Microbial Contamination of Medicinal Plants – A Review*

Wolfgang Kneifel[1]
Erich Czech[2]
Brigitte Kopp[3]

Abstract

Medicinal plants may be associated with a broad variety of microbial contaminants, which are represented by bacteria, fungi and viruses. Inevitably, this microbiological background depends on several environmental factors and exerts an important impact on the overall quality of herbal products and preparations. Risk assessment of the microbial load of medicinal plants has therefore become an important subject in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes. This study intends to contribute to this knowledge by giving a survey of published data regarding the microbial contamination of herbal plants, by dealing with methodological aspects and by considering the influence of different commonly used pharmaceutical preparation techniques on the microbiological status of the products. Finally, quality standards are discussed, which could be considered for guidelines and/or possible inclusion in the Ph.Eur. 2000.

Key words
Medicinal plants · herbal drugs · microbiological quality · review

Introduction

Medicinal plants host a wide spectrum of microorganisms with various individual properties and with considerable differences regarding qualitative and quantitative aspects. In principle, the microbial load of plants is the result of a series of influences caused by animate and inanimate sources, and microbial contaminants are easily transferred via air- and soil-borne vectors (Fig. **1**). The persistence and resistance properties of the plant microflora are determined by intrinsic as well as extrinsic factors which are due to natural, agricultural, environmental and technological influences (Table **1**). Although bacterial endospores and fungal spores can be regarded as the two dominating groups of contaminants associated with medicinal plants, a broad diversity of bacterial, fungal cells and viruses can be found either in or on the plant material [1], [2], [3], [4]. Among these microorganisms, pathogens may also occur, and this fact particularly limits the utilisation of these plants [1], [5], besides quality reduction caused by microbially induced spoilage. Moreover, in analogy to the spices and herbs used in foods, it cannot be excluded that extraneous matter and filth material originating from rodents, insects and inorganic sources (e.g., stones) may be present in some preparations [3], [6], [7], [8].

According to experience, the degree of contamination usually depends on the distance from the soil bottom the plant has been grown (for example see [9]). Certain plants (e.g., calamus, melissa, thyme, basil, fennel etc.) contain natural barriers and antimicrobial substances which exert typical inhibitory effects on microbial growth and stability. It has been estimated that around 1400 herbs and spices may possess antimicrobial agents of different chemical nature such as oils [10], [11], peptides [12], liquid

* Dedicated to Prof. Dr. Wilhelm Fleischhacker, University of Vienna, on the occasion of his 70th birthday

Affiliation
[1] Department of Dairy Research and Bacteriology, University of Agricultural Sciences, Vienna, Austria
[2] Department of Environmental Biotechnology, IFA-Tulln, Austria
[3] Institute of Pharmacognosy, University of Vienna, Austria

Correspondence
W. Kneifel · Department of Dairy Research & Bacteriology · University of Agricultural Sciences · Gregor Mendel-Str. 33 · 1180 Vienna · Austria · E-Mail: kneifel@edv1.boku.ac.at · Phone: +43–1-47654–6103 · Fax: +43–1-47654–6122

Received June 2, 2001 · **Accepted** September 22, 2001

Bibliography
Planta Med 2002; 68: 5–15 · © Georg Thieme Verlag Stuttgart · New York · ISSN 0032-0943

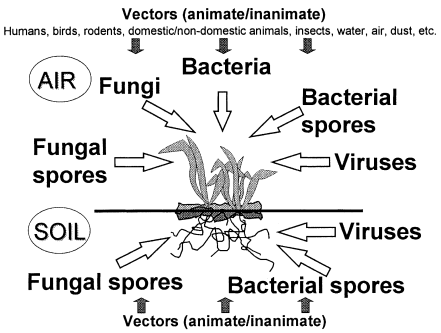

Fig. **1** Possible pathways of microbial contamination of medicinal herbal drugs.

Table **1** Factors determining the microbiological quality of medicinal plants

Intrinsic
Nature of the plant and natural barriers
Structure of the plant
Plant composition (antimicrobial compounds and agents)
Intracellular microbial contaminations
Extrinsic
Climate
Humidity
Location/position
Harvest method
Post-harvesting
Physical state (*concis*/*toto*)
Technological treatment
Packaging and storage conditions
Exogenous microbial contaminations

[13 and organic extracts [14]. Some of these biological activities have already been tapped for certain therapeutic use [15], [16] and their specific healing properties can be even rooted in history. In addition, it may also be expected that some antioxidants naturally occurring in plants may limit some microbes as well [17], [18], [19]. However, medicinal plants are originally not germ-free and thus several hygiene parameters have to be considered in routine control, especially when the plant is to be applied for medical purposes. Following this fundamental need, the evaluation of microbial contamination has increasingly become an integral part of HACCP concepts. In this context, the microbial risk inherent to herbal plants may vary with regard to the different stages of the production line and this has to be recognised in a systematic strategy of quality assurance (Table **2**). Even in/at dried herbal products some microorganisms, in particular their spores, may survive over long-term storage periods [20].

Taking into consideration the above described background situation, the intention behind this review paper is to discuss the following items:

1. to give an extensive survey of published data regarding the microbial load associated with herbal medicinal drugs;
2. to elucidate some microbiological criteria and methodological aspects which may be useful to be further integrated in modern quality assurance of medicinal plants;
3. to consider the different modes of manipulation, which are usually applied for preparing medicinal drugs such as treatment with boiling water, cold water extraction and ethanol extraction, which usually affect the microbiological nature of the products thereby obtained;
4. to use this survey as a basis for proposing tailor-made quality standards for herbal medicinal plants which may form the basis for a possible re-consideration of chapter 5.1.4 of Ph.Eur. 2000 (21) in a constructive way.

Survey of Reports about Microbial Contaminants Associated with Medicinal Plants

Microbiological data about herbal medicinal plants documented in the literature were collected. By generating three lists summarising the total aerobic mesophilic (Table **3**), enterobacterial (Table **4**) and yeast and mould (Table **5**) counts, the heterogeneous data available in the literature were compiled in order to provide a more concise survey. In these tables, an array of plants is listed along with their corresponding ranges of viable counts. As far as permissible, medians were calculated from the individual documents to allow a proper evaluation of the average microbiological situation as attributed to each plant material. It is quite obvious that pronounced differences in the total aerobic mesophilic counts (Table **3**) reflect the original and environmental criteria described above. For example, relatively low bacterial counts found with some products (e.g., Fructus Myrtilli) may be due to natural antimicrobials and probably to a generally good hygiene situation, whereas high microbial counts (e.g., Herba Urticae) may indicate less favourable hygienic conditions. Moreover, factors such as the distance of the plant from the soil and the ratio between the size of the plant surface and the sample weight may play some role. In analogy, this observation is also valid for the load with enterobacteria (Table **4**), whereas not only the total enterobacterial counts were integrated in this list but also their coliform sub-group, as far as provided by the authors. Although enterobacteria can be ubiquitously found in nature, this family possesses some indicative value towards faecal contamination. Together with the group of coliforms, it can be taken as an indicator for undesired hygiene conditions, although this conclusion has to be related to the magnitude of the viable count measured. With a few exceptions, spores of filamentous fungi rather than yeasts seem to play an important role in/at herbal plants (Table **5**).

A relatively limited number of reports exists about the presence of pathogenic microorganisms in/on herbal plants. In general, contaminations with pathogens cannot be excluded. Recently, Czech et al. [5] have screened a broad spectrum of pathogens

Table **2** Evaluation of microbiological contamination risks at different stages during the production of medicinal herbal drugs

Production steps	*Risk level*[1]
Pre-cultivation	(+)
Field cultivation	++
Harvest	++
Intermediate storage	+
Transportation	(+)
Treatments (cleaning/cutting/drying/packaging)	+
Final product (packaged/stored)	–

[1] Explanation of symbols: – usually no risk, (+) no to low risk, + low to medium risk, ++ high risk.

