Institute of Pharmacy[1], Ernst Moritz Arndt University and Institute of Hygiene of Mecklenburg-Vorpommern[2], Greifswald, Germany

Aspects of the antimicrobial efficacy of grapefruit seed extract and its relation to preservative substances contained

TH. VON WOEDTKE[1], B. SCHLÜTER[1], P. PFLEGEL[1], U. LINDEQUIST[1] and W.-D. JÜLICH[2]

Dedicated to Professor Bernhard C. Lippold, Düsseldorf, on the occasion of his 60th birthday

The antimicrobial efficacy as well as the content of preservative agents of six commercially available grapefruit seed extracts were examined. Five of the six extracts showed a high growth inhibiting activity against the test germs *Bacillus subtilis* SBUG 14, *Micrococcus flavus* SBUG 16, *Staphylococcus aureus* SBUG 11, *Serratia marcescens* SBUG 9, *Escherichia coli* SBUG 17, *Proteus mirabilis* SBUG 47, and *Candida maltosa* SBUG 700. In all of the antimicrobial active grapefruit seed extracts, the preservative benzethonium chloride was detected by thin layer chromatography. Additionally, three extracts contained the preserving substances triclosan and methyl parabene. In only one of the grapefruit seed extracts tested no preservative agent was found. However, with this extract as well as with several self-made extracts from seed and juiceless pulp of grapefruits *(Citrus paradisi)* no antimicrobial activity could be detected (standard serial broth dilution assay, agar diffusion test). Thus, it is concluded that the potent as well as nearly universal antimicrobial activity being attributed to grapefruit seed extract is merely due to the synthetic preservative agents contained within. Natural products with antimicrobial activity do not appear to be present.

1. Introduction

Over the last years, grapefruit seed extract (GSE) as well as GSE products are on the market as foodstuff supplements, naturopathic remedies and cosmetics. To this date, at least 14 popular books about GSE have been published in Germany alone. GSE is propagated by its advocates as a gentle as well as non-toxic natural product with a nearly universal healing power against a variety of ills and diseases [1–5].

Most of the GSE effects described are based on a very high antimicrobial efficacy against nearly 800 bacteria as well as 100 fungi. The authors of these books report that this data is backed by substantial experimental evidence. However, we could find only a few scientific publications concerning GSE in the international literature. These studies dealt with the preservation of food as chicken meat, fish, peanuts, fruit and vegetables [6–10] as well as several fungicide *(Aspergillus sp., Penicillium islandicum)* and antibacterial effects [11–15]. One *in vivo* study is related to the action of "citrus seed extract" on the intestinal micro-flora of patients suffering from atopic eczema [16]. However, even if these studies may address some particular aspects of the antimicrobial activity of GSE, reliable experimental data demonstrating comprehensive efficacy could not be found.

In 1996, Sakamoto et al. [17] showed that the preservative agents triclosan and methyl parabene are present in commercially available GSE. Since 1997 several official documents in Germany reported on the possible content of benzethonium chloride in GSE and warned against the sale of GSE in the pharmacy if it is not guaranteed to be free from these preserving agents [18–20]. Benzethonium chloride has only a restricted approval as a preserving agent for rinse-off cosmetics but is not permitted for any additional use in drugs or food. Beyond this fact, some suspicions arose that the antimicrobial effects of GSE may be related to preservative substances. Aside from data from the University of Münster [21], no systematic studies have yet been published concerning the contribution of preservatives to the comprehensive antimicrobial efficacy of GSE. Therefore, the aim of the present work was to test the antimicrobial efficacy of several commercially available GSE in relation to preservative substances.[3]

2. Investigations, results and discussion

GSE is a glycerolic extract from seed and juiceless pulp of grapefruits *(Citrus paradisi)* produced by a special grinding and rolling procedure. We tested six commercially available GSE (Table 1). For microbiological tests, aqueous root solutions containing 1% (v/v) GSE pure extract were prepared and used as the stock solution for a dilution series with a dilution factor of 0.5. The test concentrations applied ranged from $5 \cdot 10^{-6}$ to 1.0% (v/v) GSE pure extract.

