Screening of Chinese Medicinal Herbs for the Inhibition of *Brucella melitensis*

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Abstract

The antimicrobial activities of extracts from Chinese herbs commonly available in the local Malaysian Chinese medicine halls against three field isolates and one reference strain of *Brucella melitensis* were evaluated. A total of ten herb extracts were obtained via ethanol extraction. Antibacterial screenings were done using disc diffusion method. Herb extracts with inhibitory zones of 10 mm or more in diameter were further subjected to the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) determination. Of the 10 herbs, which were *Lunicera japonica, Flos Lonicera, Coptis chinensis, Adrographis paniculata, Isatis indigotica, Radix paeoniae rubra, Polygonum orientale, Galla chinensis, Semen plantaginis, Fructus forsythia* and *Cortex phellodendrim,* four were found to possess inhibitory effect against *Brucella melitensis* strains. The herbs are *Coptis chinensis, Radix paeoniae rubra, Galla chinensis,* and *Cortex phellodendrin.* The MIC ranged from 3.75 to 30 mg/mL. It was suggested that these four herbs are potential alternatives for the treatment or prevention of brucellosis caused by *Brucella melitensis*.

Keywords: *Brucella melitensis,* minimum inhibitory concentration (MIC), herbal extract.

Introduction

Brucellosis is a zoonotic disease caused by the bacterium from the *Brucella spp*. This disease is endemic in many areas of the world including Malaysia. It is characterized by chronic infection in animals involving many species such as cattle, swine, sheep, goat and even horses leading to abortion, infertility, genital infection and the formation of localized lesions in many parts of the body tissues (Galloway J.H,1972). Human beings are susceptible to several biotypes of this agent namely *Brucella abortus, Brucella canis, Brucella melitensis and Brucella suis* but are resistant to infection by *Brucella neotomae and Brucella ovis.* The organism is of public health importance as it can cause debilitating disease (Malta fever) like fever and chills with frequent relapses which can persist for months if left untreated. Current control measures in farms are disease surveillance and investigative studies, vaccination, test and slaughter programs to contain and eradicate the disease. With these measures, brucellosis is no longer a threat in Western Europe but is still prevalent in Malaysia and other parts of the world (Swabe, 1999). The wide practice of the application of *B. abortus* S19 vaccination of susceptible young female animals either with the full dose or the reduced dose together with the slaughter of positive animals has resulted in the significant decrease of the

overall rate of infection in countries like Egypt (Refai et al., 1990). For conventional human treatment, doxycycline and rifampin combination are used for six weeks to combat the infection. Streptomycin is included in severe cases. The tedious treatment regime and ease of transmission of this disease highly suggest a quest for alternatives or improvement on ways to manage this disease.

Ethnopharmacology is the scientific study correlating ethnic groups, their health, and how it relates to their physical habits and methodology in creating and using medicines. Since global eradication of animal brucellosis will not be realistic in the near future due to socio-economic and political factors, and since the development of a satisfactory human vaccine currently is also not achieved yet, there exists a need for optimal antibiotic treatment schedules. These would ideally minimize the percentage of treatment failures and relapses whilst simultaneously being affordable for populations of low socio-economic status as well as being convenient in order to ensure adequate patient adherence. Ethnopharmacology is a very good alternative to explore in the sense that historically both human and animal medicine have relied heavily on plant materials. Major pharmaceutical companies had started off by selling plant extracts whilst a quarter of all prescription drugs currently sold in the western world still use active ingredients derived from plants (Cox and Balick, 1997). The first stage in a drug development program using plants as the starting material is the collection and analysis of data on the uses of plants by various native cultures. Ethnobotany, ethnomedicine, folk medicine and traditional medicine can provide information that is useful as a 'pre-screen' to select plants for experimental pharmacological studies. There are a number of Chinese herbs available in Malaysia that has potential to treat brucellosis. However, the potential of the herbs has not been evaluated yet. In this study, 10 types of commonly available Chinese herbs were tested in bioassay systems that are believed to predict the action of these drugs in humans. The goal of this ethnopharmacology study is to identify drugs to alleviate human illness via analysis of plants assumed to be useful in Chinese culture throughout the world.

The objectives of this study are to determine the antibacterial effects and minimal inhibitory concentrations of Chinese crude herbs against *Brucella melitensis.*

Materials and Methods

Dried Herbs

A total of ten dried herbs were obtained from Chinese medicinal shop. The herbs were *Lunicera japonica, Flos lonicera, Coptis chinensis, Adrographis paniculata, Isatis indigotica, Radix paeoniae rubra, Polygonum orientale, Galla chinensis, Semen plantaginis, Fructus forsythia* and *Cortex phellodendrim.* The origins of these herbs are from various parts of China.