Table **3** Compilation of data (CFU/g) of total aerobic mesophilic counts analysed in medicinal drugs by different researchers. "Reported values" represent miscellaneous viable count data, regardless whether mean values or single data were considered. From these data medians were calculated based on all single and mean values as far as available

Herbal Drugs	*Reported values*	*Median*	*Min*	*Max*	*References (n)*[1]
Cortex Frangulae	3.0×10^5				3(1)
Cortex Frangulae	9.8×10^3				22(1)
Cortex Hippocastani	8.9×10^3				22(1)
Cortex Cinchonae	6.7×10^3				23(3)
Cortex Cinnamomi	1.0×10^5				3(2)
Cortex Rhois aromaticae	2.0×10^2				3(1)
Flos Aurantii	6.8×10^4	6.8×10^4	1.5×10^4	1.2×10^5	24 (2)
Flos Chamomillae	1.7×10^6	3.2×10^5	4.5×10^3	7.1×10^6	3(16); 5(5); 23(3); 24(1); 25(1); 26(3)
Flos Hibisci	4.2×10^4	4.2×10^4	1.8×10^4	6.6×10^4	3(10); 23(3)
Flos Malvae	3.0×10^7	9.8×10^5	1.6×10^2	2.3×10^8	5(5); 23(3); 24(1); 27(6)
Flos Sambuci	3.5×10^6	1.1×10^6	7.6×10^3	1.3×10^7	5(5)
Flos Tiliae	9.5×10^4	4.1×10^4	3.0×10^4	3.4×10^5	5(5); 24(1)
Flos Verbasci	2.2×10^5	1.8×10^5	1.0×10^4	4.5×10^5	5(5); 24(1); 26(1)
Flos Farfarae	8.1×10^3				24(1)
Flos Symphyti	5.8×10^4				3(2)
Flos Chamomillae romanae	6.8×10^3				24(1)
Folium Betulae	6.9×10^4	1.6×10^3	n. d.[2]	3.4×10^5	3(12); 23(3); 24(1); 26(1); 28(2)
Folium Crataegi cum flore	8.9×10^5	1.8×10^5	3.3×10^4	2.8×10^6	5(4); 22(1); 23(3); 24(1); 27(6)
Folium Malvae	3.0×10^6	3.5×10^6	2.0×10^4	3.5×10^6	5(5); 24(1); 29(2)
Folium Melissae	1.7×10^5	1.5×10^5	7.1×10^3	3.7×10^5	5(5); 24(1)
Folium Menthae pip.	1.7×10^7	8.6×10^5	1.9×10^4	1.7×10^8	3(9); 5(5); 23(3); 24(1); 25(1); 27(6); 29(2)
Folium Salviae	2.1×10^6	2.6×10^5	4.7×10^3	1.8×10^7	3(3); 5(5); 24(1); 25(1); 28(1); 29(2)
Folium Sennae	1.0×10^5	5.3×10^4	1.8×10^4	2.4×10^5	3(5); 5(4); 16(3)
Folium Uvae ursi	1.1×10^4	3.2×10^3	9.5×10^2	5.7×10^4	3(12); 5(5); 22(1); 23(4); 24(1); 29(2)
Folium Althaeae	4.2×10^7				27(6)
Folium Boldo conc.	1.0×10^4				29(1)
Folium Boldo tot.	2.9×10^3				29(1)
Folium Eucalypti conc.	1.1×10^4				29(1)
Folium Eucalypti tot.	9.4×10^3				29(1)
Folium Farfarae	1.6×10^4				26(1)
Folium Hamamelidis	2.8×10^5				22(1)
Folium Juglandis	7.3×10^6				27(6)
Folium Orthosiphonis	4.6×10^5				3(10)
Folium Plantaginis	3.3×10^3				24(1)
Folium Ribis nigri	1.5×10^5				3(8)
Folium Rubi idaei	2.0×10^0				26(1)
Folium Symphyti (fresh)	2.8×10^5				3(3)
Fructus Anisi	3.5×10^5	9.8×10^4	1.7×10^3	1.1×10^6	5(5)
Fructus Carvi	1.4×10^6	5.1×10^4	2.2×10^3	8.1×10^6	3(2); 5(5)
Fructus Foeniculi	4.1×10^6	1.6×10^5	2.4×10^4	2.5×10^7	5(5); 23(3); 25(1)
Fructus Myrtilli	3.2×10^3	2.4×10^3	2.2×10^2	9.9×10^3	5(4); 22(1); 23(3)
Fructus Sennae	3.3×10^5	2.2×10^5	6.5×10^3	8.6×10^5	3(2); 5(3); 16 (3); 24(1); 30(1)
Fructus Cynosbati	1.3×10^2				24(1)
Fructus Cynosbati powder	1.1×10^5				3(10)
Fructus Juniperi	2.4×10^2				23(3)
Herba Echinaceae	1.4×10^6	1.9×10^6	2.9×10^5	1.9×10^6	5(3)
Herba Equiseti	3.2×10^4				26(1)
Herba Hyperici	6.4×10^6	2.3×10^5	1.8×10^4	3.6×10^7	5(5); 27(6)
Herba Passiflorae	2.5×10^7	6.6×10^6	7.6×10^4	1.2×10^8	5(5); 24(1)
Herba Thymi	7.9×10^6	3.3×10^6	3.3×10^6	7.9×10^6	5(5); 23(3)
Herba Urticae	1.4×10^7	4.1×10^6	1.0×10^6	4.2×10^7	5(5); 23(3); 25(1); 26(1)
Herba Visci albi	5.6×10^4	5.6×10^4	1.0×10^3	1.1×10^5	25(1); 28(2)
Herba Absinthii	7.8×10^3				23(3)
Herba Anserinae	4.0×10^5				26(1)
Herba Boraginis	3.1×10^6				3(3)
Herba Millefolii	1.7×10^5				25(1)
Herba Solidaginis	1.8×10^6				3(8)

Table **3** Cont.

Herbal Drugs	*Reported values*	*Median*	*Min*	*Max*	*References (n)*[1]
Herba Veronicae	1.8×10^0				26(1)
Herba Violae tricoloris	1.9×10^6				22(1)
Lichen Islandicus	2.5×10^5	5.5×10^3	5.3×10^3	7.5×10^5	5(3)
Pericarpium Aurantii	2.2×10^3	2.1×10^3	7.2×10^2	4.0×10^3	5(4)
Radix Althaeae	1.6×10^5	2.5×10^4	7.4×10^2	1.0×10^6	5(5); 23(3); 24(1); 28(1)
Radix Liquiritiae	3.5×10^5	$3.4\ 10^5$	2.4×10^4	8.6×10^5	3(8); 5(4)
Radix Primulae	6.4×10^4	4.3×10^4	2.2×10^4	1.5×10^5	5(4)
Radix Valerianae	1.1×10^5	9.1×10^4	2.8×10^4	2.2×10^5	5(5); 25(1)
Radix Gentianae	4.6×10^2				23(3)
Radix Ginseng	1.7×10^6				22(1)
Radix Harpagophyti	6.9×10^5				22(1)
Radix Ipecacuanhae	1.3×10^3				23(4)
Radix Ratanhiae	1.1×10^3				23(3)
Radix Rhei	7.2×10^4				28(1)
Radix Symphyti	8.2×10^5				3(14)
Rhizoma Curcumae xanthorrhizae	1.6×10^6				23(3)
Rhizoma Graminis	2.8×10^4				3(10)
Rhizoma Zingiberis	1.1×10^4				3(3)
Semen Lini	2.6×10^7	1.3×10^5	5.0×10^1	2.3×10^8	3(502); 5(5); 23(3); 25(1)
Semen Psyllii	5.4×10^4	1.0×10^4	1.8×10^3	2.3×10^5	5(5); 24(1)
Semen Hippocastani	2.4×10^2				23(3)