The evaluation of minimum inhibitory concentrations (MIC) gave a survey of the antimicrobial activity (Table 2). Generally, GSE No. 2 did not show any growth inhibiting potency under the test conditions up to the maximum concentration of 0.1% GSE pure extract. With the other five GSE tested, MIC values between 1 and 31 ppm ($1\ \text{ppm} = 10^{-4}\%$) were found depending on the microbes, indicating relatively low activity differences between the various GSE.

As evidence for the antimicrobial activity of GSE, several publications present MIC values ranging between 2 and 20000 ppm [1, 2, 4]. Even if the sources of these data were not fully revealed, the data appeared to be based on tests that used the GSE basic concentrate Citricidal® (GSE No. 1 in our study). The published data for different test strains of *S. aureus* (2–6 ppm), *B. subtilis* (2 ppm), *P. mirabilis* (6 ppm) and *E. coli* (2–16 ppm) are in the same order of magnitude as our results. Only the published MIC value for *S. marcescens* (2000 ppm) is considerably higher than that found in our tests. This may be explained by the use of different strains of this microbe.

To establish the concentration-dependent antimicrobial activity of GSE, the agar diffusion test was used. Examining the results (Figs. 1–3), it is evident that GSE No. 2 showed no effects under these test conditions. This was also true if the original non-diluted GSE No. 2 was tested.

With the other five GSE, in most cases nearly identical dose-response curves were found. In the case of *S. aureus*, the data was linear up to 0.06% GSE pure concentration (Fig. 1). With higher GSE concentrations (i.e., up to 1%), a lower increase of the growth zones dependent on log of the GSE concentration was found. *M. flavus, B. subtilis* and *S. marcescens* showed similar curves. However, the maximum ranges of linearity of the dose-response curves were different up to 0.25% with *M. flavus* (Fig. 2), indicating a certain variability of the sensitivity of the test germs against the GSE. Even if the dose-response curves of GSE No. 4 were slightly shifted to the left in the diagrams, indicating a higher activity of this GSE related to the others, in general the differences in antimicrobial activities between the GSE appeared to be more or less negligible.

Only with *E. coli* (Fig. 3) different activities of the GSE were found. The dose-response curves of GSE No. 1 and 3 were identical as well as significantly more flat than that of GSE No. 4, 5 and 6, indicating a higher sensitivity of this test strain against GSE No. 1 and 3.

In both test models GSE No. 2 was without antimicrobial efficacy. This GSE sold as CitroBiotic® (see Table 1) was not produced from a US basic extract but rather from the company's own concentrate [22]. It was reported free of preservative substances as well as pesticide residues, as indicated in an analytical test report [23]. On the other hand, in several samples of the US concentrate Citricidal® detectable amounts of benzethonium chloride as well as triclosan were found [24]. The additive of benzethonium chloride was declared for the sample used in our tests. Consequently, because the GSE No. 3 (NutriBiotic®) was known to be made from Citricidal® [22] a preservative also had to be suspected in this GSE.

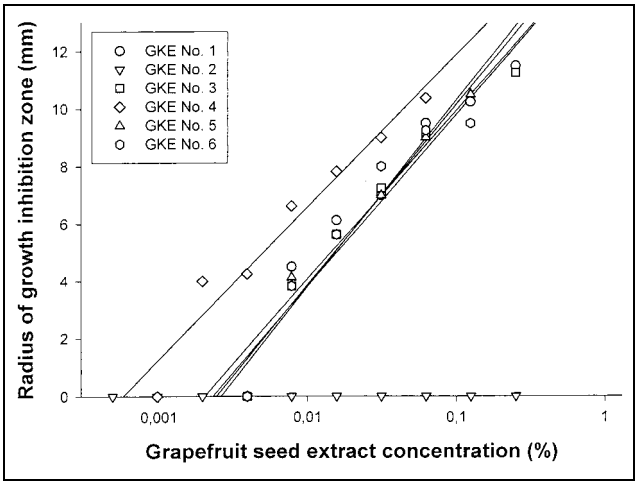

Fig. 2: Concentration dependent antimicrobial activity of different commercially available grapefruit seed extracts (GSE) measured by the agar diffusion test using *Micrococcus flavus*

By TLC analysis we were able to confirm the content of benzethonium chloride in GSE No. 1 and 3 and establish the presence of this substance also in GSE No. 4, 5 and 6. In GSE No. 1, 3 and 5 the preservative agents triclosane and methyl parabene could be detected in addition to that of benzethonium chloride (Fig. 4). The latter results were confirmed by additional TLC analyses under alternative analytical conditions for the detection of methyl parabene and triclosan, respectively.

