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Full Length Research Paper

In vitro antibacterial effect of a mumijo preparation from Mongolia

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Mumijo is a traditional medicine used in Central Asia thousands of years. Its beneficial effect is exerted on metabolic processes and on the human immune system. It is also used as an antiseptic agent. The *in vitro* antibacterial activity of a mumijo preparation was investigated with agar diffusion technique against 25 Gram-positive and 6 Gram-negative bacterial isolates representing 13 different genera. Effect of different media and pH on the antibacterial action was also studied. Different concentrations of mumijo exerted bactericid and/or bacteriostatic effect against 3 Gram-negative (*Acidovorax delafieldii*, *Serratia marcescens*, *Variovorax paradoxus*) and 15 Gram-positive bacterial isolates (*Agrococcus jenensis*, *Arthrobacter globiformis*, *Bacillus benzoevorans*, *Bacillus cereus* var. *mycoides*, *Bacillus megaterium*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*) on the complete media. The maximum inhibitory potential of mumijo was observed at neutral pH (pH=7.4). Antibacterial effect of mumijo was not observed when minimal media were used for cultivation. The experimental data revealed that mumijo has remarkable growth inhibition effect against Gram-positive bacteria on complete medium at neutral pH.

Key words: Mumijo, Gram-positive bacteria, Gram-negative bacteria, bacteriostatic effect, bactericid effect.

INTRODUCTION

Mumijo has been used as a traditional medicine in Central Asia for thousands of years. This material is the fossil deposits of snow petrel (*Pagodroma nivea*) stomach and is located in the high mountainous regions (2000-5000 m) of Central Asia (Aiello et al., 2008). The beneficial effects of mumijo are exerted on metabolic processes and on the human immune system and it also has antiseptic activity (Garedew et al., 2004; Aiello et al., 2008). Garedew et al. (2004) demonstrated with micro-calorimetric measurements that mumijo is a complex mixture of effective pharmacological substances that acts as a natural bacteriostatic agent against two Gram-positive bacteria, *Bacillus brevis* and *Bacillus subtilis* (Garedew et al., 2004). They mentioned in their study that mumijo is more effective against Gram-positive than Gram-negative bacterial isolates, but it and a comprehensive study of this feature has not been published so far. Therefore, in the present study we investigated the *in vitro* antibacterial activity of a mumijo preparation from Mongolia on different media and on different pH against 6 Gram-negative and 25 Gram positive bacterial isolates representing 13 different genera.

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MATERIALS AND METHODS

Strains and media

Bacterial isolates (Table 1) were maintained on T1 medium (4 g/L beef extract, 4 g/L peptone, 10 g/L glucose, 1 g/L yeast extract, 20 g/L agar) at 4°C. The antibacterial activity assays were performed in T1, LB (10 g/L tryptone, 2 g/L yeast extract, 10 g/L NaCl, 20 g/L agar), T3A/B (A: 2 g/L $NaNO_3$ / B: 2 g/L $(NH_4)_2SO_4$, 0,5 g/L $MgSO_4 \times 7H_2O$, 0,5 g/L KCl, 0,5 g/L K_2HPO_4, 30 g/L sucrose, 10 mg/L $ZnSO_4 \times 7H_2O$, 10 mg/L $FeSO_4 \times 7H_2O$, 3 mg/L $CuSO_4 \times 7H_2O$,

Table 1. *In vitro* antibacterial effect of different dilutions of a Mongolian mumijo preparation on complete media in agar diffusion test after incubation at 30°C for 24 and 48 h.

