Antibacterial Activity of Houttuynin Sodium Bisulphate (HSB)

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Abstract

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Houttuynin sodium bisulphate (HSB), α-hydroxyl-capryl-ethyl-sodium-sulphonate, is a product formed by reacting sodium bisulphate with houttuynin, which is obtained from a medicinal herb *Houttuynia cordata* Thunb. The antibacterial activity of HSB was measured by optical density. Minimum inhibitory concentrations at 50% and 90% inhibition level (MIC_{50} and MIC_{90}) for *Escherichia coli* varied from 0.5 to 0.6 mg/ml and from 2.6 to 2.7 mg/ml, respectively. For *Staphylococcus aureus* MIC_{50} and MIC_{90} varied from 0.2 to 0.3 mg/ml and from 0.4 to 0.5 mg/ml, respectively. In comparison with MIC of antibiotics currently used for treatment of disorders caused by *S. aureus* or by *E. coli* were MICs of HSB relatively high and general antibacterial activity very weak.

Keywords: houttuynin sodium bisulphate; *Houttuynia cordata*; antibacterial activity; *Escherichia coli*; *Staphylococcus aureus*

Houttuynin (houttuyin) sodium bisulphate (α-hydroxyl-capryl-ethyl-sodium-sulphonate, CH_3-$(CH_2)_8$-CO-CH_2-CHOH-SO_3Na) or HSB is a white crystalline powder, slightly soluble in water formed by reacting sodium bisulphate with houttuynin. Houttuynin (capryl ethaldehyde, CH_3-$(CH_2)_8$-CO-CH_2-CHO) is the main component of the volatile oil of *Houttuynia cordata*, which is unstable, insoluble in water, and not suitable for a practical drug preparation (HU & DU 1997).

H. cordata is a perennial herb from the family *Sauruaceae* that is used in traditional Chinese phytotherapy as anti-inflammatory, detoxicant, antipyretic and diuretic agent (PRÖBSTLE & BAUER 1992).

There has been reported the antibacterial activity of fractions separated by HPLC from the volatile essential oil of *H. cordata* against *Salmonella enteritidis, Shigella dysenteriae, Escherichia coli, Vibrio parahaemolyticus* and *Yersinia enterocolitica*, lower antibacterial activity against *Bacillus subtilis* and *Staphylococcus aureus* (KANG *et al*. 1997).

A preparation made from HSB offered effects similar to the combination of penicillin G with streptomycin in the treatment of bovine clinical mastitis in relieving the clinical signs and eliminating intramammary infection, in clinical trials with cows (HU & DU 1997).

In vitro determined minimum inhibitory concentrations for *Staphylococcus aureus* and *Streptococcus agalactiae* were 0.25 mg/ml in broth culture and 0.5 mg/ml in milk (HU & DU 1997).

A stimulating effect of HSB on the phagocytic functions of neutrophils from milk strippings has been reported. HSB enhanced the phagocytic index, killing index, and degree of phagocytosis and degree of killing of neutrophils (CAI *et al*. 1997). A herbal preparation from *Herba houttuyniae* showed stimulating effects on blood and milk neutrophil phagocytosis (HU *et al*. 1992)

The objective of this study was to evaluate the antibacterial activity of HSB against *Escherichia coli* and *Staphylococcus aureus*.

MATERIAL AND METHODS

Houttuynin sodium bisulphate (Beijing Second Pharmaceutical Factory, Beijing, China) was obtained from Mr. Hu Songhua (Department of Animal Science and Veterinary Medicine, Zhejiang Agricultural University, Hangzhou, Zhejiang, P. R. China).

Escherichia coli ATCC 25 922 and *Staphylococcus aureus* ATCC 25 928 reanimate from Culti-Loops® (Oxoid) was used as a bacterial strain.

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Wilkins-Chalgren broth (Oxoid) was used as a growth medium.

The HSB was dissolved in distilled water to form a preparation with a final concentration of 50 mg/ml and then autoclaved. Zero, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 and 0.8 ml of dilution were poured into sterile tubes containing 9 ml of broth (final concentration was 0, 0.55, 1.09, 1.61, 2.13, 2.63, 3.13, 3.61, 4.08 mg/ml). For each dilution five repetitions were prepared ($n = 5$). The first and second series of tubes were inoculated with 10^8 cfu of *E. coli* and *S. aureus*, respectively. For preparing the standard curve of O.D. for HSB the third series was not inoculated. All series of tubes were incubated at 37°C for 24 h. Initial O.D. was calculated at 620 nm on a spectrophotometer (Ultrospec III, Pharmacia LKB, UK). Samples were always appropriately shaken (Shaker R4, Mikrotechna) before particular mensuration. The growth of organisms was observed as turbidity with the aid of the spectrophotometer. For obtaining the final curve of inhibition, averages of standard values of O.D. for HSB were taken from calculated averages of O.D. of both bacterial strains. The difference between the calculated final and initial values was interpreted as the growth of bacteria, whereas comparison of the final values with the control values depicted the inhibitory effect of HSB on bacterial strains.

