Comparative Investigation of the Antimicrobial Activity of PADMA 28 and Selected European Herbal Drugs

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Key Words

Tibetan medicine · PADMA 28 · *Quercus cortex* · *Hamamelidis folium* · *Menthae piperitae folium* · *Anserinae herba* · *Hyperici herba* · Antibacterial and antifungal effect

Summary

Objective: PADMA 28 is a multicomponent preparation of 20 herbs, calcium sulphate, and camphor, derived from Tibetan medicine. It is usually used in the treatment of peripheral circulatory disorders, accompanied by the symptoms tingling, formication, heaviness and tenseness in arms and legs, numbness in hands and feet, and cramps in the calf. Recently, the question of whether appropriate preparations of PADMA 28 also exhibit antibacterial and antimycotic activity has often been raised. As there are as yet no experimental findings that answer this question, an in vitro study was carried out. In a parallel survey we investigated the antimicrobial properties of 5 herbal drugs which are commonly used in the traditional European folk medicine for the topical treatment of mild skin infections, wounds and eczematous skin lesions. **Methods:** The minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) of alcohol-based (tinctures) and aqueous (teas) herbal drug preparations were determined in vitro by a broth microdilution method for 5 Gram-positive and 5 Gram-negative bacteria, as well as the yeast *Candida albicans*. **Results:** The aqueous and alcohol-based PADMA 28 preparations as well as the corresponding preparations of the European herbal drugs showed an antibacterial effect against Gram-positive bacteria in vitro. These bacteria revealed a somewhat higher sensitivity to the teas prepared from the European herbal drugs (MIC: 1.3–20.0 mg/ml) than to the aqueous preparations of PADMA 28 (MIC: 5.0–40.0 mg/ml). The better antibacterial activity of the European herbal drugs is probably based on their relatively high amount of tanning agents. On the other hand, all tested plant preparations inhibited not at all or only insufficiently the growth of the Gram-negative bacteria tested and that of *Candida albicans*. The ethanolic PADMA 28 tinctures showed an improved inhibitory effect on the Gram-positive bacteria (MIC: 0.38–1.51% tincture or 0.38–1.51 mg PADMA 28/ml) compared with the aqueous preparations; this effect is comparable to the ethanolic tinctures of the tested European herbal drugs (MIC: 0.4–1.6/3.2% tincture or 0.4–1.6/3.2 mg herbal drug/ml). **Conclusion:** All tested tea preparations and alcoholic tinctures of PADMA 28 as well as those of the selected European herbal drugs exhibited evident antibacterial effects against Gram-positive bacteria in vitro. On the other hand, except for *Klebsiella pneumoniae*, all Gram-negative bacteria tested and the yeast *Candida albicans* were insensitive against the different aqueous and alcohol-based plant extracts.

Schlüsselwörter

Tibetische Medizin · PADMA 28 · *Quercus cortex* · *Hamamelis folium* · *Menthae piperitae folium* · *Anserinae herba* · *Hyperici herba* · Antibakterielle und antimykotische Wirkung

