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in vitro Antibacterial Activities of Antibiotics and Traditional Chinese Medicinal Herb Extracts on *Escherichia coli* and *Staphylococcus aureus*

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The present study has been aimed to assess the antibacterial effects of the gentamycin sulfate by agar-well diffusion method against *Escherichia coli* and penicillin against *Staphylococcus aureus*. Aspects on the antibacterial mode of action of Chinese medicinal herb extracts have also been characterized. The minimum inhibitory concentration (MIC) of gentamycin sulfate was 8 μg mL^{-1} and those of penicillin was 0.5 μg mL^{-1}, with the diameter of inhibition zone was 8.5 and 7.5 mm, respectively. And the rational pH value of the working antibiotics was 5, the reasonable preservation temperature should below 80 and 60 °C for gentamycin sulfate and penicillin, respectively. Meanwhile, both ethanolic extracts of *Forsythia suspensa* (forsythia) and *Taraxacum ohwianum* (taraxacum) had a better antibacterial effect to *S. aureus*, however *E. coli* only sensitive to *Forsythia suspensa* (forsythia). Thus, antibiotics combined with Chinese herb *Forsythia suspensa* (forsythia) may have better antibacterial effect to bacterium.

Keywords: Antibacterial activity, Antibiotics, Chinese medicinal plants.

INTRODUCTION

Human beings are often infected by microorganisms such as bacteria, yeasts and viruses in the living environment. And bacteria arised disease and infections become more and more serious these years. Thus to solve these problem has become a hot social issues, especially in medicine and food industry.

Generally, people like to select some antibacterial materials inhibit the growth and multiplication of bacteria. Antibacterial materials contain various natural and inorganic substances, *e.g.*, antibiotics, such as penicillin and gentamycin sulfate. They have antibacterial property specially for Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, which are known to be common pathogenic bacteria of the skin and intestine, respectively. However, how to rationally use antibiotics is a concern and the increasing antibiotic resistance of some pathogens is another concern[1-3].

Medicinal plants are another antibacterial materials to bacteria, which have been used as sources of medicine in virtually all cultures[4]. During the last decade, the use of traditional medicine has expanded globally and is gaining popularity. According to WHO, herbal medicines serve the health needs of about 80 % of the world's population, especially for millions of people in the vast rural areas of developing countries[5]. In China, the ancient beliefs and practice of traditional Chinese medicine have been healing people for thousands of years, it is still used by millions of people all over the world even after the development of modern scientific medicine. Chinese herbal medicine contains hundreds of medicinal substances-primarily plants. And traditional Chinese herbal medicine makes almost exclusive use of herbal combinations, more importantly, these formulas are not designed to treat symptoms of a specific illness; rather, they are tailored specifically to the individual according to the complex principles of traditional Chinese medicine. Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Traditional Chinese medicinal plants has received little attention in antibacterial activity research and development. However, the long history of use of medicinal plants in China and its huge biotic riches can be of paramount importance in future research and drug discovery.

In this study, two antibiotics *i.e.*, penicillin and gentamycin sulfate, two species of plants namely forsythia and taraxacum, having traditional claims for the treatment of various disorders were investigated its antibacterial activity and their rational service conditions on *Staphylococcus aureus* and *Escherichia coli* strains, which are known to be common pathogenic bacteria of the skin and intestine, respectively. These results would be useful to treat infections caused by bacteria and may provide clues to discover a new drug target for therapy bacterial inflammation safely.

EXPERIMENTAL

Two antibiotics penicillin and gentamycin sulfate and two chinese medicinal herbs namely *Forsythia suspensa* (forsythia) and *Taraxacum ohwianum* (taraxacum) were purchased from China national pharmaceutical group corporation, China. These two medicinal herbs plants were harvested and processed and naturally dried, according to traditional procedures.

Bacteria and culture: Two bacteria were kindly provided by China general microbiological culture collection center, they are Gram-positive *Staphylococcus aureus* (ATCC25922) and gram-negative *Escherichia coli* (ATCC 25322). The strains were cultured at 37 °C on plate count agar (PCA) medium.

Preparation of ethanolic extracts: Fresh plant samples were cleaned, freeze-dried in a freeze dry system (Christ 1-4) and ground into a fine powder by a Kenwood Multi-Mill (Kenwood, Havant, UK) and passed through a sieve (24-mesh). Dried plant samples were further air-dried in a ventilated oven at 40 °C for 24 h, then ground into a fine powder and passed through a sieve as above. Powdered sample (5 g) was extracted with 80 mL 95 % ethanol in water at room temperature (~ 23 °C) for 24 h in a shaking water bath, followed by 60 °C for 6 h. The extract was filtered by a Millipore filter with a 0.45 μm nylon membrane under vacuum at 23 °C. The filtrates were concentrated by rotavapor (R-114) and then freeze-dried by a freeze dry system. The samples were stored at 4 °C until use.