The minimum inhibitory concentrations (MIC) at the inhibition levels of 50% (MIC_{50}) and 90% (MIC_{90}) were determined by plotting a change in O.D. against the concentration of HSB. From the point on the curve depicting 50% or 90% growth compared with that of control (0% HSB), a line was plotted to meet the corresponding point on the *y*-axis (representing the O.D.). From the same point on the curve, a perpendicular was dropped to the *x*-axis (representing the concentration of HSB). The point of intersection of this perpendicular on the *x*-axis represented the concentration of HSB that inhibited 50% or 90% of the test bacterial strains and was designated as MIC_{50} or MIC_{90}.

RESULTS AND DISCUSSION

The growth of the bacterial strain at different concentrations of HSB observed at 620 nm was expressed as O.D. (Table 1). The higher the O.D., the greater the number of microorganisms (*E. coli*, *S. aureus*) or concentration of HSB (Standard).

MIC_{50} and MIC_{90} for *E. coli* varied from 0.5 to 0.6 mg per ml and from 2.6 to 2.7 mg/ml, respectively (Fig. 1). Concentrations of HSB between 0–1.09 mg/ml inhibit the growth of bacteria relatively rapidly. Up to the 1.09 mg per ml the level of HSB gradual inhibition was observed.

MIC_{50} and MIC_{90} for *S. aureus* were between 0.2 and 0.3 mg/ml and 0.4–0.5 mg/ml, respectively (Fig. 2).

For *S. aureus* MIC_{50} was about 0.3 mg/ml and MIC_{90} about 2.2 mg/ml lower than for *E. coli*.

In comparison of MIC of HSB with MIC of antibiotics currently used for treatment of disorders caused by *S. aureus* e.g., vancomycin (0.03–8.0 mg/l) or by *E. coli* e.g., ampicillin (0.25–5.0 mg/l) (HEJZLAR 1995) MICs of HSB were relatively high and general antibacterial activity very weak. HU and DU (1997) show data on MIC for *S. aureus* 0.25 mg/ml in broth culture. In our experiment, different resistance of the used strain probably caused higher MIC for *S. aureus*.

A stimulating effect of HSB (CAI *et al.* 1997) and herbal preparation from *Herba houttuyniae* (HU *et al.* 1992) on the phagocytic functions of neutrophils may explain overall therapeutic effects of HSB in bovine mastitis (HU & DU 1997).

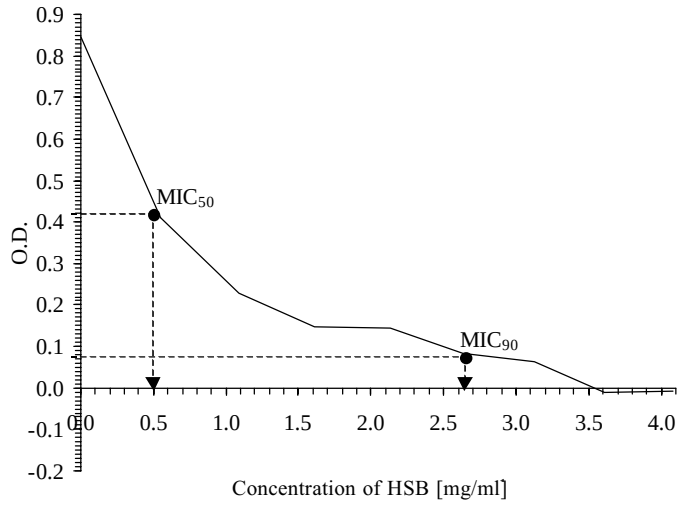

Fig. 1. Minimum inhibitory concentration of HSB at 50% (MIC_{50}) and 90% (MIC_{90}) inhibition level for *Escherichia coli*