Reference solutions of benzethonium chloride and triclosan were used to estimate concentrations by TLC analysis. Benzethonium chloride concentrations between 1.25 and 2.5% could be measured in the GSE No. 1, 2, 5 and 6. In

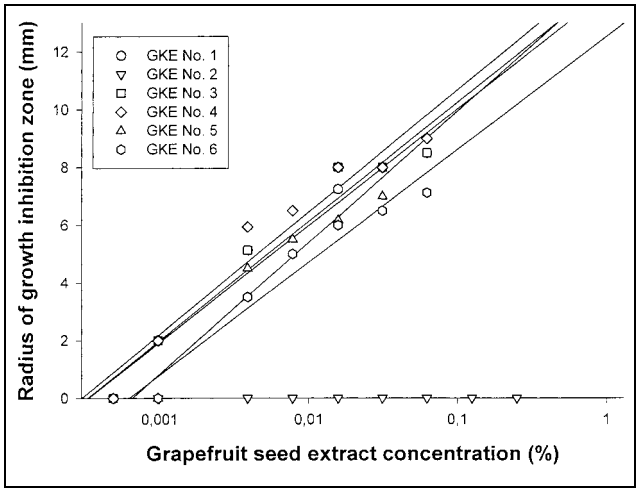

Fig. 1: Concentration dependent antimicrobial activity of different commercially available grapefruit seed extracts (GSE) measured by the agar diffusion test using *Staphylococcus aureus*

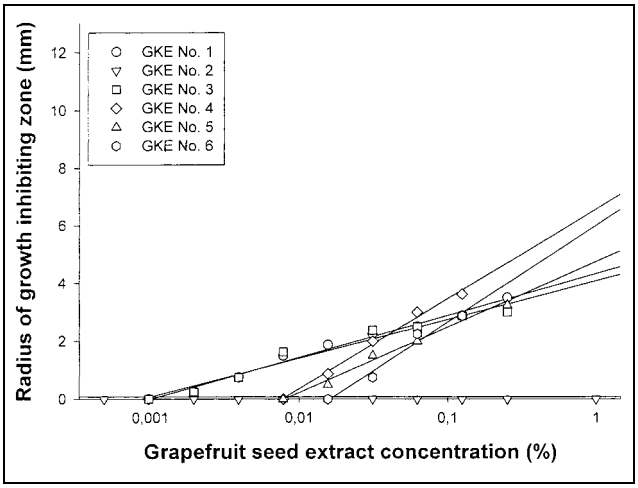

Fig. 3: Concentration dependent antimicrobial activity of different commercially available grapefruit seed extracts (GSE) measured by the agar diffusion test using *Escherichia coli*

Table 1: Commercially available grapefruit seed extracts (GSE) tested

GSE No.	Trade-mark	Firm code	Distribution
1	Citricidal®	116026	Sanitas GmbH, Steinheim, Germany
2	CitroBiotic®	L49	Sanitas GmbH, Steinheim, Germany
3	NutriBiotic®	123017	Sanitas GmbH, Steinheim, Germany
4	Grapefruit-Kern-Extrakt	P702220	Bergland-Pharma Naturheilmittel, Heimertingen, Germany
5	Grapefruit-Kern-Extrakt	no inform.	Tierra Verde, Naturstoffe aus dem tropischen Regenwald, Reutlingen, Germany
6	Grapefruit-Samen-Extrakt	36	GSE-Vertrieb, Saarbrücken, Germany