Zusammenfassung

Hintergrund: PADMA 28 ist eine traditionelle tibetische Kräutermischung, die aus 20 verschiedenen Pflanzen, Kalziumsulfat und Kampfer besteht. Die Kräutermischung wird bevorzugt bei peripheren Durchblutungsstörungen angewendet, die mit Symptomen wie Kribbeln, Ameisenlaufen, Schweregefühl in den Armen und Beinen, Wadenkrämpfen sowie Einschlafen von Händen und Füßen einhergehen. In letzter Zeit stellte sich häufiger die Frage, ob PADMA 28 auch über antibakterielle und antimykotische Eigenschaften verfügt. Da bisher zu diesem Themenkreis keine experimentellen Daten vorliegen, sollte durch In-vitro-Untersuchungen diese Lücke geschlossen werden. Parallel dazu wurde die antimikrobielle Wirkung von 5 ausgewählten pflanzlichen Drogen untersucht, die in der traditionellen europäischen Volksmedizin zur äusserlichen Behandlung von leichten Hautinfektionen, Wunden und ekzematösen Hautläsionen eingesetzt werden. **Methoden:** Die antimikrobielle Wirkung von PADMA 28 und der ausgewählten europäischen pflanzlichen Drogen wurde in vitro mit Hilfe der Mikrodilutionsmethode bei jeweils 5 Gram-positiven und 5 Gram-negativen Bakterien sowie *Candida albicans* untersucht. Zur Testung wurden alkoholische (Tinkturen) und wässrige (Tees und Kaltmazerate) pflanzliche Drogenzubereitungen eingesetzt. **Ergebnisse:** Die wässrigen und alkoholischen Zubereitungen aus PADMA 28 und einer Auswahl europäischer pflanzlicher Drogen zeigten in vitro eine antibakterielle Wirkung gegenüber Gram-positiven Bakterien. Dabei zeigte sich, dass diese Bakterien gegenüber wässrigen Zubereitungen aus den europäischen pflanzlichen Drogen etwas empfindlicher reagierten (minimale Hemmkonzentration, MHK: 1,3–20,0 mg/ml) als vergleichsweise gegenüber wässrigen Zubereitungen aus PADMA 28 (MHK: 5,0–40,0 mg/ml). Die etwas bessere antimikrobielle Wirkung der wässrigen europäischen pflanzlichen Drogenzubereitungen basiert höchstwahrscheinlich auf deren relativ hohem Gehalt an gerbenden Inhaltsstoffen. Hingegen wurde das Wachstum der getesteten Gram-negativen Bakterien und von *Candida albicans* von allen Pflanzenzubereitungen nicht oder nur ungenügend gehemmt. Die ethanolschen PADMA 28-Tinkturen (MHK: 0,38–1,51% Tinktur oder 0,38–1,51 mg PADMA 28/ml) zeigten im Vergleich zu den wässrigen Zubereitungen einen verbesserten inhibitorischen Effekt auf die Gram-positiven Bakterien und waren mit der antimikrobiellen Wirkung der getesteten europäischen Drogentinkturen (MHK: 0,4–1,6/3,2% Tinktur oder 0,4–1,6/3,2 mg pflanzliche Droge/ml) vergleichbar. Die untersuchten Gram-negativen Bakterien hingegen zeigten gegenüber allen Tinkturen keine Sensitivität. **Schlussfolgerung:** Alle getesteten Teezubereitungen und alkoholischen Tinkturen von PADMA 28 sowie diejenigen der europäischen Arzneipflanzen zeigten in vitro deutliche antibakterielle Aktivität gegen Gram-positive Bakterien. Mit Ausnahme von *Klebsiella pneumoniae* konnte das Wachstum der Gram-negativen Bakterien und des Sprosspilzes *Candida albicans* mit den verschiedenen wässrigen und alkoholischen Pflanzenextrakten nicht beeinflusst werden.

Introduction

PADMA 28 is a special mixture of herbs that is used in traditional Tibetan medicine. It consists of dried plant material from 20 different plants, as well as calcium sulphate and camphor. In the past, the original composition has been modified numerous times to its present form. Today, PADMA 28 is manufactured in Switzerland and is commercially available as tablets. In Denmark, Poland and Switzerland PADMA 28 is registered as a remedy, in other countries like Austria, the Netherlands or the USA it is sold as a food supplement (in the USA under the name PADMA Basic). The indications are symptoms of peripheral circulatory disorders such as tingling, formication, a sensation of heaviness and tension in arms and legs, numbness of hands and feet, and intermittent claudication. Initial clinical studies could demonstrate that in patients suffering from these symptoms the maximum and pain-free walking distance was significantly increased after application of PADMA 28, as well as the vascular function (decrease of the ischemic window). Further applications in the field of other chronic inflammatory disorders are also described [1, 2]. According to tradition, wound-healing properties were also attributed to the original ‘Gabur’ formula. A small ex vivo pilot study showed a positive influence of PADMA 28 on the spontaneous bactericidal activity against *Escherichia coli* and *Salmonella typhimurium* of blood serum in children suffering from recurrent respiratory tract infections [3]. In vitro tests revealed a strong antioxidant activity as well as antiproteolytic, immunomodulatory and antiinflammatory properties [4–6]. Recently, clinicians have been questioning more and more whether aqueous or alcohol-based preparations of the herbal mixture can be used for the topical treatment of mild skin infections, candidal mycosis, and vaginal infections e.g. in immunosuppressed patients. Until now in this field of study there are no experimental data available. Thus, it would be of great practical interest to evaluate the antimicrobial potential of PADMA 28 more closely. In the following survey we present the results of some preliminary screening studies in vitro. We determined the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of hot and cold water infusions as well as of an alcohol-based preparation (tincture) of PADMA 28. Furthermore, we compared these results with the antibacterial properties of selected single European herbs (St. John’s wort, hamamelis, peppermint, silverweed and European oak) and their teas and tinctures respectively, which are used traditionally in complementary medicine as antimicrobial compounds and as potent external wound-healing agents for superficial skin injuries such as grazes and slight burns [7–11].