Species	Diameter of inhibition zone (mm)											
	T1 medium						LB medium					
	24 h			48 h			24 h			48 h		
	10×	50×	100×	10×	50×	100×	10×	50×	100×	10×	50×	100×
Gram-negative isolates												
A. delafieldii (SZMC 6210)	33±2.5	13±2.3	0±0.0	26±2.5	0±0.0	0±0.0	16±0.6	0±0.0	0±0.0	13±3.2	0±0.0	0±0.0
S. marcescens (SZMC 0567)	12±1.0	11±0.6	10±0.3	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
V. paradoxus (SZMC 6203)	29±2.6	16±1.2	0±0.0	26±2.5	0±0.0	0±0.0	16±0.6	0±0.0	0±0.0	13±3.2	0±0.0	0±0.0
Gram-positive isolates												
A. jenensis (SZMC 6202)	36±2.1	19±2.1	12±0.6	34±1.5	19±0.6	0±0.0	21±0.6	0±0.0	0±0.0	17±1.2	0±0.0	0±0.0
A. globiformis (SZMC 6205)	18±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
B. benzoevorans (SZMC 6219)	18±1.0	12±0.6	0±0.0	14±0.6	0±0.0	0±0.0	12±1.2	0±0.0	0±0.0	11±2.3	0±0.0	0±0.0
B. cereus var. *mycoides* (SZMC 0042)	12±1.5	9±0.0	0±0.0	9±0.6	8±0.0	0±0.0	12±0.6	10±0.6	9±1.0	11±1.2	6±5.5	0±0.0
B. megaterium (SZMC 0571)	12±0.5	9±0.5	0±0.0	10±0.6	6±5.5	0±0.0	12±0.0	4±0.0	0±0.0	12±0.0	0±0.0	0±0.0
B. subtilis (SZMC 14624)	16±1.5	15±1.2	11±0.6	16±1.2	15±1.2	11±0.6	16±0.6	14±1.5	11±0.6	15±1.0	12±0.0	0±0.0
B. subtilis (SZMC 0209)	19±3.6	0±0.0	0±0.0	15±0.5	0±0.0	0±0.0	24±1.5	16±1.5	0±0.0	18±1.5	13±1.0	0±0.0
B. subtilis (SZMC 6334)	13±0.6	0±0.0	0±0.0	13±0.6	0±0.0	0±0.0	9±0.6	0±0.0	0±0.0	9±0.6	0±0.0	0±0.0
B.subtilis (SZMC 6332)	11±0.6	0±0.0	0±0.0	11±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
E. faecalis (SZMC 14617)	18±1.2	11±0.6	0±0.0	0±0.0	18±1.2	11±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
E. faecalis (ATTC 2912)	13±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	13±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
E. faecalis (SZMC 0227)	13±1.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	13±1.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
S. aureus (SZMC 14529)	20±1.5	0±0.0	0±0.0	18±1.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	10±0.6	0±0.0	0±0.0
S. aureus (SZMC 6271)	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	11±1.7	0±0.0	0±0.0	10±0.6	0±0.0	0±0.0
S. aureus (SZMC 14619)	11±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	9±0.6	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0

Culture collection abbreviations: ATTC, American Type Culture Collection, USA; SzMC, Szeged Microbial Collection, University of Szeged, Hungary. The diameter of the hole was 8 mm in the agar diffusion test.

20 g/L agar) and T4A/B (A: 5 g/L $NaNO_3$ / B: 5 g/L $(NH_4)_2SO_4$, 1 g/L KH_2PO_4, 0,5 g/L $MgSO_4 \times 7H_2O$, 10 g/L glucose, 20 g/L agar, 1 ml Wickerham vitamin solution) medium.

Preparation of mumijo

Mumijo preparation was made by the Microbial Biosynthesis Laboratory, Institute of Biology, Mongolian Academy of Sciences (Ulaanbaatar, Mongolia). The mumijo (collected from high mountains around Ulanbataar) was ground and milled, the powder was soaked in distilled water in 1:2 ratio in room temperature for 24 h. This solution was filtered on cheese cloth then the clear solution was concentrated with Anhydro A/S drier (Denmark) at 80°C inner and 65°C outer temperature. The dry matter content of the mumijo preparation was 50%.