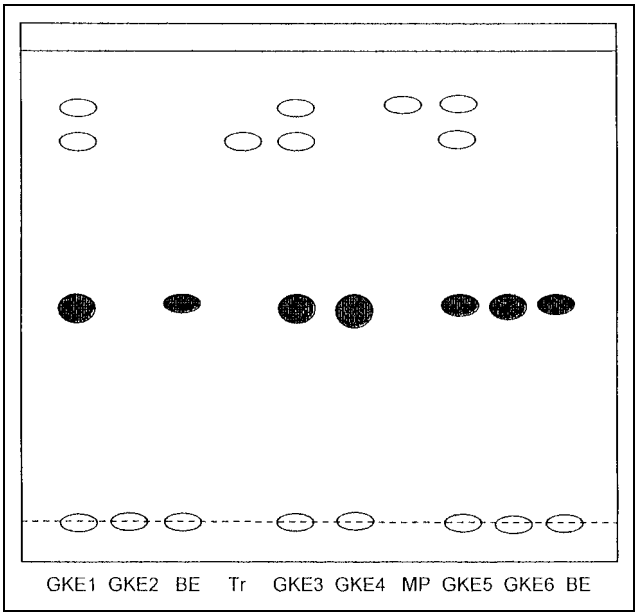

Fig. 4: TLC analysis of benzethonium chloride (BE), triclosan (Tr), and methyl parabene (MP) in commercially available grapefruit seed extracts (GSE 1–6); mobile phase: 5 volume parts water, 5 volume parts acetic acid 98%, 100 volume parts methanol; 1% (v/v) ethanolic test and reference solutions; visualization: UV (empty bands), Dragendorff's reagent (filled bands)

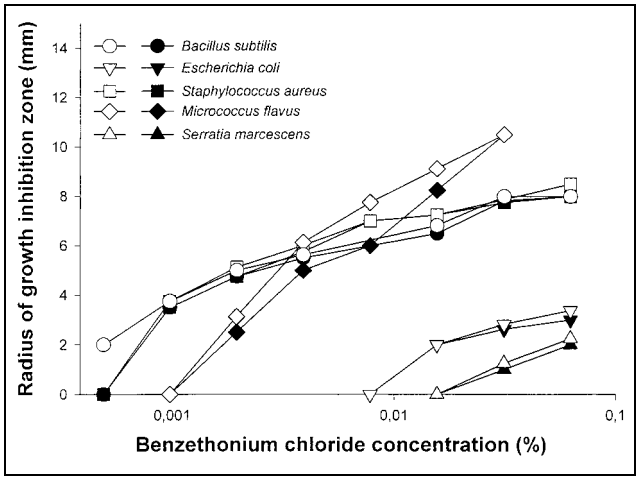

Fig. 5: Concentration dependent antimicrobial activity of benzethonium chloride solutions in water (empty symbols) as well as in 1% preservative-free grapefruit seed extract (filled symbols) measured by the agar diffusion test using several test germs

GSE No. 4 as much as 5–10% benzethonium chloride was detected. The triclosan concentration was 0.025% in GSE No. 1 and 3, respectively, and 0.0125% in GSE No. 5. According to the analytical test reports [24] in the GSE products Citricidal® and NutriBiotic® around 0.008 to 1% triclosan and 8 to 17% benzethonium chloride were found, respectively.

In the standard serial broth dilution assay, benzethonium chloride showed an antibacterial activity against all test strains, being nearly in the same order of magnitude as the GSE tested. The difference in activity against the various microbes tested was also the same as found with the GSE. Methyl parabene showed no antibacterial activity up to a maximum test concentration of 0.05%; higher concentrations being limited by the solubility of the substance. Triclosan showed a considerably higher antimicrobial activity. In contrast to benzethonium chloride, there was a greater efficacy against *E. coli* but no effect against *S. marcescens* (Table 2). These results are consistent with data from the literature [25–29] and could be further corroborated by several agar diffusion tests (data not shown). The varying concentrations of the different preservative agents is likely to be responsible for the slight differences between the antimicrobial activity of the GSE, as was found in the agar diffusion tests.

Five of the six tested GSE showed antimicrobial activity, but all effective GSE contained one to three preservative agents. Based on the dose-response curves of the various preserving agents on different microbes tested, it appeared that benzethonium chloride was responsible for most of the antimicrobial activity.