Material and Methods

Bacterial Strains

All the plant preparations were tested against 5 Gram-positive and 5 Gram-negative bacterial strains as well as against the yeast *Candida albi-*

cans. Except for *Listeria monocytogenes*, all the other bacterial strains may be found on human skin. ATCC: American Type Culture Collection; NCTC: National Collection of Type Culture, UK.

Gram-positive bacteria:

<i>Staphylococcus aureus</i>	ATCC	6538
MRSA (methicillin-resistant <i>Staphylococcus aureus</i>)	NCTC	10442
<i>Staphylococcus epidermidis</i>	ATCC	15305
<i>Streptococcus pyogenes</i>	ATCC	12344
<i>Listeria monocytogenes</i>	ATCC	9341

Gram-negative bacteria:

<i>Klebsiella pneumoniae</i>	ATCC	10031
<i>Escherichia coli</i>	ATCC	11229
<i>Pseudomonas aeruginosa</i>	ATCC	15442
<i>Acinetobacter baumannii</i> (PADMA 28 only)		clinical isolate
<i>Serratia marcescens</i> (PADMA 28 only)		clinical isolate
<i>Citrobacter freundii</i> (European herbal drugs only)	ATCC	8090
<i>Enterobacter aerogenes</i> (European herbal drugs only)	ATCC	13048

Fungus:

<i>Candida albicans</i>	ATCC	10231
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Plant Material

The PADMA 28 material was provided by PADMA AG, Schwerzenbach, Switzerland. We tested 2 different PADMA 28 compositions: ‘PADMA 28 with camphor’ was the original powdered plant mixture of the commercially available tablets, but without the excipients silicon dioxide and sorbitol; composition and dosage per tablet (403.0 mg): *Aegle marmelos* (Bengal quince) fruit (20.0 mg); *Pimenta dioica* (allspice) fruit (25.0 mg); *Aquilegia vulgaris* (columbine) aerial part (15.0 mg); *Calendula officinalis* (marigold) flower (5.0 mg); *Elletaria cardamomum* (cardamon) fruit (30.0 mg); *Syzygium aromaticum* (clove) flower bud (12.0 mg); *Saussurea lappa* (saussurea) root (40.0 mg); *Hedychium spicatum* (ginger lily) rhizome (10.0 mg); *Lactuca sativa* (lettuce) leaf (6.0 mg); *Cetraria islandica* (Iceland moss) thallus (40.0 mg); *Glycyrrhiza glabra* (licorice) root (15.0 mg); *Azedarachta indica* (margosa) fruit (35.0 mg); *Terminalia chebula* (myrobalan) fruit (30.0 mg); *Plantago lanceolata* (ribwort) aerial part (15.0 mg); *Polygonum aviculare* (knotgrass) aerial part (15.0 mg); *Potentilla aurea* (golden cinquefoil) aerial part (15.0 mg); *Pterocarpus santalinus* (red randalwood) wood (30.0 mg); *Sida cordifolia* (heart-leaved sida) aerial part (10.0 mg); *Valeriana officinalis* (valerian) root (10.0 mg); *Aconitum napellus* (monkshood) tuber (1.0 mg); *Calcii sulfas pulv.* (gypsum; 20.0 mg); dextrocaphora (natural camphor; 4.0 mg). ‘PADMA 28 without camphor’ has the same composition, but without the camphor.

