ANTIBACTERIAL ACTIVITY OF FRESH PLANT MATERIAL OF *HOUTTUYNIA CORDATA* THUNB. AGAINST *PSEUDOMONAS AERUGINOSA*

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Houttuynia cordata is a perennial herb from the family *Saururaceae* that is used in traditional Chinese phytotherapy as anti-inflammatory, detoxicant, antipyretic and diuretic agent. The antibacterial activity of fresh plant material against *Pseudomonas aeruginosa* was measured by optical density. Minimum inhibitory concentration at 50% inhibition level (MIC_{50}) was 9.8% w/v and level of inhibition at concentration 10% (w/v) of tested plant material was 50.8%.

Houttuynia cordata; antibacterial activity; *Pseudomonas aeruginosa*

INTRODUCTION

Houttuynia cordata is a perennial herb from the family *Saururaceae* that is used in traditional Chinese phytotherapy as anti-inflammatory, detoxicant, antipyretic and diuretic agent (Pröbstle, Bauer, 1992). Decocted leaves are used for treatment of dysentery, enteritis, gastritis, hemoptysis, laryngitis and pharyngitis. Crushed aerial parts applied externally are used for treatment of abscesses, anal prolapse and hemorrhoids (Duke, 1985).

Cepharanone B, aristolactam A II, piperolactam, norcepharadione B (Pröbstle, Bauer, 1992), phytol, stigmast-4-ene-3,6-dione isolated from aerial parts of *H. cordata* show *in vitro* inhibitory activity to the cyclooxygenase (Pröbstle et al., 1993). Methyl *n*-nonyl ketone, lauryl aldehyde, and capryl aldehyde isolated from aerial parts of *H. cordata* inactivated *in vitro* herpes simplex virus type I, influenza virus and human immunodeficiency virus type I (Hayashi et al., 1995).

It has been reported the antibacterial activity of fractions separated by HPLC from the volatile essential oil of *H. cordata* against *Salmonella enteri-*

tidis, Shigella dysenteriae, Escherichia coli, Vibrio parahaemolyticus and *Yersinia enterocolitica*, lower antibacterial activity against *Bacillus substilis* and *Staphylococcus aureus*. No inhibitory effect against *Listeria monocytogens* was reported (K a n g et al., 1997).

Product of reaction of houttuynin with sodium bisulphite (houttuynin sodium bisulphate, HSB, CH_3-$(CH_2)_8$-CO-CH_2-CHOH-SO_3Na) is effective drug for the treatment of bovine clinical mastitis (H u , D u , 1997).

Pseudomonas aeruginosa is an aerobic, motile, Gram-negative rod; its outstanding bacteriologic feature is the production of colorful water-soluble pigments. It is commonly found free living in moist environments, but is also a pathogen of plants, animals, and humans. It is the most consistently resistant of all the medically important bacteria to antimicrobics (R y a n , 1990).

The objective of this study was to evaluate the antibacterial activity of fresh aerial material of *H. cordata* against *Pseudomonas aeruginosa*.

MATERIAL AND METHODS

Tested plants *H. cordata* were grown from seeds obtained in 1998 with compliments by service Index seminum from Japan (Botanical Garden, Tohoku University Kawauchi). They were grown in glasshouses of the Institute of Tropical and Subtropical Agriculture (ITSA) of the Czech University of Agriculture (CUA) in Prague. Voucher specimens have been deposited at ITSA CUA in Prague.

Bacterial strain *Pseudomonas aeruginosa* ATCC 27 853 was grown and maintained on Wilkins-Chalgren broth (Oxoid) medium.

Determination of inhibition effect was measured by method of sensitivity of food pathogens to *Allium sativum* (K u m a r , B e r w a l , 1998), modified by the authors.

Zero, 0.1, 0.5 and 1 g (0, 1, 5, 10% w/v) of cut ups of fresh plants material were weighted out in tubes and sterilized for 2 minutes by 0.2% solution of $HgCl_2$ (Sigma-Aldrich). For each concentration three repetitions were prepared ($n = 3$). Further, the plant material was rinsed 5 times with saline and 5 ml of sterile Wilkins Chalgren broth was poured into tubes. These tubes were inoculated with 10^8 cfu of bacteria and incubated at 37 °C for 24 h. Initial O.D. was measured at 620 nm using spectrophotometer (Ultrospec III, Pharmacia LKB, UK). Samples were always appropriately shaken (Shaker R4, Mikrotechna) before particular mensuration. The growth of organisms was observed with the aid of the spectrophotometer as turbidity. The difference between the final and initial readings was interpreted as the growth of bacteria, whereas comparison of the final readings with the control readings depicted the inhibitory effect of *H. cordata* on bacterial culture.

The minimum inhibitory concentration (MIC) of *H. cordata* at an inhibition level of 50% (MIC_{50}) was determined by plotting change in O.D. against the concentration of plant material. From the point on the curve depicting 50% growth compared with that of control (0% plant material), 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 plant material). The point of intersection of this perpendicular on the *x*-axis represented the concentration of plant material that inhibited 50% of the test bacteria and was designated as MIC.

Statistical relationship between inhibition of bacterial strain and concentration of fresh plant material was determined as correlation coefficient (r). A *t*-test was used to compare the measured values of inhibitory effect for 10% and 0% of fresh plant material.