Herb Extract Preparation

Thirty grams of herbs were macerated with 80% ethanol (500 mL) using a blender and left for five days at room temperature. The mixture was filtered and evaporated at 60ºC to obtain the dried herb extracts.

Brucella Isolates

One reference strain (*Brucella melitensis* 16M), one isolate from goat (*Brucella melitensis* 293) and two isolates from sheep (*Brucella melitensis* 183 and *Brucella melitensis* 4611) were used.

Antibacterial Screening (Disc Diffusion Method)

Extract concentration of 30 mg/mL was made by mixing 30 mg crude extract with one millilitre (1 mL) of 3% DMSO. Seventy microlitres (70 µL) of each extract was added to blank discs. The herb extract infused discs were dried at 60ºC in an oven overnight. Negative control were prepared by adding 70 µL of 3% DMSO to blank discs and dried at 60ºC overnight while Clavumox disc was used as positive control. Twenty mililitre Mueller Hinton Agar (MHA) plate were seeded with 20 ul suspension of test microorganism in 0.5 McFarland concentrations. One herb extract infused disc, one positive control disc and one negative control disc were put in each MHA plate. Triplicates were done. Inhibition zones diameters were read using the Aura Image (Oxoid). Extract with inhibition zone diameter of ten millimetres (10 mm) or more were subjected to MIC determination.

Determination of MIC of herb extracts on Brucella melitensis

Two-fold serial dilution methods were used. Briefly, 30 mg/mL crude extracts were filtered through 0.25 µm millipore filter for sterilization. The extracts were serially diluted starting from 30 mg/mL up to 0.015 mg/mL. Dilutions were made in 96-well microplates. Two hundred microlitres (200 µL) of 30 mg/mL extracts were dispense in the 1st column and 100 µL *Brucella* broth medium in the 2nd to 12th well. Two-fold serial dilution was done by pipetting 100 of extract from the 1st well into the 2nd well and so on. Twenty microliter (20 µL) of *Brucella* inoculum was added to every well. Streptomycin (0.05 mg/mL) was used as positive control while the negative control was made up of medium and inoculums. Triplicates were done for all four isolates. The plates were wrapped in parafilm and incubated at 37 ºC for three days. The growth of inoculum was determined by turbidity. Clear wells indicate absence of bacteria growth. The MIC of herbs was the lowest concentration in the medium that completely inhibit visible growth.

Determination of MBC of herb extracts on Brucella melitensis

The MBC of herb extract was carried out by inoculating ten microlitres (10 µL) of mixture from each well on MHA plate. Inoculated MHA plates were incubated at 37ºC for three days. The lowest concentration that yielded no growth after subculturing was taken as MBC.

Results and Discussion

The antimicrobial screening results of the herb extracts are shown in Table 1. Four herbs showed inhibitory effects on the organism namely, *Coptis chinensis, Radix paeoniae rubra, Galla chinensis and Cortex phellodendrim. Galla chinensis* showed the largest inhibition zone which were, 39 mm for *Brucella melitensis* 16M, 36 mm for *Brucella melitensis* 293, 33 mm for *Brucella melitensis* 183 and 40 mm for *Brucella melitensis* 4611. *Cortex phellodendrim* showed the smallest inhibition zone which is, 13 mm for *Brucella melitensis* 16M, 12 mm for *Brucella melitensis* 293, 12 mm for *Brucella melitensis* 83 and 15 mm for *Brucella melitensis* 4611. The larger the inhibition zone the stronger the inhibitory effect of the tested sample. As *Galla chinensis* has the largest inhibitory zone, it means that it has the strongest inhibitory effect on *Brucella melitensis* and has high potential to be used as an antimicrobial agent against this microorganism. In fact, the inhibitory zone of *Galla chinensis* is almost similar to Clavumox which has the highest zone of 45 mm thus, making it a potential alternative drug for management of infections. The other six herbs did not show any inhibitory zone thus, they do not have the potential to be used as anti *Brucella* agents. Among the four *Brucella i*solates, *Brucella melitensis* 293 is the most resistant to the inhibitory effect of the herbs because the inhibitory zones are the smallest which are 14 mm for *Coptis chinensis*, 14 mm for *Radix paeoniae rubra,* 32 mm for *Galla chinensis* and 12 mm for *Cortex phellodendrin.*

Table 1. Inhibition Zone Diameter in mm of Herb Extracts on Four Strains of *Brucella melitensis*

Herbs	***Brucella melitensis* 16M**	***Brucella melitensis* 293**	***Brucella melitensis* 183**	***Brucella melitensis* 4611**
Lunicera japonica	0	0	0	0
Flos lonicera	0	0	0	0
Coptis chinensis	22	14	29	22
Andrographis paniculata	0	0	0	0
Isatis indigotica	0	0	0	0
Radix paeoniae rubra	18	14	18	16
Galla chinensis	39	32	33	40
Semen plantaginis	0	0	0	0
Fructus forsythia	0	0	0	0
Cortex phellodendrim	13	12	12	15
Clavumox	44	42	45	45
3% DMSO Infused Blank Disc	0	0	0	0

The antimicrobial activities of different extracts tested are shown in Tables 2 to 5. Bactericidal activity was confirmed by determining the MBC:MIC ratio. The MBC:MIC ratio describes a relationship between the minimum *in vitro* bactericidal concentration and the MIC of an antibiotic. If the MBC:MIC ratio of a pathogen is between 1:1 to 2:1, the drug is considered to be bactericidal against that pathogen.