The dried material of the 5 indigenous plants (*Hyperici herba*, *Anserinae herba*, *Hamamelidis folium*, *Menthae piperitae folium*, and *Quercus cortex*) was obtained from Caelo GmbH, Hilden, Germany.

Preparation of the Test Solutions

Tea

A volume of 100 ml boiling water (aqua purificata) was poured onto 8.0 g of each of the PADMA 28 powders and onto 4.0 g of each of the single dried herbs, respectively. The aqueous PADMA 28 preparations were allowed to soak for 10 min and were then filtered. The filtrate was centrifuged for 20 min at 6000 rpm to clarify the solution. Before testing, the supernatant was filtered under sterile conditions (pore Ø: 0.22 µm). The teas of the European plants were treated in the same way, except for the centrifugation step.

Cold Maceration

A volume of 100 ml cold water was poured onto 8.0 g of each of the PADMA 28 powders. In accordance with the latest European Pharmacopoeia, the preparations were allowed to soak for 30 min with occasional shaking. After filtration the filtrate had to be centrifuged for 20 min at

Table 1. Antimicrobial activity of aqueous tea preparations of Padma 28 against a selection of Gram-positive and Gram-negative bacteria as well as *Candida albicans*

	Hot infusion (tea)				Cold maceration			
	Padma 28 with camphor		Padma 28 without camphor		Padma 28 with camphor		Padma 28 without camphor	
	MIC ¹	MBC ²	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria								
<i>Staphylococcus aureus</i>	5.0	10.0–20.0	5.0	10.0–20.0	5.0	10.0–20.0	5.0	10.0–20.0
MRSA	5.0	5.0–10.0	5.0	5.0–10.0	5.0	5.0–10.0	5.0	5.0–10.0
<i>Staphylococcus epidermidis</i>	5.0	10.0–20.0	5.0	10.0–20.0	5.0	10.0–20.0	5.0	10.0–20.0
<i>Streptococcus pyogenes</i>	20.0	40.0	20.0	40.0	20.0	>40.0	20.0	>40.0
<i>Listeria monocytogenes</i>	40.0	>40.0	40.0	>40.0	40.0	>40.0	40.0	>40.0
Gram-negative bacteria								
<i>Klebsiella pneumoniae</i>	5.0–10.0	5.0–10.0	5.0–10.0	5.0–10.0	5.0–10.0	5.0–10.0	5.0–10.0	5.0–10.0
<i>Escherichia coli</i>	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0
<i>Pseudomonas aeruginosa</i>	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0
<i>Acinetobacter baumannii</i>	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0
<i>Serratia marcescens</i>	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0
Fungus								
<i>Candida albicans</i>	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0	>40.0

¹ MIC = Minimum inhibitory concentration (mg Padma 28/ml).

² MBC = Minimum bactericidal concentration (mg Padma 28/ml).

6000 rpm. Before the supernatant was added to the test system it was filtered under sterile conditions (pore Ø: 0.22 µm).

Tinctures

The tinctures were prepared according to the latest edition of the European Pharmacopoeia. The PADMA 28 mixtures as well as each of the European dried herbs were mixed with ethanol 70% (V/V) in a 1 to 10 ratio (10 g herbal drug per 100 ml ethanol) and allowed to stand for 5 days in the dark at room temperature with occasional shaking; then they were filtered.

Camphor Test Solutions

PADMA 28 contains camphor in a concentration of 4.0 mg per tablet and 4.0 mg in 403.0 mg drug mixture, respectively. For the aqueous and ethanolic PADMA 28 preparations we used always 8.0 g and 10.0 g of the plant powder. Therefore, to investigate if the camphor proportion in PADMA 28 has any effect on the bacterial growth, test solutions of 80.0 mg camphor per 100 ml water and 100.0 mg camphor per 100 ml ethanol 70% (V/V) were produced. Aliquots of both solutions were tested for their antimicrobial activity.