Experimental designs: In order to determine the minimal inhibitory concentration (MIC) of gentamycin sulfate to *E. coli* and the minimum inhibitory concentration of penicillin to *S. aureus*, gentamycin sulfate was diluted by phosphate buffered saline (PBS, pH 7.0-7.2) to the final concentration of 0, 20, 40, 60 and 80 μg mL^{-1} and penicillin concentration of 0, 0.2, 0.4, 0.6 and 0.8 μg mL^{-1}.

For determination the effect of different pH value of antibiotics to antibacterial activity, the 80 μg mL^{-1} gentamycin sulfate and 0.8 μg mL^{-1} penicillin was adjusted at pH value to 3, 4, 5, 6, 7, 8 and 9 using HCl or NaOH to keep 1 h and then adjusted back to the primary value to test antibacterial activity, respectively.

For determination the effect of different temperature of antibiotics to antibacterial activity, both 80 μg mL^{-1} gentamycin sulfate and 0.8 μg mL^{-1} penicillin were under 40, 50, 60, 70, 80, 90 and 100 °C on dry bath incubator kept 0.5 and 1 h, respectively and then cooled back to the room temperature to test antibacterial activity, respectively.

The freeze dried extract samples of herbs were dissolved in phosphate buffered saline solution to the final forsythia concentration of 80, 120, 160 and 200 mg mL^{-1} and the taraxacum concentration of 80, 160, 240 and 320 mg mL^{-1} and sterilized by filtration through 0.22 μm sterilizing Millipore express filter (Millex-GP, Bedford, OH).

Determination of antibacterial activity: An agar-well diffusion method was employed for determination of antibacterial activities (NCCLS, 1999). Both bacterium were suspended in sterile water and diluted to ~10^6 CFU mL^{-1}. The suspension (100 μL) was spread onto the surface of plate count agar medium. Wells (4.6 mm in diameter) were cut from the agar with a sterile borer and 60 μL prepared antibiotics and extract herbs solutions above were delivered into them. Negative controls were prepared using phosphate buffered saline solution. The inoculated plates were incubated at 35 °C for 24 h. Antibacterial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested bacteria. Diameter of inhibition zone was expressed in millimeters. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Minimum inhibitory concentrations of gentamycin sulfate and penicillin to the bacteria: The range of concentrations tested was 0 to 80 μg mL^{-1} and 0 to 0.8 μg mL^{-1} for gentamycin sulfate and penicillin, respectively. There was a significant variation in the antibacterial activities (diameter of inhibition zone values) of two antibiotics to the corresponding bacteria. For Gram-negative *E. coli*, the diameter of inhibition zone values of gentamycin sulfate were between 8.5 and 17.2 mm and those of penicillin to Gram-positive *S. aureus* were between 7.5 mm and 17.7 mm (Fig. 1). Moreover, the gentamycin sulfate concentration less than 8 μg mL^{-1} and the penicillin concentration less than 0.5 μg mL^{-1} have no inhibitory activity. So the minimum inhibitory concentration value of gentamycin sulfate to *E. coli* was 8 μg mL^{-1} and those of penicillin to *S. aureus* was 0.5 μg mL^{-1}.