[1] (n), number of samples examined in the corresponding study.
[2] n.d., non-detectable.

and indicator germs. It was shown that these microorganisms are relatively rarely found, with the exceptions of *Bacillus cereus* and *Clostridium perfringens*. However, these two spore-formers usually do not appear in magnitudes representing a real toxicity potential. In the 1980's, Leimbeck [31] detected *E. coli* and *Pseudomonas aeruginosa* in many of the samples and therefore suggested to treat the drugs with boiling water for decontamination. In other studies, alternative treatments have been introduced [22], [23], [26], [27], [32]. Since herbal plants frequently carry a considerable amount of moulds, the generation of mycotoxins, especially as cold water maceration products are concerned, should be taken into consideration [5]. Hitokoto et al. [33] have shown that moulds like *Penicillium*, *Aspergillus*, *Rhizopus*, *Mucor*, *Cladosporium* and *Aureobasidium* spp. can be found quite often in association with herbal drugs, but mycotoxin producers were only present around the level of 2%. On the contrary, Kumar and Roy [34] have detected considerable risk levels of aflatoxins in several herbal medicinal samples of different taxa. From these findings we may conclude that environmental conditions (climate, humidity, hygiene etc.) largely contribute to the mycotoxin problem.

Microbial Quality Standards, Class Plans and Sampling Guidelines

The Ph.Eur. 2000 [21] provides different guidances and tolerance levels which can be applied for evaluating the microbiological quality of herbal medicinal plants. In this version, fundamental distinctions are made by categories, depending on the purpose of medical application and the preparation technique. Herbal products can be assigned mainly to categories 3B, 4A and 4B. In contrast to other chapters outlined in the Ph.Eur. 2000, these evaluation criteria do not form a mandatory part, but are used as a recommendation for target levels. Unfortunately, this chapter neither contains any advice regarding sampling plans (e.g., answers to the question "How many samples have to be drawn from a lot ?") nor does it suggest typical attributive class plans, which are frequently applied in modern food quality assessment.

The European Herbal Infusion Association (EHIA) basically follows the ISO guidances, which comprise sampling plans and standardized protocols for aerobic mesophilic bacteria, yeasts and moulds, *E. coli and Salmonella* [35]. In principle, those methods established for the examination of foods are used to assess the microbiological quality of herbal infusions. The EHIA usually tolerates higher microbiological threshold levels [35] than given in the Ph. Eur. 2000.

The examination of the microbiological quality is commonly based on general and specific evaluation criteria, each of them having a more or less pronounced indicative meaning, which further enables practical conclusions (Table **6**). In principle, most quality aspects of medicinal plants can be compared with those considered in the area of food microbiology, since spices and herbs, tea, vegetables, cereals may exhibit similar microbiological tendencies. Differences in the guidelines proposed by different associations were presented in detail by Kolb [35]. However, medicinal drugs possess several important differences to the food area: besides their content of specific compounds of particular pharmaceutical and medical relevance with dose-dependent properties, these products are necessarily not consumed for "conventional purposes" because they do not primarily fulfil a nutritive or relishing function. Moreover, the consumers of

Table **4** Compilation of data (CFU/g) of Enterobacteriaceae and coliform counts analysed in medicinal drugs by different researchers. "Reported values" represent miscellaneous viable count data, regardless whether mean values or single data were considered. From these data medians were calculated based on all single and mean values as far as available

Herbal Drugs	*Reported values (Enterobacteriaceae)*	*Median*	*Min*	*Max*	*Reported values (Coliforms)*	*Median*	*Min*	*Max*	*References (n)*[1]
Cortex Frangulae					2.0×10^3				3(1)
Cortex Cinchonae	5.0×10^1								23(3)
Cortex Cinnamomi					1.5×10^3				3(2)
Cortex Rhois aromaticae					1.0×10^2				3(1)
Flos Aurantii	2.5×10^1	2.5×10^1	n. d.[2]	2.5×10^1					24(2)
Flos Chamomillae	2.6×10^5	8.0×10^4	n. d.	1.0×10^6	2.9×10^5	1.8×10^5	1.0×10^4	9.7×10^5	3(16); 5(5); 23(3); 24(1); 26(3)
Flos Hibisci	n. d.				5.0×10^2				3(10); 23(3)
Flos Malvae	3.4×10^5	1.3×10^4	n. d.	1.7×10^6	3.1×10^5	1.2×10^5	5.0×10^1	1.0×10^6	5(5); 23(3); 24(1); 27(6)
Flos Sambuci	5.3×10^5	8.9×10^4	5.0×10^1	2.4×10^6	5.2×10^5	8.9×10^4	5.0×10^1	2.4×10^6	5(5)
Flos Tiliae	2.2×10^3	9.5×10^2	5.0×10^1	7.1×10^3	2.2×10^3	1.1×10^3	1.0×10^2	5.6×10^3	5(5); 24(1)
Flos Verbasci	2.2×10^4	7.1×10^2	5.0×10^0	1.3×10^5	2.7×10^4	1.0×10^3	2.0×10^2	1.3×10^5	5(5); 24(1); 26(1)
Flos Farfarae	n. d.								24(1)
Flos Symphyti					2.4×10^3				3(2)
Flos Chamomillae romanae	n. d.								24(1)
Folium Betulae	n. d.				3.8×10^3				3(12); 23(3); 24(1)
Folium Crataegi cum flore	1.5×10^5	3.9×10^4	n. d.	7.1×10^5	1.8×10^5	6.9×10^4	2.2×10^4	5.7×10^5	5(4); 23(3); 24(1)
Folium Malvae	5.4×10^5	4.8×10^4	5.0×10^1	3.0×10^6	6.2×10^5	2.7×10^4	1.6×10^4	3.0×10^6	5(5); 24(1)
Folium Melissae	4.4×10^4	3.2×10^4	n. d.	1.1×10^5	4.5×10^4	5.0×10^4	5.0×10^1	1.1×10^5	5(5); 24(1)
Folium Menthae pip.	2.1×10^4	5.0×10^2	5.0×10^1	9.3×10^4	2.6×10^4	5.8×10^3	5.0×10^1	9.3×10^4	3(9); 5(5); 23(3); 24(1); 25(1)
Folium Salviae	2.3×10^5	1.3×10^3	5.0×10^1	1.4×10^6	2.0×10^5	1.8×10^4	5.0×10^1	1.1×10^6	3(3); 5(5); 24(1); 25(1)
Folium Sennae	2.9×10^4	1.5×10^4	5.0×10^1	9.6×10^4	2.6×10^4	1.0×10^4	5.0×10^1	9.6×10^4	3(5); 5(4); 16(3)
Folium Uvae ursi	3.6×10^1	5.0×10^1	n. d.	5.0×10^1	2.8×10^2	5.0×10^1	5.0×10^1	1.4×10^3	3(12); 5(5); 23(4); 24(1)
Folium Orthosiphonis					5.3×10^3				3(10)
Folium Plantaginis	5.0×10^0								24(1)
Folium Ribis nigri					2.4×10^3				3(8)
Folium Symphyti (fresh)					1.4×10^4				3(3)
Fructus Anisi	2.0×10^5	3.6×10^3	5.0×10^1	9.6×10^5	1.1×10^5	3.6×10^3	5.0×10^1	5.1×10^5	5(5)
Fructus Carvi	1.1×10^5	6.5×10^4	4.0×10^3	4.0×10^5	7.1×10^4	3.9×10^4	8.0×10^2	2.7×10^5	3(2); 5(5)
Fructus Foeniculi	9.2×10^4	1.3×10^4	5.0×10^1	5.8×10^5	9.8×10^4	1.5×10^4	5.0×10^1	4.5×10^5	5(5); 23(3); 25(1)
Fructus Myrtilli	4.0×10^1	5.0×10^1	n. d.	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5(4); 23(3)
Fructus Sennae	1.9×10^4	1.3×10^3	5.0×10^1	5.0×10^4	5.6×10^3	2.6×10^3	5.0×10^1	1.7×10^4	3(2); 5(3); 16(3); 24(1)
Fructus Cynosbati	n. d.								24(1)
Fructus Cynosbati powder					6.1×10^3				3(10)
Fructus Juniperi	n. d.								23(3)
Herba Echinaceae	3.7×10^5	1.1×10^4	8.0×10^2	1.1×10^6	3.0×10^5	7.4×10^3	6.0×10^2	8.9×10^5	5(3)
Herba Equiseti	5.0×10^2								25(1)
Herba Hyperici	5.1×10^4	6.7×10^4	4.3×10^3	9.5×10^4	4.2×10^4	4.0×10^4	3.4×10^3	9.5×10^4	5(5)
Herba Passiflorae	4.2×10^4	4.8×10^2	5.0×10^1	2.0×10^5	7.2×10^3	5.0×10^1	5.0×10^1	3.5×10^4	5(5); 24(1)
Herba Thymi	4.4×10^5	1.8×10^5	5.0×10^1	1.8×10^6	4.1×10^5	2.5×10^5	1.5×10^4	1.4×10^6	5(5); 23(3)
Herba Urticae	7.8×10^5	5.3×10^4	n. d.	3.2×10^6	8.5×10^5	1.9×10^5	2.0×10^3	2.3×10^6	5(5); 23(3); 25(1)
Herba Visci albi	5.0×10^2								25(1)
Herba Absinthii	n. d.								23(3)
Herba Boraginis					7.0×10^3				3(3)
Herba Millefolii	5.0×10^2								25(1)
Herba Solidaginis					1.1×10^4				3(8)
Lichen Islandicus	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5(3)
Pericarpium Aurantii	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	5(4)
Radix Althaeae	4.1×10^4	5.0×10^1	n. d.	2.4×10^5	5.1×10^4	1.5×10^3	5.0×10^1	2.1×10^5	5(5); 23(3); 24(1)
Radix Liquiritiae	3.6×10^3	3.9×10^3	1.7×10^3	5.0×10^3	3.1×10^3	2.9×10^3	1.0×10^3	4.9×10^3	3(8); 5(4)
Radix Primulae	1.7×10^4	1.4×10^3	1.0×10^2	6.6×10^4	1.7×10^4	1.2×10^3	1.0×10^2	6.6×10^4	5(4)
Radix Valerianae	6.3×10^3	2.8×10^2	5.0×10^1	3.1×10^4	6.7×10^3	5.0×10^1	5.0×10^1	2.7×10^4	5(5); 26(1)
Radix Gentianae	n. d.								23(3)
Radix Ipecacuanhae	n. d.								23(4)
Radix Ratanhiae	n. d.								23(3)
Radix Symphyti					2.9×10^4				3(14)