Geometric Dilutions of Test Solutions

For the geometric dilution of all test preparations physiological saline solution (NaCl 0.9%) was used. The aqueous preparations were tested in concentrations of 40.0 mg, 20.0 mg, 10.0 mg, 5.0 mg, 2.5 mg, 1.25 mg, 0.625 mg and 0.3125 mg PADMA 28/ml, and 20.0 mg, 10.0 mg, 5.0 mg, 2.5 mg, 1.25 mg, 0.625 mg, 0.3125 mg and 0.165 mg herbal drug/ml, respectively. The alcohol-based tincture dilutions ranged from 50% (V/V) tincture to 0.05% (V/V) and were tested in parallel to a dilution of ethanol 70% (V/V).

Test Protocols

Test Method

The determination of MIC and MBC based on a modified broth microdilution method according to the DIN regulation 58940, Part 8 and Appendix 1, 1997, the official and obligatory German instruction for antibiotic sensitivity testing.

Test Equipment

The tests were performed in sterile 96-well microtitre plates (Greiner Labortechnik, Frickenhausen, Germany). 100 µl test or control solution was pipetted into each well and mixed with every 100 µl of the bacterial test inoculum. Each bacterium and each preparation was tested in duplicate and repeated twice. If there was a declination of the results, the test was repeated again.

Culture Media

Except for *Streptococcus pyogenes*, the bacteria tested were cultured in Iso-Sensitest Broth (Oxoid GmbH, Wesel, Germany). The streptococci were cultured in Brain Heart Infusion Broth (Merck, Darmstadt, Germany), and for the yeast *Candida albicans* Sabouraud Broth (BBL, USA) was used.

Inoculum

To obtain an appropriate exponential growth and a sufficient number of cells, the microorganisms were cultured overnight in an incubator at 37 °C. After approx. 20 h the final inocula of the bacterial strains were adjusted to 5×10^5 colony-forming units (CFU) per ml and 5×10^3 CFU/ml for *Candida albicans*, respectively. The final number of bacteria of each inoculum was checked by the spiral plater counting method (Spiral Systems, USA).

Incubation

The inoculated microtitre plates were incubated at 37 °C for 20 ± 2 h.

Sterility and Growth Controls

As sterility controls, aliquots of the growth and dilution media were incubated without bacteria in every test plate. Additionally, wells without a test substance, but only inoculum, were prepared to control the bacterial growth during the incubation period.

Evaluation of Test Results

After the incubation time, cell growth was visible as a precipitate or flocculation at the bottom of the wells. Both the MIC and the MBC were determined. The MIC is defined as the lowest concentration of the test sub-

Table 2. Antimicrobial activity of aqueous tea preparations of 5 European herbal drugs against a selection of Gram-positive and Gram-negative bacteria as well as *Candida albicans*

	<i>Quercus cortex</i>		<i>Hamamelidis folium</i>		<i>Menthae piperitae folium</i>		<i>Anserinae herba</i>		<i>Hyperici herba</i>	
	MIC ¹	MBC ²	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria										
<i>Staphylococcus aureus</i>	2.5–5.0	2.5–5.0	2.5	2.5	5.0	5.0	5.0	5.0	2.5	2.5
MRSA	2.5	2.5	1.3	2.5	1.3–2.5	1.3–2.5	2.5	2.5	2.5	2.5
<i>Staphylococcus epidermidis</i>	1.3	2.5–5.0	1.3	2.5	2.5	2.5	2.5	5.0	2.5	5.0
<i>Streptococcus pyogenes</i>	10.0	10.0–20.0	10.0	20.0	20.0	>20.0	20.0	>20.0	10.0 – >10.0	>10.0
<i>Listeria monocytogenes</i>	5.0	5.0–10.0	2.5	5.0	2.5–5.0	5.0	5.0	5.0–10.0	2.5	5.0
Gram-negative bacteria										
<i>Klebsiella pneumoniae</i>	2.5–5.0	5.0–10.0	5.0	5.0–10.0	5.0	5.0	2.5–5.0	2.5–5.0	10.0–20.0	10.0–20.0
<i>Escherichia coli</i>	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0
<i>Pseudomonas aeruginosa</i>	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0
<i>Citrobacter freundii</i>	20.0	20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0
<i>Enterobacter aerogenes</i>	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0
Fungus										
<i>Candida albicans</i>	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0	>20.0

¹ MIC = Minimum inhibitory concentration (mg herbal drug/ml).