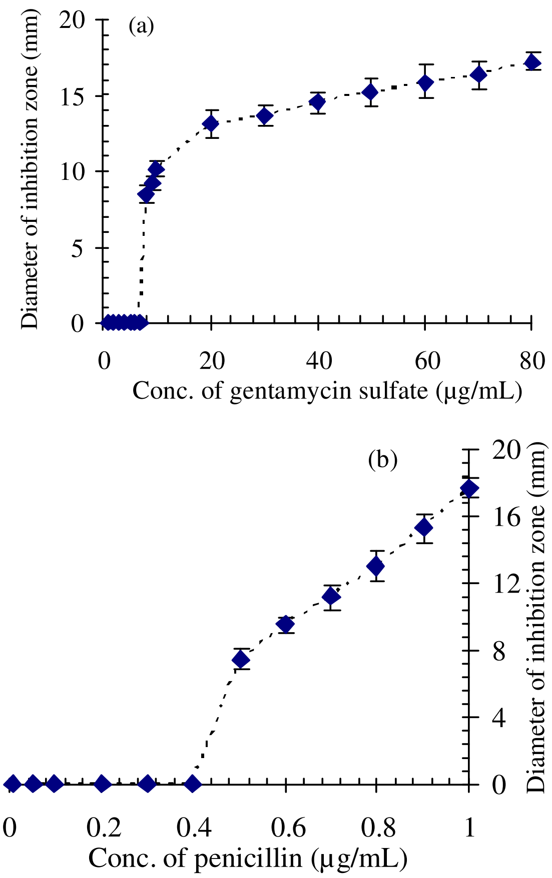

Fig. 1. Antibacterial activity of gentamycin sulfate and penicillin at different concentrations against (a) *Escherichia coli* and (b) *Staphylococcus aureus*, respectively.

Relationship between antibacterial activity and antibiotic concentration: The correlations between antibacterial activity and antibiotic concentration were shown in Fig. 2. In general, with the concentration of antibiotics increased both of the diameter of inhibition zone values also increased. The R^2 values of gentamycin sulfate and penicillin concentration for *E. coli* and and *S. aureus* was 0.8872 and 0.9956, respectively.

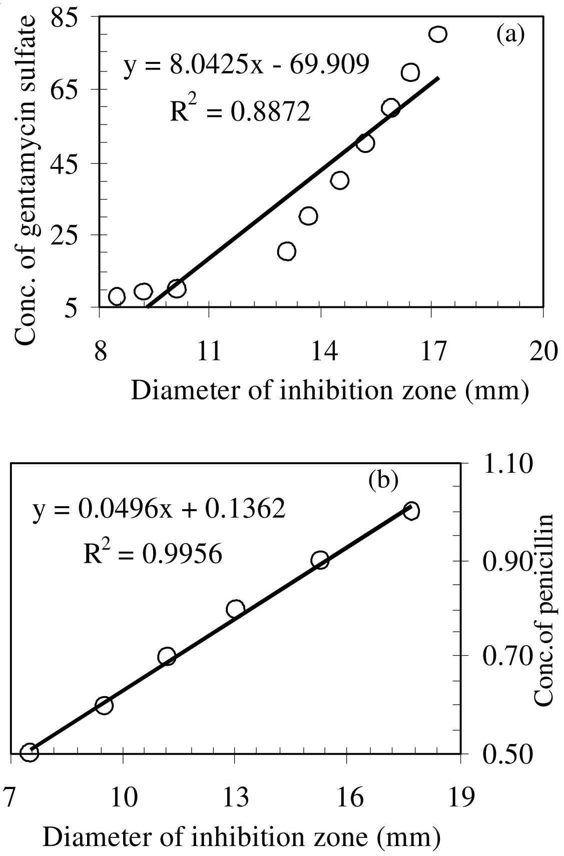

Fig. 2. Relationship between diameter of inhibition zone (mm) and concentration of gentamycin sulfate and penicillin (μg mL^{-1}) for (a) *Escherichia coli* and (b) *Staphylococcus aureus*, respectively

Influence of different pH value on the antibacterial activity: The effect of different antibiotic pH value on antibacterial activity was determined (Fig. 3). The diameter of inhibition zone values of both antibiotics against bacteria were increased and then decreased with pH values from 3 to 9. The diameter of inhibition zone values were between 15 mm to 17.4 mm of *E. coli* and 14.1 mm to 18.9 mm of *S. aureus* and both of the maximum diameter of inhibition zone value was under the pH value 5, indicating the pH value of the working antibiotics against bacteria should be around 5.

Influence of different temperature on the antibacterial activity: The effect of different antibiotic temperature on antibacterial activity was determined (Fig. 4). The diameter of inhibition zone values of gentamycin sulfate against *E. coli* decreased along with the increased treatment temperature, the turning point was around 90 °C and before 80 °C the difference of diameter of inhibition zone values were not significantly with the value of about 17 mm. However, the change trend of diameter of inhibition zone values of penicillin against