Table **4** Cont.

Herbal Drugs	*Reported values (Enterobacteriaceae)*	*Median*	*Min*	*Max*	*Reported values (Coliforms)*	*Median*	*Min*	*Max*	*References (n)[1]*
Rhizoma Curcumae xanthorrhizae	5.0×10^1								23(3)
Rhizoma Graminis					1.5×10^3				3(10)
Rhizoma Zingiberis					5.0×10^2				3(3)
Semen Lini	1.1×10^5	2.0×10^2	n. d.	4.9×10^5	1.1×10^5	7.1×10^4	5.0×10^1	4.9×10^5	3(502); 5(5); 23(3); 25(1)
Semen Psyllii	5.1×10^3	2.8×10^2	5.0×10^1	2.9×10^4	2.8×10^3	5.0×10^1	5.0×10^1	1.3×10^4	5(5); 24(1)

[1] n, number of samples examined in the corresponding study.
[2] n.d., non-detectable.

medicinal plants are usually not healthy, but rather people who undergo some form of therapeutic treatment. This means that on the one hand toxicological factors and on the other hand higher risk levels and hazard classes have to be considered. Furthermore, diverse physical processing techniques performed during the pharmaceutical technology have to be taken into account. For example, treatment with boiling water is some kind of extraction procedure for relevant compounds but also should aim at microbial decontamination.

Several approaches have been made to optimise the design of sampling plans, that form the basis for a reliable evaluation of samples and for making decisions whether a produced or packaged lot should be accepted or rejected [36], [37], [38]. According to modern hygiene standards, so-called "attributive class plans" usually prescribe a uniform strategy for the examination and evaluation of a defined number of samples with a certain mass [39]. These plans allow attributive conclusions ("good" vs."bad" or "lower than" vs. "higher than", "acceptable" vs. "non-acceptable") and should be based on a risk assessment which allows one to clearly discriminate among accepted and rejected lots. In the case of medicinal plants, the classical pathogens such as Salmonellae or Listeriae and, according to recent experiences, also EHEC and *Campylobacter*, are not tolerated at all in any sample. For that reason usually portions of 10 or 25 grams or larger amounts (depending on the risk dimension) should be examined following standardised methodologies. The only acceptable result thereby obtained should be "not detectable in ... grams". Hence, these so-called 2-class plans follow a very strict strategy. In addition, so-called 3-class plans offer some kind of compromise within product evaluation, since the triple distinction between "acceptable", "still acceptable or tolerated" and "non-acceptable" samples is facilitated. In this context, the following criteria have to be regarded: n...total number of examined samples; c...number of tolerated samples exceeding the tolerance level m but not the maximum level M, which must not be exceeded by any of the samples. Although these plans may lack some clarity [38], they are sensitive tools, if general quality parameters (e.g., total aerobic mesophilic, coliform, endospore counts, etc.) are considered. They also intend to recognise the accuracy of the used method as affected by possible variations of the measured values. Nevertheless, not only the "non-accepted" samples but also the number of samples which can be "still accepted or tolerated" have to be based on profound experiences.

Under practical conditions, most methodological instructions usually prescribe sampling plans which follow some correlation between the product lot size and the number of samples, which have to be drawn for testing. This relationship has led to the understanding that bigger lots have to be examined using a higher number of samples than small-lot size productions. Nevertheless, other key factors such as the targeted product safety, methodological selectivity and possible methodical variations are not considered in a sufficient way. By outlining this difficulty, Hildebrandt [40] has described a simplified function to demonstrate that the overall quality of an examination can only be efficiently enhanced by exponentially increasing the number of samples (n): $\sqrt{n}$ = safety × selectivity × variation. From this function we may conclude that sample numbers over five do not offer some marked advantage, unless enhanced safety factors and hazard potentials have to be taken into account. Busse [41] has also critically illuminated the problematic situation of applying attributive class plans for screening a defined number of samples for pathogens in bigger lots and also the rationale behind defining variables of 3-class plans.

Methodological Aspects

In the Ph.Eur. 2000 [21], most media and diluents used in microbiological routine examination are specified. They can be applied for diverse materials and products. However, in the light of the above described situation, recent methodological developments and additionally emerged microbial pathogens have induced some need for re-considering the methodological principles and testing criteria. In their screening paper, Czech et al. [5] have introduced an array of methods that may be used to assess the microbiological quality of medicinal plants. As far as possible, these authors followed the basic instructions outlined in the Ph.Eur. 2000, but some aspects were also derived from current food examination protocols or from methods used in medical routine examination. A general flow diagram of a microbiological quality control program for plant samples is presented in Fig. **2**.