² MBC = Minimum bactericidal concentration (mg herbal drug/ml).

stance at which the microorganisms tested do not show any visible growth. The MBC is defined as the lowest concentration of the test substance at which 99.9% of the microorganisms have been killed. For its determination a sample of 10 µl broth was removed from each well, dropped onto a blood-agar plate for bacteria or an SPS-agar plate (Sabouraud agar supplemented with penicillin and streptomycin; Merck) for *Candida albicans* and incubated for further 24 h at 37 °C. After that incubation period the lowest concentration without any colony growth on the agar plate was defined as the MBC.

Results

Tea Preparations and Cold Maceration of PADMA 28

The efficacy range and intensity of the bacterial growth inhibition was the same for both aqueous preparations, the hot infusion and the cold maceration (table 1).

At a concentration of 5.0 mg PADMA 28/ml the growth of the staphylococcal bacteria was inhibited. To inhibit bacterial growth of *Streptococcus pyogenes*, a concentration of 20.0 mg/ml was required, for *Listeria monocytogenes* even 40.0 mg/ml. The MBC was found to be one or two concentration steps higher than the MIC. In contrast to this, the growth of the Gram-negative bacteria and of *Candida albicans* was not affected even at concentrations of 40.0 mg/ml. *Klebsiella pneumoniae*, a rod-shaped Gram-negative bacterium of the family Enterobacteriaceae, was the only germ that reacted sensitively to both the tea preparation and the cold maceration of 10.0 mg PADMA 28/ml.

Since the antibacterial potential of both aqueous preparations was identical, the quality and quantity of the plant compounds responsible for the effect seem to be independent of whether the extract is prepared with hot water and a short extraction time or with cold water but a threefold longer extraction time.

Furthermore, no significant differences in efficacy were observed between the traditional PADMA 28 mixture with camphor and the drug mixture without camphor. In addition, the individually prepared aqueous camphor preparations did not show any effect on either the bacterial or the yeast growth.

Tea Preparations of Selected European Drugs

In a parallel study we tested the antimicrobial potential of tea preparations of *Quercus cortex*, *Hamamelidis folium*, *Menthae piperitae folium*, *Anserinae herba* und *Hyperici herba* (table 2). Like the PADMA 28 preparations, these teas made from single herbal drugs inhibited the growth of Gram-positive bacteria whereas, except for *Klebsiella pneumoniae*, the Gram-negative microorganisms as well as the *Candida* strain remained unaffected.

Interestingly, the MIC and MBC values of the hot single European herbal drug infusions were one to two dilution steps lower compared with the preparations of the Tibetan remedy. With respect to the aqueous preparation of PADMA 28 the Gram-positive bacterium *Listeria monocytogenes* exhibited an evidently higher sensitivity (about 8-fold) against the tea preparations of the European herbal drugs (MIC: 2.5–5.0 mg herbal drug/ml) (tables 1, 2). The somewhat better antimicrobial potency of the European herbal drugs compared with PADMA 28 may probably be explained by the fact that these European plants typically contain tanning agents. Low-molecular-weight tanning agents are water soluble and well known for their antibacterial effect.

