited by all tested extracts. The commercial products were analyzed by high pressure liquid chromatography and thin layer chromatography. All samples contained at least one preserving agent. These substances were identified as either benzethonium chloride, benzalkonium chloride, methyl parabene or propyl parabene. Freshly prepared extracts from seeds of grapefruits (*Citrus paradisi*) did not inhibit the germination of *Venturia inaequalis*. It was therefore concluded that the antifungal effect of grapefruit seed extracts is caused by the added preservatives.

Introduction

Apple scab is the most important disease affecting apple growing in temperate climates. Especially in organic cultures only a few permitted substances are able to control scab effectively. These compounds include lime sulphur, sulphur and copper witch. A large number of alternative products (250) were compared with regards to their fungicidal activity against apple scab. These assays were performed by means of a standardized apple seedling greenhouse test. Several GSE-products revealed a pronounced antifungal activity.

For many years the use of grapefruit seed extracts (GSE) as highly effective antimicrobial agents has been praised by lay media. The supposed activity encompasses inhibition of bacteria, fungi and viruses as well as several unicellular parasites (Uhlenbrock, 1996). However, only a limited number of scientific reports deal with these effects (Woedtke et al., 1999, Takeoka et al., 2001).

In 1997 the Central Laboratory of German Pharmacists (Zentrallaboratorium Deutscher Apotheker) detected benzethonium chloride in several commercial GSE. Based on these findings, the Drug Commission of the German Pharmacists Association (Deutsche Arzneimittelkommission, AMK) issued a statement, that GSE

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potentially containing benzethonium chloride must not be sold by pharmacies any more (AMK, 1998).

Woedtke et al. (1999) examined six commercial grapefruit seed extracts and detected high activity against *Bacillus subtilis*, *Micrococcus flavus*, *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, *Proteus mirabilis* and *Candida maltosa* in five of the products investigated. All active samples contained one to three preserving agents, namely benzethonium chloride, triclosan, and methyl parabene. In contrast, freshly prepared extracts from seeds and juiceless pulp of *Citrus paradisi* didn't show any antimicrobial activity. Thus, the authors concluded that the antimicrobial activity of commercially available grapefruit seed extracts was due to the added preservatives.

The commercial products are labeled by the manufacturers as glyceric extracts containing ascorbic, lactic and citric acid as antioxidants. No other preserving additives are indicated and no data about the extraction method are given. According to Nutri Team Inc. (Vermont, USA) GSE is obtained from an aqueous solution of dried and ground grapefruit seeds and pulp, which is distilled, then spray dried and re-dissolved in glycerin. The obtained liquid is mixed with ammonium chloride, ascorbic acid and catalysts, which allegedly convert the polyphenols of the seeds and pulp into quaternary ammonium compounds (www.nutriteam.com/gsewhat.html). The supporters of commercial GSE products claim that these GSE-derived quaternary ammonium compounds differ from the chemically identical synthetic compounds benzethonium chloride and benzalkonium chloride by their lack of toxicity to animal life. However, none of these pseudo-scientific statements has ever been validated by scientific data.