Influence of Different Preparation Techniques on the Microbiological Quality

Medicinal herbal drugs are applied in different forms, and manipulation and processing factors largely determine the micro-

Table **5** Compilation of data (CFU/g) of yeast and mould counts analysed in medicinal drugs by different researchers. "Reported values" represent miscellaneous viable count data, regardless whether mean values or single data were considered. From these data medians were calculated based on all single and mean values as far as available

Herbal Drugs	*Reported values (Yeasts)*	*Median*	*Min*	*Max*	*Reported values (Moulds)*	*Median*	*Min*	*Max*	*References (n)*[1]
Cortex Frangulae					5.8×10^3				22(1)
Cortex Hippocastani					4.5×10^3				22(1)
Cortex Cinchonae[2]					2.1×10^3				23(3)
Flos Aurantii[2]					9.8×10^3	9.8×10^3	1.5×10^3	1.8×10^4	24(2)
Flos Chamomillae	1.4×10^5	4.3×10^4	2.8×10^3	5.2×10^5	2.5×10^3	1.1×10^3	3.2×10^2	1.0×10^4	5(5); 26(3)
Flos Chamomillae[2]					5.5×10^2	5.5×10^2	1.7×10^2	9.2×10^2	23(3); 24(1)
Flos Hibisci[2]					4.7×10^1				23(3)
Flos Malvae	3.4×10^2	5.0×10^1	5.0×10^1	1.2×10^3	2.4×10^6	2.0×10^4	1.0×10^2	1.2×10^7	5(4); 27(6)
Flos Malvae[2]					1.0×10^3	1.0×10^3	n. d.	2.0×10^3	23(3); 24(1)
Flos Sambuci	8.3×10^4	4.0×10^3	5.0×10^1	2.6×10^5	1.0×10^4	4.0×10^3	5.0×10^2	4.0×10^4	5(5)
Flos Tiliae	7.0×10^2	5.0×10^1	5.0×10^1	2.0×10^3	4.5×10^4	3.0×10^3	3.0×10^3	1.3×10^5	5(3)
Flos Verbasci	4.5×10^3	4.0×10^3	3.0×10^2	1.2×10^4	9.0×10^3	8.5×10^3	1.0×10^2	1.8×10^4	5(5); 26(1)
Folium Betulae					4.1×10^3	4.1×10^3	2.0×10^3	6.1×10^3	26(1); 28(2)
Folium Crataegi cum flore	3.1×10^3	3.0×10^3	5.0×10^1	6.3×10^3	8.6×10^6	1.2×10^4	9.1×10^3	4.3×10^7	5(3); 22(1); 27(6)
Folium Malvae	1.7×10^4	5.3×10^2	5.0×10^1	7.0×10^4	3.5×10^4	4.2×10^3	8.2×10^2	1.3×10^5	5(4)
Folium Malvae[2]					1.6×10^5	1.0×10^4	2.0×10^3	4.7×10^5	24(1); 29(2)
Folium Melissae	6.5×10^2	5.0×10^1	5.0×10^1	2.0×10^3	1.0×10^4	1.2×10^4	9.0×10^2	2.0×10^4	5(5)
Folium Menthae pip.	3.1×10^4	1.2×10^4	5.0×10^1	1.0×10^5	2.0×10^6	1.3×10^5	1.0×10^2	1.1×10^7	5(5); 27(6)
Folium Menthae pip.[2]					9.0×10^3	8.5×10^3	1.0×10^3	2.0×10^4	23(3); 24(1); 25(1); 29(2)
Folium Salviae	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	4.7×10^4	3.0×10^4	1.9×10^3	1.3×10^5	5(4); 28(1)
Folium Salviae [2]					3.8×10^4	2.5×10^4	3.0×10^3	1.0×10^5	24(1); 25(1); 29(2)
Folium Sennae	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	6.6×10^3	2.0×10^3	9.1×10^2	1.7×10^4	5(3)
Folium Uvae ursi	6.5×10^2	5.0×10^1	5.0×10^1	3.0×10^3	9.2×10^3	2.0×10^3	5.0×10^1	3.2×10^4	5(5); 22(1)
Folium Uvae ursi[2]					3.4×10^2	2.8×10^2	1.0×10^1	8.0×10^2	23(4); 24(1); 29(2)
Folium Althaeae					3.5×10^7				27(6)
Folium Boldo conc.[2]					1.7×10^3				29(1)
Folium Boldo tot.[2]					1.3×10^2				29(1)
Folium Eucalypti conc.[2]					6.0×10^3				29(1)
Folium Eucalypti tot.[2]					1.0×10^2				29(1)
Folium Farfarae					2.2×10^3				26(1)
Folium Hamamelidis					2.9×10^4				22(1)
Folium Juglandis					2.0×10^7				27(6)
Folium Plantaginis[2]					1.3×10^3				24(1)
Folium Rubi idaei					6.3×10^2				26(1)
Fructus Anisi	1.7×10^4	1.7×10^4	5.0×10^1	8.2×10^4	1.1×10^3	2.0×10^2	5.0×10^1	3.5×10^3	5(5)
Fructus Carvi	1.6×10^4	5.0×10^1	5.0×10^1	7.7×10^4	3.0×10^3	5.0×10^1	5.0×10^1	1.5×10^4	5(5)
Fructus Foeniculi	1.4×10^2	5.0×10^1	5.0×10^1	5.0×10^2	7.3×10^2	4.0×10^2	5.0×10^1	2.2×10^3	5(5)
Fructus Myrtilli	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	3.6×10^3	2.3×10^2	1.0×10^2	1.4×10^4	5(3); 22(1)
Fructus Sennae	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	2.2×10^3	4.0×10^2	1.0×10^2	6.0×10^3	5(3)
Fructus Sennae[2]					2.5×10^4	5.0×10^3	9.0×10^2	7.0×10^4	16(3); 24(1); 30(1)
Fructus Cynosbati [2]					3.0×10^1				24(1)
Fructus Juniperi					n. d.				23(3)
Herba Echinaceae	1.0×10^2	5.0×10^1	5.0×10^1	2.0×10^2	3.3×10^3	9.1×10^2	9.0×10^2	8.2×10^3	5(3)
Herba Equiseti					1.1×10^2				26(1)
Herba Hyperici	6.3×10^4	2.0×10^4	5.0×10^1	2.1×10^5	4.7×10^6	1.6×10^5	4.0×10^4	2.3×10^7	5(4); 27(6)
Herba Passiflorae	8.0×10^1	5.0×10^1	5.0×10^1	2.0×10^2	2.9×10^3	2.0×10^2	1.0×10^2	1.4×10^4	5(5)
Herba Thymi	4.8×10^3	4.6×10^3	5.0×10^1	1.0×10^4	5.5×10^4	3.2×10^4	5.0×10^3	1.5×10^5	5(4)
Herba Urticae	5.0×10^3	1.1×10^3	5.0×10^1	2.0×10^4	1.3×10^5	5.3×10^4	6.3×10^2	5.3×10^5	5(5); 26(1)
Herba Visci albi					8.9×10^3				28(2)
Herba Absinthii[2]					2.0×10^1				23(3)
Herba Anserinae					1.8×10^0				26(1)
Herba Millefolii					4.0×10^2				25(1)
Herba Veronicae					1.6×10^2				26(1)
Herba Violae tricoloris					3.2×10^3				22(1)
Lichen Islandicus	3.6×10^3	6.0×10^2	5.0×10^1	1.0×10^4	2.1×10^4	1.4×10^3	3.0×10^2	6.0×10^4	5(3)
Pericarpium Aurantii	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	1.4×10^2	1.0×10^2	1.0×10^2	2.7×10^2	5(1)

Table **5** Cont.