PADMA 28 Tinctures

The efficacy of the alcohol-based PADMA 28 preparations (tinctures) can only be evaluated in direct comparison with an

Table 3. Antimicrobial activity of alcoholic tinctures of Padma 28¹ against a selection of Gram-positive and Gram-negative bacteria as well as *Candida albicans*

	Padma 28 with camphor		Padma 28 without camphor		Ethanol 70 % (V/V) (alcohol control)	
	MIC ²	MBC ³	MIC	MBC	MIC	MBC
Gram-positive bacteria						
<i>Staphylococcus aureus</i>	0.38–0.76	1.51–3.13	0.38–0.76	1.51–3.13	12.5	25.0
<i>MRSA</i>	1.51	1.51–3.13	1.51	1.51–3.13	12.5	25.0
<i>Staphylococcus epidermidis</i>	0.38–0.76	3.13	0.38–0.76	3.13	6.3	25.0
<i>Streptococcus pyogenes</i>	0.38–0.76	0.76	0.38–0.76	0.76	6.3	6.3
<i>Listeria monocytogenes</i>	1.51	3.13	1.51	3.13	3.2	12.5
Gram-negative bacteria						
<i>Klebsiella pneumoniae</i>	3.13–6.25	3.13–6.25	3.13–6.25	3.13–6.25	6.3	6.3
<i>Escherichia coli</i>	6.25	6.25–12.5	6.25	6.25–12.5	6.3	12.5
<i>Pseudomonas aeruginosa</i>	3.13–6.25	6.25	3.13–6.25	6.25	3.2	12.5
<i>Acinetobacter baumannii</i>	3.13–6.25	3.13–6.25	3.13–6.25	3.13–6.25	6.3	12.5
<i>Serratia marcescens</i>	6.25	6.25	6.25	6.25	6.3	6.3
Fungus						
<i>Candida albicans</i>	12.5	12.5	12.5	12.5	25.0	25.0

¹ Tincture 1:10; 1 vol.% ethanolic tincture = 1 mg herbal drug/ml.

² MIC = Minimum inhibitory concentration (% tincture, V/V).

³ MBC = Minimum bactericidal concentration (% tincture, V/V).

alcohol-based control solution of the same ethanol concentration, i.e. 70% (V/V), which was used to prepare the tinctures. Regarding the efficacy of the tinctures against the Gram-negative bacteria and the yeast the results of the ethanol control (MIC: 6.3–25.0% (V/V) ethanol) clearly show that the antibacterial activity is solely a result of the alcohol.

In contrast, the effect of the PADMA 28 tinctures on the staphylococci (MIC: 0.38–1.51% tincture or 0.38–1.51 mg Padma 28/ml), *Streptococcus pyogenes* (MIC: 0.38–0.76% tincture or 0.38–0.76 mg Padma 28/ml), and *Listeria monocytogenes* (MIC: 1.51% tincture or 1.51 mg Padma 28/ml) was remarkable (table 3). With regard to mg Padma 28/ml broth it was plain to see that less than a tenth of the amount of the herbal mixture was effective, compared to the aqueous Padma 28 preparation (tables 1, 3). This means that the plant compounds that are responsible for the antibacterial activity show more lipophilic properties and can be enriched to a far higher concentration in an ethanol preparation than might be possible after an aqueous extraction procedure.

The presence of camphor in the Tibetan herb mixture did not affect the antimicrobial activity of PADMA 28 tinctures at all.

Tinctures from Selected European Herbal Drugs

The tinctures of the single European herbal drugs show similar results compared with the Tibetan remedy. The growth of the Gram-negative bacteria and *Candida albicans* was not more strongly inhibited by the alcohol-based plant preparations than by ethanol itself (table 4). On the other hand, the Gram-positive bacteria proved to be very sensitive to the tinctures. In comparison with the PADMA 28 results, the MIC and MBC values of the ethanolic tinctures of the selected European herbal drugs were more or less within the same range

of antibacterial activity (MIC: 0.4–1.6/3.2% tincture or 0.4–1.6/3.2 mg herbal drug/ml).

Discussion

The present study offers some interesting aspects about the antibacterial activity of some aqueous and ethanolic plant preparations in vitro: on the one hand PADMA 28, a complex powdered herb mixture, and on the other hand 5 single European herbal drugs used moreover in complementary medicine for the topical treatment of mild skin infections and eczematous skin lesions. On the basis of the amount of tanning agents the European herbal drugs are known as antimicrobial agents. Therefore, the comparative investigation of the antimicrobial activity of PADMA 28 and selected European herbal drugs should help to value and classify the antimicrobial effectiveness of the Tibetan remedy.