Antibacterial activity assay

The effect of mumijo on the growth of bacterial isolates was examined in different culture media (LB, T1, T3A, T3B, T4A and T4B; pH=7.0) and at different pH. An agar diffusion technique was used to estimate the size of inhibition of bacterial growth by different concentrations of mumijo. Solid culture media (LB, T1, T3A, T3B, T4A and T4B; pH=7.0) were overlaid with 1 ml of similar broth that

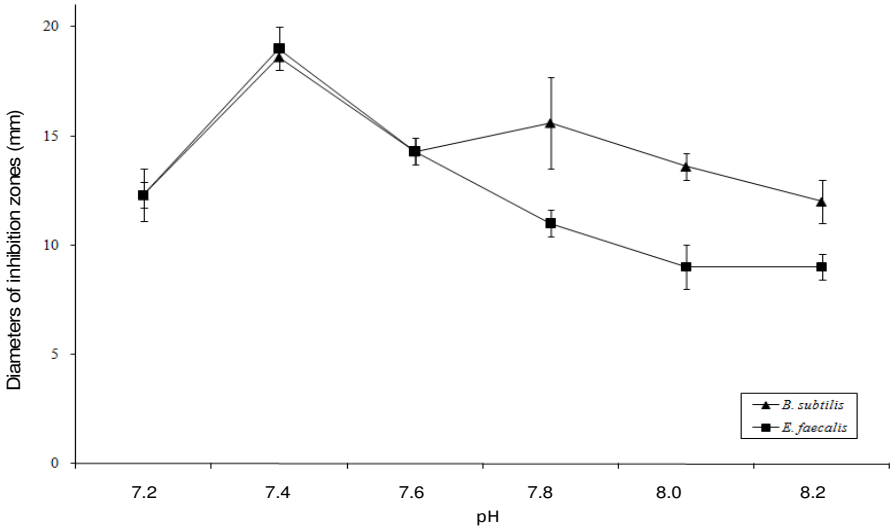

Figure 1. pH-dependence of the antimicrobial activity of 10-fold diluted mumijo preparation in TRIS/HCl buffer (pH=7.2-8.2) against *B. subtilis* (SZMC 14624) and *E. faecalis* (SZMC 14617).

were inoculated with 10^6 bacterial cell/ml. After drying 100 µl aliquots of a serial mumijo dilution (10, 50, 100 and 200 fold dilution in sterile distilled water) were filled into wells. Sterile distilled water was used as negative control.

The two most susceptible microorganisms (*B. subtilis* and *E. faecalis*) cultured on the most suitable medium (T1) were used in the investigation of pH-dependence of antibacterial effect.

In the preliminary experiments, pH values set by 20% NaOH and 30% HCl at pH=5, 6, 7, 8, 9 were used. Based on the results of these investigations, in further experiments mumijo was solved (10 fold) in TRIS/HCl buffer (pH=7.2, 7.4, 7.6, 7.8, 8.0 and 8.2). In this case sterile buffers were used as negative controls. The diameters of the inhibition zones were measured after incubation at 30°C for 24 and 48 h. All experiments were repeated three times.

RESULTS

Mongolian mumijo was ineffective on minimal media (T3A/B and T4A/B) but showed substantial antibacterial effect on complete media (T1 and LB). Growth of the most investigated Gram-positive bacteria (*Agrococcus jenensis* SZMC 6202; *Arthrobacter globiformis* SZMC 6205; *Bacillus benzoevorans* SZMC 6219; *Bacillus cereus* var. *mycoides* SZMC 0042; *Bacillus megaterium* SZMC 0571; *B. subtilis* SZMC 14624, SZMC 0209, SZMC 6334,SZMC 6332; *E. faecalis* SZMC 14617, ATTC 2912, SZMC 0227; *Staphylococcus aureus* SZMC 14529, SZMC 6271, SZMC 14619) was inhibited by mumijo, at the same time only three investigated Gram-negative bacteria (*Acidovorax delafieldii* SZMC 6210, *Serratia marcescens* SZMC 0567, *Variovorax paradoxus* SZMC 6203) proved to be sensitive (Table 1). Mumijo was ineffective against the other Gram-negative (*Escherichia coli* SZMC 0582, *Eubacter gergoviae* SZMC 6222, *Pseudomonas aeruginosa* SZMC 0568) and Gram-positive (*Enterococcus hirae* SZMC 14623, *Micrococcus luteus* SZMC 6207, SZMC 0197, *S. aureus* SZMC 0579, SZMC 14623, ATCC 25923, *Staphylococcus epidermidis* SZMC 14531, *Streptococcus agalactiae* SZMC 14533, *Streptococcus pneumoniae* ATTC 49619, *Streptococcus pyogenes* SZMC 14535) strains involved in this study. In our *in vitro* tests inhibition zones were clearly detectable in cases of the 10, 50 and 100 fold dilutions of mumijo preparation and antibacterial effect were not observed in case of the 200 fold dilution. In cases of some isolates the diameters of the inhibition zones disappeared after incubation of 48 h indicated that the mumijo acted bacteriostatically against them. These data are summarized in Table 1.