Herbal Drugs	*Reported values (Yeasts)*	*Median*	*Min*	*Max*	*Reported values (Moulds)*	*Median*	*Min*	*Max*	*References (n)*[1]
Radix Althaeae	2.5×10^2	5.0×10^1	5.0×10^1	1.0×10^3	2.7×10^3	1.7×10^3	3.0×10^1	7.3×10^3	5(5); 28(1)
Radix Althaeae[2]					1.2×10^6	2.5×10^2	1.5×10^2	3.5×10^2	23(3); 24(1)
Radix Liquiritiae	1.3×10^3	5.0×10^1	5.0×10^1	3.8×10^3	2.3×10^5	2.5×10^4	3.2×10^3	6.6×10^5	5(3)
Radix Primulae	3.8×10^2	1.0×10^2	5.0×10^1	1.3×10^3	2.1×10^3	6.0×10^2	1.0×10^2	7.2×10^3	5(4)
Radix Valerianae	1.4×10^3	5.0×10^1	5.0×10^1	5.5×10^3	1.2×10^3	6.0×10^2	1.0×10^2	3.6×10^3	5(4)
Radix Gentianae[2]					4.0×10^0				24(3)
Radix Ginseng					2.9×10^3				22(1)
Radix Harpagophyti					1.2×10^5				22(1)
Radix Ipecacuanhae[2]					1.1×10^1				23(4)
Radix Ratanhiae[2]					9.4×10^1				26(3)
Radix Rhei					1.0×10^4				28(1)
Rhizoma Curcumae xanthorrhizae[2]					8.7×10^5				26(3)
Semen Lini	8.0×10^1	5.0×10^1	5.0×10^1	2.0×10^2	1.8×10^2	2.0×10^2	5.0×10^1	3.0×10^2	5(5)
Semen Psyllii	5.0×10^1	5.0×10^1	5.0×10^1	5.0×10^1	7.2×10^2	1.0×10^2	5.0×10^1	2.3×10^3	5(5)
Semen Hippocastani[2]					9.3×10^2				23(3)

[1] n, number of samples examined in the corresponding study.
[2] Yeasts and moulds were not specified separately.

Table **6** Indicative meaning of groups of microorganisms usually examined in microbiological routine control of medicinal herbal plants

Group	*Meaning*
Total aerobic mesophilic count	General hygiene and quality parameter
Enterobacteria	General hygiene and quality parameter, indicator for faecal contamination, but also ubiquitously present
Coliforms	Indicator for faecal contamination, but also ubiquitously present
Enterococci	Indicator for faecal contamination, but also ubiquitously present
Pseudo- and aeromonades	Indicator for enhanced spoilage potential, mainly from water-borne sources
Coagulase-positive staphylococci	Indicator for pathogens of human origin
Aerobic and anaerobic sporeforming bacteria	bacteria, typical soil microflora
Lactobacilli	Possible indicator for spoiled plant material
Yeasts and moulds	Ubiquitously present microorganisms, in part indicator for possible mycotoxigenic potential
Pathogens	Occurrence bears high health risk, to be avoided (*Salmonella*, *Listeria monocytogenes*, *E. coli*, EHEC[1], *Campylobacter*, *Yersinia*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, mycotoxin producers)

[1] EHEC...enterohaemorrhagic strains of *E.coli*.

biological quality of the final products [2], [3]. The application of hot water extraction (herbal infusion, herbal tea) usually compensates for microbiological contaminations, since it can be expected that boiling water markedly reduces the viable counts by several log units and also inactivates possible pathogens [28]. However, those drugs which are subjected to cold water extraction (herbal maceration) may host a considerable amount of microbes, and the extraction procedure carried out at ambient temperature usually enables microbial multiplication. Herbal tinctures, which are made by ethanol extraction should, in general, provide good hygiene conditions, but the result depends on the alcoholic concentration applied.

With these presumptions, a series of tests were performed to examine the influence of cold water maceration on the microbiological situation of a selection of herbal medicinal drugs. For this purpose, we prepared a 1% (w/v) mixture of the plant in sterilised tap water, followed by an extraction (standing) period for 24 hours at 22 °C. After this treatment the contents were mixed by shaking and the maceration process was stopped by applying a cooking step for 10 seconds. The whole procedure was accompanied by a microbiological monitoring of the supernatant samples (Fig. **3**). It is evident from the distribution of the viable counts that during the extraction procedure at 22 °C a pronounced microbial propagation takes place, so that even extremely high populations can be measured in the macerations before the heating step. During this period, the total aerobic count and also the enterobacterial counts developed towards a magnitude which would not permit the direct use of this product. The spore counts differed with regard to their basic load in the individual samples. As expected, the final boiling procedure largely improved the hygiene situation: enterobacteria decreased to the detection limit (non-detectable in 0.1 ml), and total areobic counts as well as spore counts yielded results lower than 10^4

CFU per ml of the maceration liquid. Based on this finding we would strongly recommend the application of a final heat treatment as an obligatory step. However, possible changes in the pharmacological properties during heat treatment may play some role and have therefore to be considered.

Another test series with selected plants was carried out to study the particular microbiological situation in herbal ethanolic tinctures. Tinctures were prepared by maceration of 20 grams of drug samples with 80 ml ethanolic solutions at different concentrations, according to the Ph.Eur. 2000. All samples were examined 6 days after preparation. Some correlation between ethanol concentration and total aerobic mesophilic counts was observed, although only the higher ethanol concentrations (60, 70, 80%) led to marked decontaminating effects (Fig. **4**). Only that product which basically had a high bacterial endospore count (Herba Passiflorae) showed relatively high residual counts. Thus, products rich in spores should not be used for the preparation of tinctures.

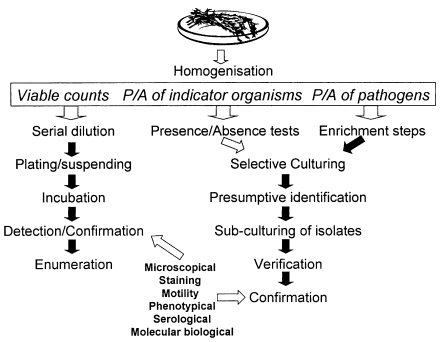

Fig. **2** General structure of a protocol for the microbiological examination of medicinal herbal drugs. P/A Presence/Absence testing.

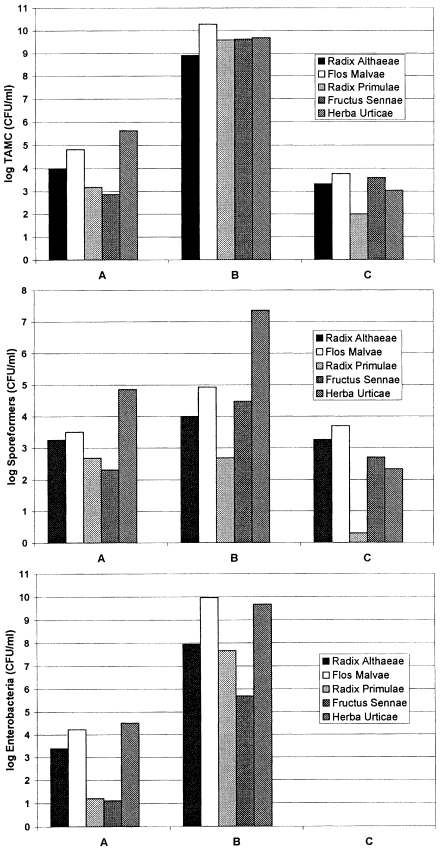

Fig. **3** Changes of microbiological quality parameters (TAMC... total aerobic mesophilic count, sporeformer count, enterobacterial count) during the maceration procedure of selected herbal drugs with cold water. A...initial counts of the maceration, B...counts determined after storage of the maceration for 24 hours at 22 °C, C... residual counts determined after a final boiling procedure.