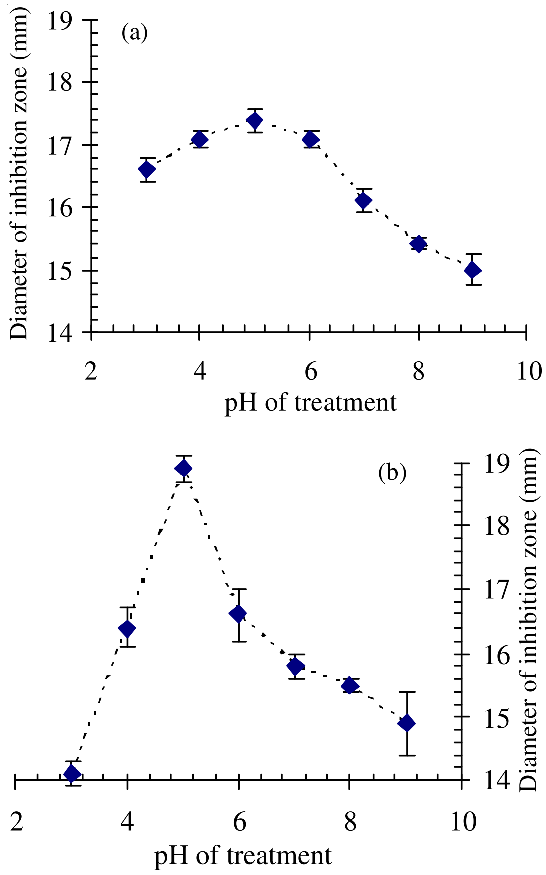

Fig. 3. Antibacterial activity of gentamycin sulfate and penicillin after different pH values treatment against (a) *Escherichia coli* and (b) *Staphylococcus aureus*, respectively

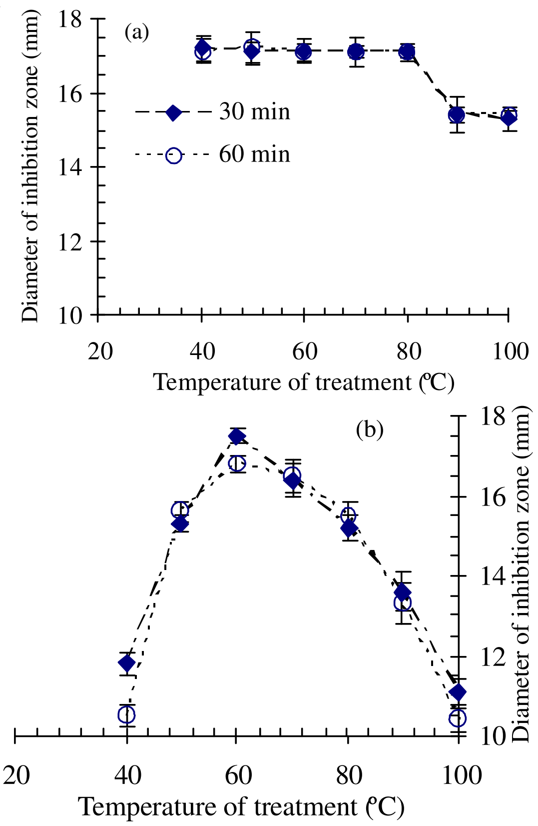

Fig. 4. Antibacterial activity of gentamycin sulfate and penicillin after different temperature treatment against (a) *Escherichia coli* and (b) *Staphylococcus aureus*, respectively

S. aureus was increased and then decreased with the increased treatment temperature and the maximum diameter of inhibition zone value was 17.5 mm under the treatment temperature 60 °C. Under two different treatment time, both of the antibacterial activity was no obvious difference, these showed up as the same diameter of inhibition zone value change trend.

Antibacterial activity of forsythia and taraxacum: Two chinese medicinal herbs *Forsythia suspensa* (forsythia) and *Taraxacum Ohwianum* (taraxacum) were used to determine their antibacterial activity against *E. coli* and *S. aureus*. There was a significant variation in the antibacterial activities (diameter of inhibition zone values) of the two extracts (Table-1).

TABLE-1
ANTIBACTERIAL ACTIVITY OF
FORSYTHIA AND TARAXACUM

Test	Concentration (mg mL^{-1})	Inhibition zone (mm)[a]	
		Escherichia coli	*Staphylococcus aureus*
Forsythia	80	–	–
	120	8.6 ± 0.21	7.5 ± 0.23
	160	12.7 ± 0.45	11.4 ± 0.43
	200	+	+
Taraxacum	80	–	–
	160	–	–
	240	–	6.5 ± 0.12
	320	–	10.4 ± 0.63

[a]Average of three replicates; + indicates complete inhibition and – indicates no inhibition.

For *E. coli*, the values of diameter of inhibition zone of forsythia extract was 8.6 and 12.7 mm under the concentration of 120 and 160 mg mL^{-1}, respectively. Under the concentration of 80 mg mL^{-1}, there was no obvious inhibition. However under the concentration of 200 mg mL^{-1} the *E. coli* was completely inhibition. So the minimum inhibitory concentration of forsythia for *E. coli* was 120 mg mL^{-1}. Moreover, the taraxacum extract almost had no inhibition under any concentration.