To determine if the matrix of grapefruit seed natural substances influences the antimicrobial efficacy of benzethonium chloride, we prepared dilutions of this substance either in water or in preservative-free GSE Nr. 2. Comparing the results of the agar diffusion tests, there is practically no difference in the antimicrobial activity of benzethonium chloride in these two dilution series (Fig. 5). Thus, the natural matrix substances of GSE do not contribute to the antimicrobial effects of the GSE.

No antimicrobial efficacy could be found in various self-made GSE preparations. None of the preservative agents found in the commercial GSE could be detected by TLC in the self-made GSE preparations. A comparison of the self-made GSE with the GSE No. 2 by TLC showed the same flavonoid patterns, indicating a comparable extraction of natural grapefruit seed substances with our procedure.

In summary, the results of this study show that for five commercially available GSE a high antimicrobial efficacy was found, which would explain the reported healing power of GSE. However, the same GSE contained considerable quantities of the preservative agent benzethonium chloride. In three of the five antimicrobial active GSE, the preservative agents triclosan and methyl parabene were also detected.

Table 2: Minimum inhibitory concentrations (MIC) [%] of commercially available grapefruit seed extracts (GSE) and preservative agents

Test substance	*S. aureus*	*B. subtilis*	*P. mirabilis*	*E. coli*	*S. marcescens*	*C. maltosa*
GSE No. 1	2.0×10^{-4}	3.9×10^{-4}	1.6×10^{-3}	3.9×10^{-4}	3.1×10^{-3}	3.1×10^{-3}
GSE No. 2	>0.1	>0.1	>0.1	>0.1	>0.1	>0.1
GSE No. 3	2.0×10^{-4}	2.0×10^{-4}	1.6×10^{-3}	3.9×10^{-4}	3.1×10^{-3}	3.1×10^{-3}
GSE No. 4	9.8×10^{-5}	3.9×10^{-4}	1.6×10^{-3}	7.8×10^{-4}	1.6×10^{-3}	1.6×10^{-3}
GSE No. 5	2.0×10^{-4}	3.9×10^{-4}	3.1×10^{-3}	7.8×10^{-4}	3.1×10^{-3}	3.1×10^{-3}
GSE No. 6	2.0×10^{-4}	2.0×10^{-4}	3.1×10^{-3}	3.1×10^{-3}	3.1×10^{-3}	3.1×10^{-3}
Benzethonium chloride	9.8×10^{-5}	9.8×10^{-5}	1.6×10^{-3}	1.6×10^{-3}	3.1×10^{-3}	–
Triclosan	$<1.6 \times 10^{-5}$	3.1×10^{-5}	1.25×10^{-4}	3.1×10^{-5}	$>5 \times 10^{-4}$	–
Methylparabene	>0.05	>0.05	>0.05	>0.05	>0.05	–

Benzethonium chloride was detected in all GSE that contained preservative agents. This substance is allowed only for rinse-off cosmetics up to a concentration of 0.1%. Consequently, it seems reasonable to demand that only preservative-free GSE preparations be permitted for commercial sale [20].

Nevertheless, the only commercially available GSE with no detectable preservatives also had no detectable antimicrobial activity. The same was true for self-made extracts from grapefruit seed and juiceless pulp. Several lines of experimental data provided evidence that the antimicrobial effects being attributed to GSE were based merely on the activity of synthetic preservative agents. It is unlikely that a natural active substance is formed during the extraction procedure, which has structural similarity with the synthetic preservative [30].

It is concluded that the supposed antiseptical effects of GSE are a result of the added synthetic preservative agents. Most of the therapeutic indications being acclaimed to GSE correspond to the known efficacies of benzethonium chloride, triclosan and methyl parabene [26–29, 31–40].

3. Experimental

3.1. Test materials

GSE No. 1 and 2 (Table 1) were made available to us by the Sanitas GmbH, Steinheim, Germany. The other GSE were purchased locally. GSE No. 1 (Citricidal®) is a GSE basic concentrate produced by Bio/Chem Research, Lakeport, CA, USA, containing 60% pure extract portion and 40% glycerol. GSE No. 3 (NutriBiotic®) was made from this Citricidal® basic extract [22]. To the best of our knowledge, most of the GSE commercially available at least until the end of 1997 is made from 33% of this or a corresponding basic concentrate from the USA, and diluted with 67% glycerol giving a pure grapefruit seed extract portion of 20% (v/v) in the commercially available GSE.