In addition to these procedures, alternative methods such as treatment with ethylene oxide or radiation with ionic rays lead to decontamination effects [42]. These methods can be seen as a compromise between ensuring the microbiological safety of the product and avoiding consumer's risk and special legal permissions are required in many countries.

Conclusions and Future Perspectives

Taking into account the pronounced variability within the hygiene situation of herbal medicinal plants, it is difficult to propose uniform criteria for the evaluation of microbiological quality parameters. However, in front of the growing importance of thresholds, tolerance and target levels as well as specifications in daily business, the guidances outlined in the Ph.Eur. 2000 should be carefully re-considered, since there is an urgent need for having available standards for allowing legal decisions. These guidelines should be applicable not only for conventionally but also for biologically produced plants. Especially the latter ones are known to be treated with natural fertilizers (e.g., stinging nettle broth) containing considerably higher microbial levels and also preferred by many insects, which often carry contaminants.

Although the establishment of 3-class plans may bear problems, this strategy offers the advantage of giving a more detailed insight into sample quality. By considering the currently available literature and also the experience with influences of different preparation procedures on the microbiological situation, the following conclusions can be pointed out:

1. In general, excellent hygiene quality as indicated by the absence of pathogens in at least 10 grams of product and a low level of microbial contamination (i.e., low total aerobic mesophilic, endospore and enterobacterial counts) should be the main goal for each producer of medicinal plants. This also forms the optimum prerequisite for providing herbal plants of high quality and with desired therapeutic benefits. This requirement is of particular importance for those products and preparations which are assigned to category 3 of Ph.Eur. 2000.
2. In agreement with the Pharmacopoeia, a clear distinction should be made in terms of considering the impact of differ-

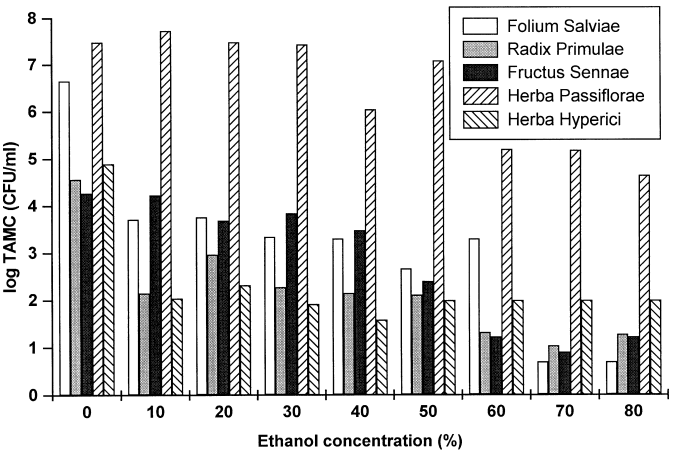

Fig. **4** Total aerobic mesophilic counts (TAMC) of tinctures of selected herbal drugs at different ethanolic concentrations, 6 days after preparation.

ent preparation techniques of the drugs. Agreement should be obtained among expert panels if a product with a certain microbiological load can be used for certain applications, or if it would be necessary to obligatorily carry out some treatment with boiling water or with alternative methods. Heating and ethanolic extraction do not yield sterile drugs, but usually products with relatively low viable counts (expectedly, of approximately < 10^4 per ml). The residual microflora thereby obtained is represented mainly by bacterial spores, enterobacteria usually do not survive these conditions.

3. Standardised methodological protocols describing in detail the techniques of microbiological examination should be approved and validated, so that comparable laboratory conditions ensure the agreement between different assessors.
4. Following the above described targets, an attempt was made to propose some alternative/additional criteria and revised threshold levels of the microbiological guidances as given in the Ph.Eur. 2000 (Table **7**). The overview gained from the literature available has been taken as a basis for generating this classification. We suggest that this evaluation scheme be treated as a recommendation and a basis for discussion. Being aware that following this proposal would cause additional work load and costs, it should also be taken into consideration that this effort has some preventive importance and helps to avoid costs arising from quality problems and consumer complaints. Alternatively, the strategy of a two-step examination could be followed: 1) analysis of a bulk sample proportionally drawn from all (n) samples, followed by 2) analysis of the individual samples, if the bulk sample has indicated results ranging around or over the proposed thresholds. However, the decision whether this strategy is acceptable or not depends on the homogeneity of the material to be considered and should be carefully evaluated.

Acknowledgements

We gratefully acknowledge the helpful comments of Gudrun Abel (Neumarkt), C. Erdelmeier (Karlsruhe), D. Flamme (Neumarkt), R. Franke (Bruckmühl), C. Franz (Vienna), G. Franz (Regensburg), and K. Rahn (Karlsruhe).

References

[1] Frank B. Mikroorganismen in Drogen. Der mikrobiologische Status von Drogen und Drogenzubereitungen und seine Beurteilung. Deutsche Apotheker Zeitung 1989; 129: 617 – 23
[2] Wichtl M. Allgemeiner Teil. In : Teedrogen und Phytopharmaka. Ein Handbuch für die Praxis auf wissenschaftlicher Grundlage. 3. Auflage Stuttgart: Wissenschaftliche Verlags-GesmbH, 1997: 5 – 31
[3] Schilcher H. Rückstände und Verunreinigungen bei Drogen und Drogenzubereitungen. Planta Medica 1982; 44: 65 – 77
[4] McKee LH. Microbial contamination of spices and herbs: a review. Lebensmittel-Wissenschaft und Technologie 1995; 28: 1 – 11
[5] Czech E, Kneifel W, Kopp B. Microbiological status of commercially available medicinal herbal drugs – a screening study. Planta Medica 2001; 67: 263 – 9
[6] Kalinovic I, Rozman V. Infestation of stored medicinal plants and herbal tea by insects and mites. Plant Protection Science 2000; 36: 21 – 2
[7] Kneifel W, Berger E. Microbiological criteria of random samples of spices and herbs retailed on the Austrian market. Journal of Food Protection 1994; 57: 893 – 901
[8] Farrell KT (editor). Spices, Condiments and Seasonings, second edition. New York: Chapman & Hall, 1990
[9] Alonzo V, Monforte MT, Tumino G, Ragusa S, Bisignano G. A note on the microbial contamination of medicinal plants. Pharmeuropa 1994; 6: 47 – 55

Table **7** Proposed microbiological examination and evaluation criteria for relevant categories of herbal medicinal drugs according to the Ph. Eur. 2000 (only brief descriptions of preparations/categories are given)