For *S. aureus*, the values of diameter of inhibition zone of forsythia extract was 7.5 and 11.4 mm under the concentration of 120 and 160 mg mL^{-1}, respectively. Under the concentration of 80 mg mL^{-1}, there was no obvious inhibition, however under the concentration of 200 mg mL^{-1} the *S. aureus* was completely inhibition. So the minimum inhibitory concentration of forsythia for *S. aureus* was 120 mg mL^{-1}. Moreover, the diameter of inhibition zone values of taraxacum extract was 6.5 and 10.4 mm, respectively. There was no inhibition under the concentration less than 240 mg mL^{-1}, thus the minimum inhibitory concentration of taraxacum for *S. aureus* was 240 mg mL^{-1}.

Both of the antibiotics tested in this study exhibited high antibacterial activity against the corresponding bacteria and there was a highly positive relationship between the concentration of antibiotics and antibacterial activity. To some extent, these results were similar to those of previous studies. Gentamycin is an aminoglycoside antibiotic that is synthesized by *Micromonospora*. It is active against most Gram-negative bacteria and some Gram-positive and gentamycin sulfate inhibits bacterial protein synthesis. The combination of the *E. coli* serum antibody and the gentamycin sulfate have been evaluated that the antibacterial activity of gentamycin sulfate increased 1000 times and the clinical curative effect enhanced 100 times than it alone, this means higher efficacy and safety of gentamycin sulfate to eradicate *E. coli in vitro*[6]. The antibacterial activity of standard antibiotics such as ampicillin and cloxacillin was also tested and they showed moderate to good antibacterial activity for *S. aureus*, whereas they were ineffective against *E. coli* and *Pseudomonas aeruginosa*[7]. Further, this study has shown a link between the concentration of antibiotics and their antibacterial activity and the present result indicated that the minimum inhibitory concentration value of gentamycin sulfate to *E. coli* was 8 μg mL^{-1} and those of penicillin to *S. aureus* was 0.5 μg mL^{-1}. Moreover, the pH value of the working antibiotics against bacteria should be around 5 and if the gentamycin sulfate treated higher than 80 °C, its antibacterial activity will be lower, however those of the penicillin was 60 °C. These results may provide certain basis on the preservation and use of antibiotics.

Of the two herb extracts tested in this study, forsythia exhibited high antibacterial activities against both of bacterium, taraxacum only showed antibacterial activities against *S. aureus*. Ethanolic extracts of the compound Chinese medicine with the proportion of the honeysuckle: forsythia: the licorice as 2:2:1 were shown antivirus and antibacterial properties, *S. aureus*, *E. coli* and *Candida albicans* were effectively inhibited by them[8]. The activity test indicated that only ethanol extracts of *Taraxacum mongolicum* exhibited antibacterial activity against some bacteria, such as *S. aureus* and its isolated strain from air, *E. coli* and *P. aeruginosa*[9]. However, in this study, the results did not completely follow the trend described above, they indicated that ethanolic extracts of forsythia alone could inhibit both of *S. aureus* and *E. coli*. Moreover, *T. ohwianum* (taraxacum) had no antibacterial activity against *E. coli*, as *T. ohwianum* and *T. mongolicum* were same different taraxacum species, may be different species had different antibacterial activity.

Our results suggest that Gram-positive bacteria were generally more sensitive to the herb extracts than Gram-negative bacteria. This was consistent with the previous studies on other spices and herbs[10-13]. A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria, Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria[14]. Moreover, both bacterium (*S. aureus* and *E. coli*) were more sensitive to forsythia extracts than taraxacum, this suggested that there might be some particular antibacterial substances in forsythia extracts and the exact mechanism should be studied in future research.

Conclusion

The present study showed that there was obvious antibacterial effect of gentamycin sulfate to *E. coli* with the minimum inhibitory concentration value 8 μg mL^{-1} and those of penicillin to *S. aureus* was 0.5 μg mL^{-1} and the most rational pH value of the working antibiotics against bacteria should be around 5, the reasonable preservation temperature should below 80 and 60 °C for gentamycin sulfate and penicillin, respectively. Meanwhile, both ethanolic extracts of *Forsythia suspensa* (forsythia) and *Taraxacum ohwianum* (taraxacum) had a better antibacterial effect to *S. aureus*, however *E. coli* only sensitive to *Forsythia suspensa* (forsythia). Thus, antibiotics combined Chinese herb *Forsythia suspensa* (forsythia) may have better antibacterial efficacy to bacterium.

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