Self-made GSE were prepared from commercially available grapefruits by cold as well as hot extraction of seeds and juiceless pulp by using glycerol, water and ethanol as well as mixtures of these solvents.

For reference purposes, benzethonium chloride (Hyamine® MicroSelect, >99%, Fluka Chemie AG, Buchs, Switzerland), triclosan (2,4,4′-trichlor-2′-hydroxydiphenylether; Synopharm GmbH, Barsbüttel, Germany) and methyl parabene (methyl 4-hydroxybenzoat; Berlin-Chemie, Berlin, Germany) were used.

3.2. Microbiological tests

Three gram-positive (*Bacillus subtilis* SBUG 14, *Micrococcus flavus* SBUG 16, *Staphylococcus aureus* SBUG 11) and three gram-negative (*Serratia marcescens* SBUG 9, *Escherichia coli* SBUG 17, *Proteus mirabilis* SBUG 47) bacteria strains as well as one yeast strain (*Candida maltosa* SBUG 700) were used.

Minimum inhibitory concentration (MIC) values were determined by standard serial broth dilution assay. All test strains were grown in nutrition medium (Tryptic soy broth, BAG-Biologische Analysensysteme GmbH, Lich, Germany) containing a defined concentration of the test substance and incubated over 18 h at the optimum growth temperature for the different test germs (*M. flavus*: room temperature; *C. maltosa*: 30 °C; all others: 37 °C). A sample was deemed free of viable germs if the nutrient solution appeared clear on visual inspection after incubation. The MIC was the lowest concentration of the test substance that suppressed cell growth [41].

The agar diffusion method was performed according to the European Pharmacopoeia [42]. Cavities of 9 to 11 mm diameter were prepared in inoculated agar plates (20 ml, 9 cm diameter, thickness of the resulting agar layer about 5 mm; Tryptic soy agar, BAG-Biologische Analysensysteme GmbH, Lich, Germany). The same volume of the respective test solution was added to each cavity. After a 2 h period of diffusion at about 4 °C, the agar plates were incubated 18 h at the optimum growth temperature for the different test microorganisms (see above). The radii of the growth inhibition zones (defined as the distance between the cavity border and the beginning of the microbe growth area on the plate) were measured, resulting in dose-response curves of the tested substances.

3.3. TLC analysis [43]

From each of the solutions of 1% of the test substance in 96% ethanol (Rudolf Walter, Rostock, Germany) 5 µl were applied to TLC aluminium sheets (Silica gel 60 F_{254}, E. Merck, Darmstadt, Germany) with the aid of micropipettes (Blaubrand® intraMARK, Brand, Germany). After a 15-min pre-activation time the TLC sheets were developed at room temperature. The mobile phase (MP) as well as detection procedure were varied depending on the substance.

To quantify the TLC-results, we used reference solutions of the respective substances with graduated concentrations.

3.3.1. Benzethonium chloride TLC [44]

MP: 5 volume parts (VP) water, 5 VP acetic acid 98% (Riedel-de-Haën AG, Seelze, Germany), 100 VP methanol (Brenntag Chemiepartner GmbH, Mülheim/Ruhr, Germany); reference: 1 mg/ml benzethonium chloride ethanolic (96%) solution; visualization: a) UV-detection, b) pink-coloured spots after spraying with a vanillin reagent [45] followed by 10 min of heating at 100 °C, c) orange-coloured spots after spraying with potassium iodobismuthate solution (Dragendorff's reagent) [46].

3.3.2. Triclosan TLC [26]

MP: 80 VP n-hexan (Applichem, Darmstadt, Germany), 20 VP aceton (Phenolchemie, Gladbeck, Germany); reference: 1 mg/ml triclosan acetonic solution; visualization: a) spraying with dichloroquinonechlorimide solution [47] and sodium acetate solution [48], b) blue-coloured spots after heating for 10 min at 100 °C.