For *Brucella melitensis* 16, all herbs fulfill the ratio except for *Coptis chinensis* which has a ratio of 4:1 and *Cortex phellodendrin* which did not show any effects at the tested concentration.

Table 2. MIC and MBC of herb extracts on *Brucella melitensis*16

Herbs	***Brucella melitensis 16M***		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	7.5	30	1: 4
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis 293*, all herbs fulfill the ratio except for *Cortex phellodendrin* which did not show any inhibitory effects at the tested concentration.

Table 3. MIC and MBC of herb extracts on *Brucella melitensis* 293

Herbs	***Brucella melitensis 293***		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	3.75	7.5	1: 2
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis* 183, the results were the same as for *Brucella melitensis* 293.

Table 4. MIC and MBC of herb extracts on *Brucella melitensis* 183

Herbs	*Brucella melitensis 183*		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	3.75	7.5	1: 2
Radix paeoniae rubia	7.5	7.5	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

For *Brucella melitensis* 4611, the MIC-to-MBC ratio was the same with the previous 2 strains. However, MIC and MBC values were higher.

Table 5. MIC and MBC of herb extracts on *Brucella melitensis* 4611

Herbs	*Brucella melitensis 4611*		
	MIC (mg/mL)	**MBC(mg/mL)**	**MIC-to-MBC ratio**
Coptis chinensis	7.5	15	1: 2
Radix paeoniae rubia	3.75	3.75	1: 1
Galla chinensis	3.75	3.75	1: 1
Cortex phellodendrin	nd	nd	nd

nd - not determined because sample was not active at the highest concentration

From the results, 2 herbs showed bactericidal activity which are, *Radix paeoniae rubia* and *Galla chinensis*. *Cortex phellodendrin* did not show bactericidal activity at the highest concentration tested while *Coptis chinensis* an MBC-to-MIC ratio that exceeded the bactericidal ratio of 1:2.

In comparison to previous work by Zhu et al*.,* (2009) who assessed the herbs' effects on *Ureaplasma urealyticum*, the findings are similar where *Coptis chinensis, Radix paeoniae rubra, Galla chinensis and Cortex phellodendrim* also yielded potential inhibitory effect on *Brucella melitensis.* However, the MICs of these herbs on the two different bacteria are different. Taking *Brucella melitensis* 16M as a comparison to *Ureaplasma urealyticum,* the MICs of *Coptis chinensis* on *Brucella melitensis* 16M is 7.5 mg/mL while on *Ureaplasma* is 20 mg/mL, MICs of *Radix paeoniae rubia* on *Brucella melitensis* 16M is 7.5 mg/mL while on *Ureaplasma* is 2.5 mg/mL, MICs of *Galla chinensis* on *Brucella melitensis* 16M is 3.75 mg/mL while on *Ureaplasma* is 1.25 mg/mL. *Cortex phellodendrin* did not yield any detectable MIC on *Brucella melitensis* at the highest tested concentration of 30 mg/mL while it yielded an MIC of

5 mg/mL on *Ureaplasma urealyticum*. From these comparisons and studies, it shows that these herbs can be developed into useful antimicrobials that can act against more than one type of bacteria.

References

Cox and Balick (1997). Plants, People and Culture: The Science of Ethnobotany. 1st edition. Henry Holt & Company, USA.

Galloway, J.H. (1972). Farm Animal Health and Disease Control. 1st edition,Lea & Febiger., Philadelphia.

Refai, M., El-Gibaly, S. and Adawi, A. (1990). Initiation of a national brucellosis control program in Egypt. In: Adams, L. G. (Ed.), Advances in Brucellosis Research. Texas A&M University, Texas USA.

Swabe, J. (1999). Animals, Disease and Human Society – Human-animal relations and the rise of veterinary medicine. 1st edition, Routledge., NY.

Zhu, C. T., Dong, C. L., Kong, Z. Y., Liu, L. X. and Yao, Y. Y. (2009). Mocrodilution inhabitation test of Chinese herbs to assess their effect against clinical strains of Ureaplasma urealyticum in vitro. *J Nanjing Med Uni* **23**: 143-145.