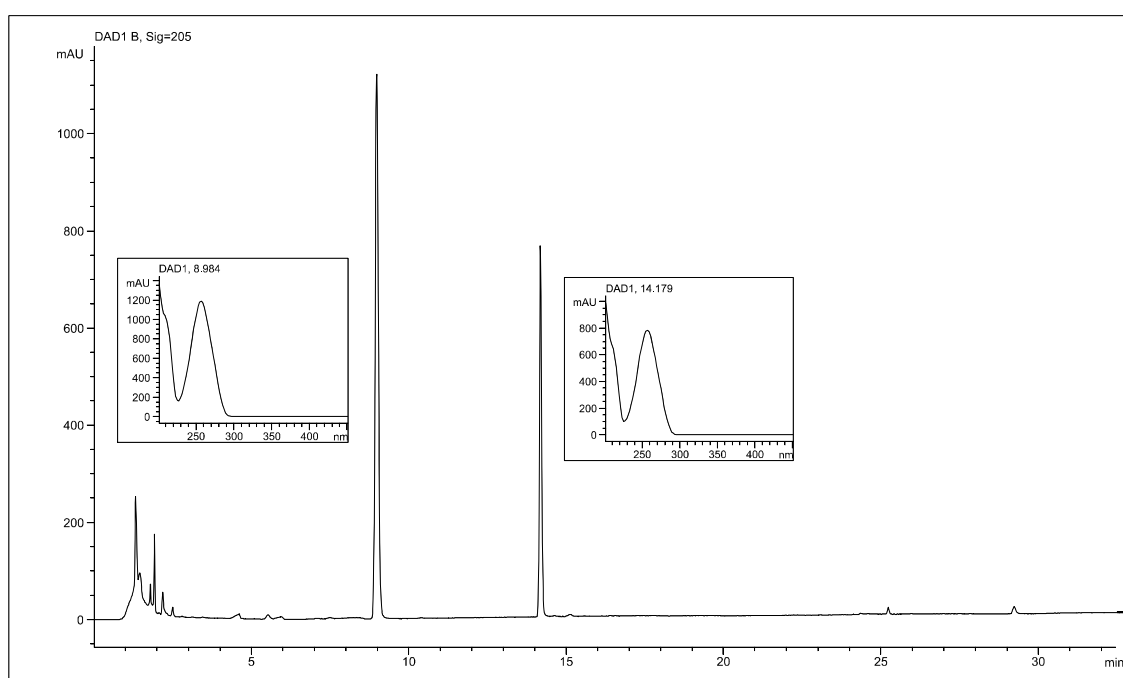
The presence of quaternary ammonium compounds in a commercial grapefruit seed extract was proven by Takeoka et al. (2001) employing high pressure liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS), nuclear magnetic resonance (NMR) spectroscopy and proton-induced X-ray emission (PIXE) analysis. The main compound of the chloroform layer of the commercial product was identified as benzethonium chloride.

Results

In the present study, four commercial grapefruit seed extracts were tested against conidia of *Venturia inaequalis* by means of a standardized germination test (Olaya and Köller, 1999). All four GSE products were partitioned successively with petrol ether, dichloromethane and methanol, obtaining a total of 12 phases (three for each product). After evaporating the solvents *in vacuo*, each phase was tested in concentrations of 2.5 mg/ml, 0.125 mg/ml, 0.013 mg/ml and 0.001 mg/ml. After an incubation period of 48 h at room temperature conidial germination was examined by microscopy. Additionally, an untreated control was analyzed. All samples were analyzed in triplicate. Inhibition rates of each tested concentration were determined employing Abbott's formula. All tested dichloromethane phases showed 100% growth inhibition in concentrations down to 0.125 mg/ml. In the same concentration range, petrol ether and methanol phases were less active.

To characterize and compare the extracts, analytical HPLC and TLC methods were established. The obtained chromatograms indicated the presence of preserving agents in all four commercial products. The identity of these substances was confirmed by comparison with reference compounds.. Two products contained both methyl parabene and propyl parabene as main compounds (Fig. 1), one benzethonium chloride, and one benzalkonium chloride.

Fig. 1: HPL-chromatogram of a commercial grapefruit seed extract containing methyl parabene and propyl parabene



HPLC-method: FM A: H₂O, FM B: Acetonitril; linear gradient: 0 min. 20% B; 5 min 20% B; 20 min. 75% B; 25 min. 95% B. 35 min. STOP; flow rate 1.000 ml/min.; column Zorbax SB-C18 4.6 x 150 mm, 3.5 µm; injection volume 10 µl; detection wave length 205 nm.

To clarify, whether the antifungal activity of the extracts was exclusively an effect of the added preserving agents, two different accessions of *Citrus paradisi* seeds were extracted and the extracts analyzed for antifungal activity. The first accession was successively extracted with dichloromethane and methanol, the second one successively with petrol ether, dichloromethane and methanol. *Venturia inaequalis* bio-assay results showed no inhibiting activity against the fungus for any of the extracts investigated in concentrations up to 2.5 mg/ml.

Discussion

The presented results clearly indicate that the antifungal activity of commercial grapefruit seed extracts is due to the contents of well known chemical preserving agents. No hint was found that natural compounds from the seeds of *Citrus paradisi*

have any antifungal activity. These results fit perfectly well with those by Woedtke et al. (1999), who analyzed the antibacterial and antifungal activity of five commercial GSE and who were also able to demonstrate, that the activity was due to a number of added synthetic preservatives and not to natural compounds contained in grapefruit seeds. The presence of preserving substances was in no case indicated on the GSE product labels.

Conclusively, the usage of GSE in alternative farming is to be discouraged. All products investigated so far are of dubious origin and composition and the accompanying product data of the liquids are misleading.

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