3.3.3. Methyl parabene TLC [49]

MP: 20 VP acetic acid 98%, 80 VP n-pentan (Applichem, Darmstadt, Germany); reference: 1 mg/ml methyl parabene methanolic solution; visualization: UV-detection.

3.3.4. Flavonoid TLC [50]

MP: 84 VP ethyl acetate (Mallinckrodt-Baker B.V., Deventer, The Netherlands), 8 VP formic acid (Laborchemie Apolda, Germany), 8 VP water; reference: 1 mg/ml quercetin methanolic solution, 1 mg/ml rutosid methanolic solution.

[3] First results have been published in German in Pharm. Ztg. **144**, 476 (1999)

Acknowledgements: We thank Mr. A. Grüttner, Sanitas GmbH, Steinheim, Germany, for making available test substances as well as informations concerning preparation as well as test results of grapefruit seed extracts. The technical assistance of Mrs. Regina Stober, Institute of Pharmacy, is gratefully acknowledged. We thank Prof. Patrick J. Bednarski, Greifswald, for help in the preparation of the manuscript.

References

1 Uhlenbrock, S.: Pharm. Ztg. **141**, 3882 (1996)
2 Sharamon, S.; Baginski, B. J.: Das Wunder im Kern der Grapefruit, 22. Ed., Windpferd Verlagsgesellschaft mbH, Aitrang 1997
3 Sharamon, S.; Baginski, B. J.: Heilen mit Grapefruitkern-Extrakt, 3. Ed., Windpferd Verlagsgesellschaft mbH, Aitrang 1997
4 Aypar, C.: Krankmachende Pilze, Viren & Bakterien: Was tun? Ratgeber Grapefruitkern-Extrakt, 2. Ed., Kern-Verlag GmbH, Olten 1997
5 Kluge, H.: Heilen mit dem Extrakt des Grapefruitsamens, 1. Ed., Südwest Verlag GmbH & Co KG, München 1997
6 Choi, D. J.; Seo, I. W.; Cho, S. H.; Joo, I. S.: Bull. Korean Fisheries Soc. **23**, 289 (1990)
7 Seo, I. W.; Cho, S. H.; Lee, K. H.: J. Korean Agricult. Chem. **36**, 265 (1993)
8 Ranzani, M. R.; Fonseca, H.: Food Add. Contamin. **12**, 343 (1995)
9 Calori-Domingues, M. A.; Fonseca, H.: Food Add. Contamin. **12**, 347 (1995)
10 Xiong, H.; Li, Y.; Slavik, M. F.; Walker, J. T.: J. Food Protect. **61**, 272 (1998)
11 Fiorentin, L.; Barioni-Junior, W.: Arq. Bras. Med. Vet. Zootec. **43**, 227 (1991)
12 Choi, J. D.; Seo, I. W.; Cho, S. H.; Joo, I. S.: J. Korean Agricult. Chem. Soc. **33**, 169 (1990)
13 Park, S. W.; Jeon, J. H.; Kim, H. S.; Joung, H.: J. Korean Soc. Horticult. Sci. **36**, 179 (1995)
14 Choi, J. D.; Seo, I. W.; Cho, S. H.: Bull. Korean Fisheries Soc. **23**, 197 (1990)
15 Sundheim, G.; Langsrud, S.: Int. Biodeterior. Biodegrad. **36**, 441 (1995)
16 Ionescu, G.; Kiehl, R.; Wichmann-Kunz, F.; Williams, Ch.; Bäuml, L.; Levine, S.: J. Orthomolecular Med. **5**, 155 (1990)
17 Sakamoto, S.; Sato, K.; Maitani, T.; Yamada, T.: Eisei Shikenjo Hokoku **114**, 38 (1996)
18 Information of the Arzneimittelkommission Deutscher Apotheker: Pharm. Ztg. **142**, 2762 (1997)