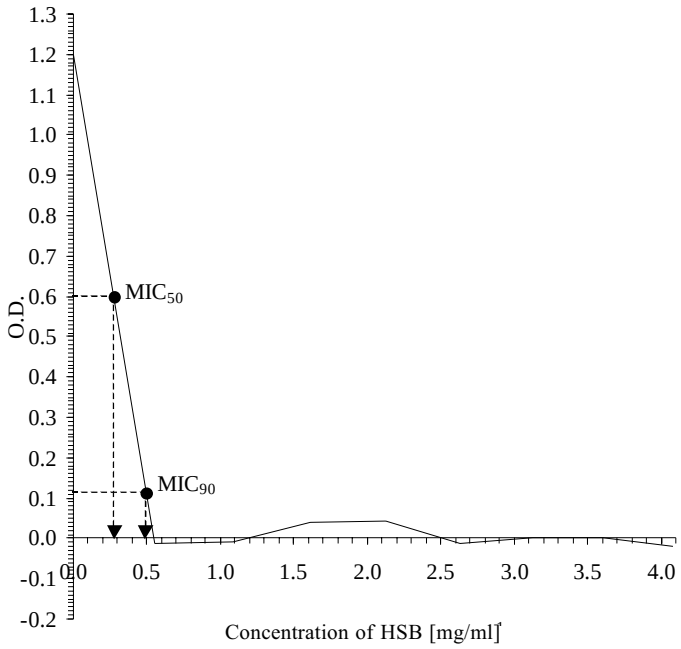

Fig 2. Minimum inhibitory concentration of HSB at 50% (MIC_{50}) and 90% (MIC_{90}) inhibition level for *Staphylococcus aureus*

Table 1. Inhibitory effect of HSB at growth of bacterial strains

Concentration of HSB (mg/ml)	Averages of reading values of O.D. ± SD			Calculated values of O.D.	
	E. coli	*S. aureus*	standard of HSB	*E. coli*	*S. aureus*
0.00	0.862 ± 0.027	1.216 ± 0.044	0.016 ± 0.003	0.846	1.200
0.55	0.540 ± 0.066	0.117 ± 0.007	0.130 ± 0.020	0.410	–0.013
1.09	0.495 ± 0.060	0.259 ± 0.029	0.268 ± 0.050	0.227	–0.009
1.61	0.568 ± 0.069	0.458 ± 0.077	0.420 ± 0.048	0.148	0.038
2.13	0.670 ± 0.085	0.569 ± 0.054	0.526 ± 0.007	0.144	0.043
2.63	0.802 ± 0.056	0.707 ± 0.033	0.719 ± 0.056	0.083	–0.012
3.13	0.934 ± 0.022	0.874 ± 0.042	0.871 ± 0.022	0.063	0.003
3.61	0.981 ± 0.008	0.994 ± 0.038	0.992 ± 0.021	–0.011	0.002
4.08	1.186 ± 0.057	1.173 ± 0.038	1.194 ± 0.023	–0.008	–0.021

OD – optical density; S – standard deviation; number of repetitions $n = 5$

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Abstract

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Houttuynin sodium bisulfit (1-hydroxy-3-oxododekanyl sulfonan sodný, HSB) je produkt reakce huttuyninu s hydrogen siřičitanem sodným a je jednou z hlavních složek éterického oleje z nadzemní části léčivky *Houttuynia cordata*. Přes svoji výraznou antimikrobiální aktivitu je nevhodný pro přípravu léčivých preparátů. Ověřovali jsme antibakteriální účinky HBS proti *Escherichia coli* a *Staphylococcus aureus*. Růst obou bakteriálních kmenů při různých koncentracích HSB naměřený při 620 nm byl vyjádřen pomocí hodnot O.D. (tab. 1). Minimální inhibiční koncentrace pro úroveň 50 a 90 % (MIC_{50} a MIC_{90}) se pohybovaly pro *E. coli* od 0,5 do 0,6 mg/ml, resp. od 2,6 do 2,7 mg/ml. Pro *S. aureus* byla stanovena MIC_{50} v rozmezí 0,2–0,3 mg/ml a MIC_{90} od 0,4 do 0,5 mg/ml. Vodný roztok HSB tedy vykázal slabší inhibiční účinek proti *E. coli* než proti *S. aureus.* V porovnání s hodnotami MIC antibiotik používaných při léčbě onemocnění způsobených *S. aureus* nebo *E. coli* lze stanovené rozsahy hodnoty MIC HSB označit za poměrně vysoké a celkovou antibakteriální aktivitu za velmi slabou.

Klíčová slova: houttuynin sodium bisulphate; *Houttuynia cordata*; antibakteriální aktivita; *Escherichia coli*; *Staphylococcus aureus*

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