Categories	*Parameter*[1]	*Criteria*[2]
Category 3		
A Preparations for oral and rectal application		This sub-category is of limited relevance for herbal medicinal drugs
B Preparations for oral application, antimicrobial treatment impossible or TAMC > 10^3 per ml or g tolerated	*Salmonella*	2-CP, sample size: 10 g, n = 5, c = 0, m = M = 0
	E. coli	2-CP, sample size: 1 g, n = 5, c = 0, m = M = 0
	Staph. aureus	2-CP, sample size: 1 g, n = 5, c = 0, m = M = 0
	Enterobacteria	3-CP, n = 5, c = 1, m = 10^2/g, M = 10^3/g
	TAMC	3-CP, n = 5, c = 2, m = 10^3/g, M = 10^4/g
	Yeasts and moulds	3-CP, n = 5, c = 2, m = 10^2/g, M = 10^3/g
Category 4		
A Preparations including treatment with boiling water	*Salmonella*	2-CP, sample size 10 g, n = 5, c = 0, m = M = 0
	E. coli	3-CP, n = 5, c = 1, m = 10, M = 10^2/g
	Enterobacteria	3-CP, n = 5, c = 2, m = 10^5/g, M = 10^6/g
	TAMC	3-CP, n = 5, c = 3, m = 10^6/g, M = 10^7/g
	Yeasts and moulds	3-CP, n = 5, c = 3, m = 10^4/g, M = 10^5/g
B Preparations not including treatment with boiling water	*Salmonella*	2-CP, sample size: 10 g, n = 5, c = 0, m = M = 0
	E. coli	2-CP, sample size: 1 g, n = 5, c = 0, m = M = 0
	Staph. aureus	2-CP, sample size: 1 g, n = 5, c = 0, m = M = 0
	Enterobacteria	3-CP, n = 5, c = 1, m = 10^2/g, M = 10^3/g
	TAMC	3-CP, n = 5, c = 2, m = 10^4/g, M = 10^5/g
	Yeasts and moulds	3-CP, n = 5, c = 2, m = 10^3/g, M = 10^4/g

Explanation of superscripts:

[1] Parameters: Detection of (presumptive) pathogens (*Salmonella*, *E.coli*, *Staphylococcus aureus*) according to standardised protocols (enrichment, selective plating, presumptive detection); examination of microbiological quality parameters (enterobacteria, TAMC = total aerobic mesophilic count, yeasts an moulds) according to cultural plate count techniques of serially diluted samples.

[2] Criteria: 2-CP: 2-class attributive plans; 3-CP: 3-class attributive plans; n: total number of samples examined, c: number of tolerated samples exceeding lower threshold level m, M: upper threshold level, which must not be exceeded by any of the samples; viable counts are CFU/g.

[10] Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E, Palmas F. *In-vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Letters in Applied Microbiology 1999; 29: 130 – 5
[11] Aureli P, Costantini A, Zolea S. Antimicrobial activity of some plant essential oils against *Listeria monocytogenes*. Journal of Food Protection 1992; 55: 344 – 8
[12] Cammue BPA, De Bolle MFC, Schoffs HME, Terras FRG, Thevissen K, Osborn RW, Rees SB, Broekaert WF. Gene-encoded antimicrobial peptides from plants. In: Boman HG, Marsh J, Goode JA, editors. Antimicrobial peptides. Chichester: John Wiley & Sons Ltd, 1994: 91 – 106
[13] Cutter CN. Antimicrobial effect of herb extracts against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella typhimurium* associated with beef. Journal of Food Protection 2000; 63: 601 – 7
[14] Nychas GJE, Tassou CC. Traditional preservatives – Oils and spices. In: Encyclopedia of Food Microbiology. San Diego, San Francisco, New York: Academic Press, 1999: 1717 – 22
[15] Dorman HJD, Deans SG. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology 2000; 88: 308 – 16
[16] Härtling C. Untersuchungen über den mikrobiellen Zustand von Laxantien auf pflanzlicher Basis. Pharmazeutische Zeitung 1983; 128: 1006 – 8
[17] Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chemistry 2000; 69: 167 – 74
[18] Yanishlieva NV, Marinova EM, Gordon MH, Raneva VG. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chemistry 1999; 64: 59 – 66
[19] Mansour EH, Khalil AH. Evaluation of antioxidant activity of some plant extracts and their application to ground beef patties. Food Chemistry 2000; 69: 135 – 41
[20] Völker L, Müller U, Zürner I, Nagel M. Entkeimung von Gewürzen. Grundlage und Notwendigkeit. Lebensmitteltechnik 1998; 9: 56 – 8
[21] European Pharmacopoeia. Kapitel 5.1.4 Mikrobiologische Qualität pharmazeutischer Zubereitungen. 3. Ausgabe, Nachtrag, 2000: 370 – 1
[22] Sincholle D, Cotta M, Guedon D, Coll R. Plantes médicinales et décontamination. Pharmaceutica Acta Helvetica 1987; 62: 14 – 8
[23] Graf E, Scheer R. Keimzahlverminderung in Drogen und Hilfsstoffen. Pharmazeutische Industrie 1980; 42: 732 – 44
[24] Favet J. Etude de la contamination microbienne d'une vingtaine de drogues végétales. Pharmaceutica Acta Helvetica 1992; 67: 250 – 8
[25] Härtling C. Beitrag zur Frage des mikrobiellen Zustandes pflanzlicher Drogen – Fakten und Folgerungen. Pharmazeutische Zeitung 1987; 132: 643 – 4
[26] Delincée H, Ammon J, Billeskov I, Bomar MT, Ehlermann DAE. Keimreduzierung bei Drogen: Nachweis der Bestrahlung. Pharmazeutische Zeitung 1990; 135: 648 – 52
[27] Brantner A, Lücke W. Influence of physical parameters on the germ-reducing effect of microwave irradiation on medicinal plants. Pharmazie 1995; 50: 762 – 6
[28] Thonke M, Khang ND, Dressel H. Zum Keimgehalt pflanzlicher Drogen. Pharmazie 1991; 46: 284 – 6
[29] Devleeschouwer MJ, Dony J. Normés microbiologiques des drogues d'origine végétale et leurs mélanges. Journal de Pharmacie de Belgique 1979; 34: 260 – 6
[30] Friedrich H, Schneider D. Untersuchungen über den mikrobiologischen Status von Fructus Sennae TV (Tinnevelly Sennesfrüchte) des Handels. Deutsche Apotheker Zeitung 1975; 115: 1463 – 5
[31] Leimbeck R. Teedrogen – Wie steht es mit der mikrobiologischen Qualität? Deutsche Apotheker Zeitung 1987; 127: 1221 – 6
[32] Dehne LI, Pfister M, Bögl KW. Neuere physikalische Verfahren zur Haltbarmachung von Lebensmitteln. Bundesgesundheitsblatt – Gesundheitsforschung – Gesundheitsschutz 2000; 43: 33 – 40
[33] Hitokoto H, Morozumi S, Wauke T, Sakai S, Kurata H. Fungal contamination and mycotoxin detection of powdered herbal drugs. Applied and Environmental Microbiology 1978; 36: 252 – 6
[34] Kumar S, Roy AK. Occurrence of aflatoxin in some liver curative herbal medicines. Letters in Applied Microbiology 1993; 17: 112 – 4
[35] Kolb N. Microbiological status of untreated herbal materials. Deutsche Lebensmittel-Rundschau 1999; 95: 263 – 9
[36] Smelt JPPM, Quadt JFA. A proposal for using previous experience in designing microbiological sampling plans based on variables. Journal of Applied Bacteriology 1990; 69: 504 – 11
[37] Dahms S, Hildebrandt G. Some remarks on the desingn of three-class sampling plans: A review. Journal of Food Protection 1998; 61: 757 – 61
[38] Hildebrandt G, Böhmer L, Dahms S. Three-class attribute plans in microbiological quality control: a contribution to the discussion. Journal of Food Protection 1995; 58: 784 – 90
[39] Baird RM. Monitoring microbiological quality: conventional testing methods. In: Denyer S, Baird R, editors. Guide to Microbiological Control in Pharmaceuticals New York: Ellis Horwood Publishers, 1990: 125 – 45
[40] Hildebrandt G. Probenahme und Prüfpläne. In: Baumgart J, editor. Mikrobiologische Untersuchung von Lebensmitteln. Hamburg: Behr's Verlag, 1993: 1 – 15
[41] Busse M. Über den Missbrauch von Probenahmeplänen. Deutsche Molkerei-Zeitung 1989; 110: 1370 – 7
[42] Kabelitz L. Are the current requirements regarding the microbiological purity of medicinal plants practicable? Pharmeuropa 1997; 9: 570 – 5