RESULTS AND DISCUSSION

The growth of the bacterial strain in different concentrations of *H. cordata* observed at 620 nm was expressed as O.D. (Table I). The higher the O.D., the greater the number of microorganisms. Level of inhibition *P. aeruginosa* at concentration 10% (w/v) of tested plant material was 50.8%, compared with control.

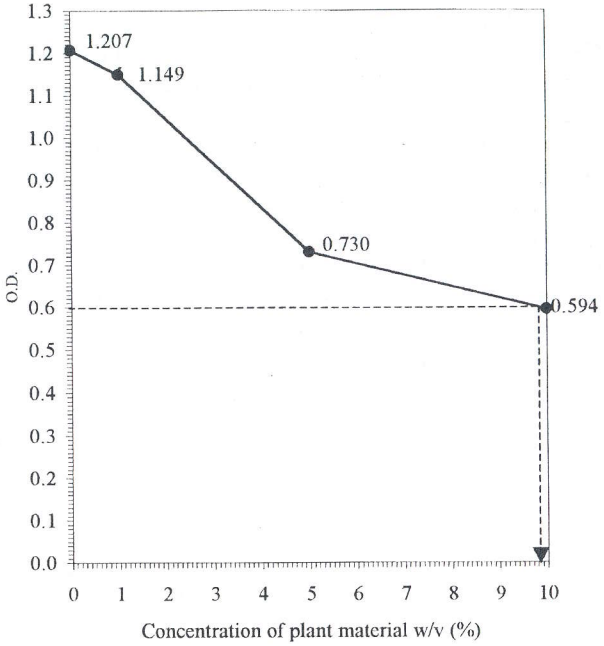
Calculated value of correlation coefficient ($r = -0.96$) is possible to interpret as statistical significant relationship between inhibition of bacterial strain and concentration of fresh plant material. Difference of measured values of inhibitory effect between 10% and 0% of fresh plant material is possible to interpret as statistical significant ($p = 0.02$).

Minimum inhibitory concentration of *H. cordata* at 50% inhibition level (MIC_{50}) for *P. aeruginosa* was 9.8% (w/v) (Fig. 1). Concentration of plant

I. Inhibitory effect of different concentrations of plant material of *Houttuynia cordata* at growth of *Pseudomonas aeruginosa*

Concentration of plant material w/v (%)	Average value O.D. ± SD
0	1.207 ± 0.161
1	1.149 ± 0.189
5	0.730 ± 0.097
10	0.594 ± 0.089

OD – optical density
SD – standard deviation
number of repetitions $n = 3$



1. Minimum inhibitory concentration plant material of *Houttuynia cordata* at 50% inhibition level (MIC_{50}) for *Pseudomonas aeruginosa*

material between 1% and 5% inhibit growth of bacteria relative rapidly. Up to the 5% level of plant material gradual inhibition was observed.

In comparison with conclusions of our previous experiments, *P. aeruginosa* was more resistant than all tested strains of *Escherichia coli, Salmonella enteritidis* and *Staphylococcus aureus* (Kokoška, Rada, 1998). Opposite of literate data (Kang et al., 1997), stronger inhibition of *H. cordata* against Gram-negative bacteria than against Gram-positive bacteria was not confirmed. Together with antiviral and other specific effects, for example inhibitory activity of some enzymes, antibacterial effect participates probably in general anti-inflammatory activity of *H. cordata*.

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Antibakteriální účinek čerstvého rostlinného materiálu *Houttuynia cordata* Thunb. proti *Pseudomonas aeruginosa*.

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Houttuynia cordata je vytrvalá bylina z čeledi *Saururaceae*, která je používána v tradiční čínské fytoterapii jako protizánětlivý, detoxikační, antipyretický a diuretický prostředek.

Cílem práce bylo ověřit a podrobněji prozkoumat předpokládané antibakteriální účinky čerstvé rostlinné hmoty z nadzemní části *H. cordata* proti *Pseudomonas aeruginosa*, jako jednomu z původců závažných bakteriálních onemocnění.

Růst *Pseudomonas aeruginosa* při různých koncentracích *H. cordata* naměřený při 620 nm byl vyjádřen pomocí hodnot O.D. (tab. I). Vyšší hodnota O.D. odpovídá většímu počtu mikroorganismů. Při koncentraci 10 % (w/v) rostlinného materiálu došlo k inhibici o 50,8 % oproti nárůstu mikroorganismů stanovenému v kontrole.

Minimální inhibiční koncentrace nadzemní hmoty *H. cordata* pro inhibiční úroveň 50 % (MIC_{50}) byla odečtena graficky (obr. 1). MIC_{50} = 9,8 % (w/v).

Pro stanovení závislosti inhibice jednotlivých bakteriálních kmenů na koncentraci rostlinného materiálu byla vypočtena hodnota korelačního koeficientu (r). Vypočtenou hodnotu r = –0,96 lze považovat za ukazatele statisticky významné závislosti mezi oběma sledovanými veličinami. Také výsledek t-testu potvrdil statisticky prů-

kazný rozdíl mezi hodnotami naměřenými pro 0% a 10% koncentraci rostlinného materiálu ($p = 0,02$).

Antibakteriální účinek se spolu s účinkem antivirovým a dalšími specifickými účinky, jako je např. schopnost inhibovat aktivitu některých enzymů, pravděpodobně podílí na celkovém protizánětlivém působení *H. cordata*.

Houttuynia cordata; antibakteriální účinek; *Pseudomonas aeruginosa*

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