Irrespective of the preparation procedure, all the plant preparations tested showed clear antibacterial effects against Gram-positive bacteria. The growth of the Gram-negative bacteria and the yeast *Candida albicans* was not inhibited, with the exception of *Klebsiella pneumoniae*. In principle the antimicrobial activity of PADMA 28 teas and tincture was comparable with the corresponding preparations of the selected European herbal drugs except for *Listeria monocytogenes*. This Gram-positive bacterium was much more sensitive against the tea preparations of the European herbal drugs (MIC: 2.5–5.0 mg/ml) than against the aqueous preparations of PADMA 28 (MIC: 40.0 mg/ml).

Extracting the multicomponent mixture PADMA 28 with ethanol (70 vol.%), the tincture showed a remarkable in-

Table 4. Antibacterial activity of ethanolic tinctures of 5 European herbal drugs against a selection of Gram-positive and Gram-negative bacteria as well as *Candida albicans*

	<i>Quercus cortex</i>		<i>Hamamelidis folium</i>		<i>Menthae piperitae folium</i>		<i>Anserinae herba</i>		<i>Hyperici herba</i>		Ethanol 70 % (V/V) (alcohol control)	
	MIC ¹	MBC ²	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria												
<i>Staphylococcus aureus</i>	1.6	1.6	0.4	0.8	1.6	1.6	1.6	0.8 – 1.6	1.6	1.6	12.5	25.0
MRSA	1.6	1.6	0.4	0.8	0.8	0.8 – 1.6	1.6	1.6 – 3.2	0.8 – 1.6	3.2	12.5	25.0
<i>Staphylococcus epidermidis</i>	0.8	3.2	0.4	0.8 – 1.6	1.6	1.6	0.8 – 1.6	3.2	1.6	6.3	6.3	25.0
<i>Streptococcus pyogenes</i>	1.6–3.2	3.2	0.8	0.8 – 1.6	0.8	0.8	0.8 – 1.6	0.8 – 1.6	1.6	1.6	6.3	6.3
<i>Listeria monocytogenes</i>	1.6	3.2	0.8	0.8 – 1.6	0.8	0.8 – 1.6	0.8	0.8	0.8	1.6	3.2	12.5
Gram-negative bacteria												
<i>Klebsiella pneumoniae</i>	1.6	3.2	1.6	3.2	0.8	0.8	1.6	1.6	1.6	3.2	6.3	6.3
<i>Escherichia coli</i>	6.3	12.5	6.3	6.3	6.3	6.3 – 12.5	6.3	12.5	6.3	12.5	6.3	12.5
<i>Pseudomonas aeruginosa</i>	3.2	6.3	3.2	6.3	3.2	6.3 – 12.5	3.2	6.3	3.2	6.3	3.2	12.5
<i>Citrobacter freundii</i>	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	12.5
<i>Enterobacter aerogenes</i>	6.3	12.5	6.3	12.5	6.3	6.3	6.3	12.5	6.3	12.5	6.3	12.5
Fungus												
<i>Candida albicans</i>	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5	25.0	25.0

1 vol. % ethanolic tincture = 1 mg herbal drug/ml.

¹ MIC = Minimum inhibitory concentration (% V/V).

² MBC = Minimum bactericidal concentration (% V/V).

hibitory effect on the Gram-positive bacteria (MIC: 0.35–1.39 mg/ml), which was 6–25 times lower than that of the aqueous extracts. Both PADMA 28 mixtures, with and without camphor, showed identical growth inhibitory effects, and even for the alcoholic tincture no inhibiting influence of the camphor amount could be observed.

For a simple tea preparation of PADMA 28 the water temperature and the time of extraction had no influence on its antibacterial activity. But with regard to a practical use a hot

water preparation should always be preferred because of a lower microbial contamination.

As there are many different staphylococci strains that form a main part of the normal skin flora, these may become pathogenic after a skin injury. Therefore it seems to be promising to tentatively test, in vivo, the aqueous and ethanolic preparations of PADMA 28 for the topical treatment of mild skin infections, for example at the fingers, toes or soles of the feet.

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