The pH dependence of the antibacterial effect of 10-fold diluted mumijo was investigated in T1 medium at different pHs (pH=5-9) against the two most susceptible bacterial isolates which are important in practical respects. *B. subtilis* is known as food poison causing bacterium (Apetroaie-Constantin et al., 2009). *E. faecalis* can cause serious life-threatening infections (e.g. endocarditis; bladder, prostate, epididymal, nervous system infections) in consequence of the wide distribution of multi-resistant strains all over the world (Sava et al., 2010). The antibacterial effect of mumijo was observed only at pH=6 and 7 after 24 and 48 h of incubation at 30°C in case of *B. subtilis* SZMC 14624 (diameters of the inhibition zones were 12±1.2 and 16±1.7 at pH=6 and 7, respectively) and *E. faecalis* SZMC 14617 (diameters of the inhibition zones were 13±1.0 and 19±1.0 at pH=6 and 7, respectively). Based on these data mumijo was diluted (10 fold) in TRIS/HCl buffers (pH=7.2 to 8.2) to determine the optimal pH of mumijo dilution in respect of the antibacterial effect. This pH was at pH=7.4 in case of the both investigated bacteria (Figure 1). These data suggested that the antibacterially active compounds of

mumijo are effective at pH=6-8.

DISCUSSION

Mumijo is a widely and clinically used natural, human medicine in the oriental region of the world (e.g. Russia and China). Its beneficial effect on health recovery is demonstrated in patients with different infections, gastric and intestinal ulcers, fractures and after radiation (Garedew et al., 2004; Aiello et al., 2008). These features of this material are connected with its immune-stimulating and antiallergic activity (Aiello et al., 2008). The antitoxic and cholagogic properties of the dry mumijo extract are also revealed (Frolova et al., 1998). Spassow (1994) observed the nootropic activity of mumijo and Aiello et al. (2008) its strong neuroprotective effect in cortical neurons against apoptosis. The antibacterial activity of mumjo has not been intensively studied so far.

Data about the antibacterial effect of mumijo in the literature were available only in case of two *Bacillus* isolates (Garedew et al., 2004). In our *in vitro* study the different concentration of a mumijo preparation showed remarkable antibacterial effect. Effectiveness against Gram-negative bacteria and the pH dependence of the antibacterial activity has not been revealed so far. In our *in vitro* study mumijo inhibited the growth of both type of bacteria, among them some potential human pathogens (e.g. *B. subtilis*, *E. faecalis*, *S. aureus*). This observation suggests the possible application of mumijo in treatment of bacterial infections. It is hypothesized that the differences between the antibacterial activity of mumijo in the complete and minimal medium might be based on the different uptake mechanism of the bacterial cells which are activated by different nutrients. The antibacterial compounds of mumijo might get into the cell in an uptake mechanism activated by only the complete media.

Conclusion

Our study revealed that mumijo has an antibacterial effect. With further investigation of this complex mixture, mumijo would be promising agent in food and pharmaceutical industry in the near future.

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