19 Information of the Arzneimittelkommission Deutscher Apotheker: Pharm. Ztg. **143**, 7 (1998)
20 Information of the Arzneimittelkommission Deutscher Apotheker: Pharm. Ztg. **143**, 2612 (1998)
21 Forster, J.; Luckmann, D.; Meier, H.; Steckel, J.: Grapefruit, Seminary Paper, Institute of Pharmaceutical Technology of the Münster University, summer semester 1997
22 Personal information by letter from Sanitas GmbH, Steinheim, Germany, 30. 9. 1997
23 Bremer Umweltinstitut – Gesellschaft für Schadstoffanalytik und -begutachtung mbH: Test report from 1. 9. 1997 by order of Sanitas GmbH, Steinheim, Germany
24 Bremer Umweltinstitut – Gesellschaft für Schadstoffanalytik und -begutachtung mbH: Test report from 6. 6. 1997 by order of Sanitas GmbH, Steinheim, Germany
25 Schönberger, R.; Schönberger, H.; in: Weuffen, W.; Berencsi, G.; Gröschel, D.; Kemter, B.; Kramer, A.; Krasilnikow, A. P. (Eds.): Handbuch der Antiseptik II/2, 1. Ed., p. 115, Verlag Volk und Gesundheit, Berlin 1983
26 Räuchle, A.; in: Weuffen, W.; Berencsi, G.; Gröschel, D.; Kemter, B.; Kramer, A.; Krasilnikow, A. P. (Eds.): Handbuch der Antiseptik II/3, 1. Ed., p. 527, Verlag Volk und Gesundheit, Berlin 1987
27 Wallhäußer, K. H.: Praxis der Sterilisation – Desinfektion – Konservierung – Keimidentifizierung – Betriebshygiene, 4. Ed., p. 489, Georg Thieme Verlag, Stuttgart, New York 1988
28 Wallhäußer, K. H. [27], p. 517
29 Wallhäußer, K. H. [27], p. 553
30 Sharamon, S.; Baginski, B. J.: Information letter of the Grapefruit Seed Forum, demanded according to a hint in [2], Summer 1997
31 Beilfuß, W.; Bückler, L.; Eigener, U.; Harke, H.-P.; Sturm, U.; in: [26], p. 265
32 van Bronswijk, J. E.; Koekkoek, H. H.: J. Med. Entomol. **8**, 748 (1971)
33 Gaffar, A.; Solis-Gaffar, M. C.; Tavss, E.; Marcussen, H. W.; Rustogi, H. W.: J. Dent. Res. **60**, 1897 (1981)
34 Tompkins, D. S.; Johnson, P.; Fittall, B. R.: J. Hosp. Infect. **12**, 51 (1988)
35 Jenkins, S.; Addy, M.; Newcomber, R.: J. Clin. Periodont. **17**, 698 (1990)
36 Bendig, J. W. A.: J. Hosp. Infect. **15**, 143 (1990)
37 Kwochka, K. W.; Kowalski, J. J.: Am J. Vet. Res. **52**, 115 (1991)
38 Webster, J.; Faoagali, J. L.; Cartwright, D.: J. Paediatr. Child Health **30**, 59 (1994)
39 Kjaerheim, V.; Barkvoll, P.; Waaler, S. M.; Rolla, G.: J. Clin. Periodontol. **22**, 423 (1995)
40 Barkvoll, P.; Rolla, G.: J. Clin. Periodontol. **22**, 485 (1995)
41 Süßmuth, R.; Eberspächer, J.; Haag, R.; Springer, W.: Biochemisch-mikrobiologisches Praktikum, 1. Ed., p. 144, Georg Thieme Verlag, Stuttgart, New York 1987
42 Ph. Eur. 1997 – European Pharmacopoeia, Third Edition, p. 104, Council of Europe, Strasbourg 1996
43 Ph. Eur. 1997 [42], p. 30
44 Ph. Eur. 1997 [42], p. 454
45 Ph. Eur. 1997 [42], p. 268
46 Ph. Eur. 1997 [42], p. 249
47 Ph. Eur. 1997 [42], p. 209
48 Ph. Eur. 1997 [42], p. 245
49 Pachaly, P.: Dünnschichtchromatografie in der Apotheke, 1. Ed., p. 94, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart 1982
50 Pachaly, P. [49], p. 149

Received February 17, 1999
Accepted February 25, 1999

Dr. rer. nat. Thomas von Woedtke
Institute of Pharmacy
Ernst Moritz Arndt University
Friedrich-Ludwig-Jahn-Straße 17
D-17487 Greifswald