ANTIMICROBIAL ACTIVITY OF THE EXTRACTS FROM LONICERA HYPOGLAUCA MIQ. ETHNOPHARMACOLOGICAL COMMUNICATION

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ABSTRACT

\textit{Lonicera hypoglauca} Miq. is used in the traditional medicine for the treatment of sore throat infection, respiratory tract infection and intestinal tract diseases indicating antimicrobial activity. To validate the traditional medicinal claim, in vitro antimicrobial activity of the extracts was screened against eleven human pathogenic bacteria and fungi. The ethyl acetate (EE) and n-butanol (BE) extracts of the flower of \textit{Lonicera hypoglauca} Miq were tested against five Gram-positive bacteria, five Gram-negative bacteria and five fungi species. Antimicrobial activity was determined by the tube-dilution method. The EE and BE extracts showed antimicrobial activity against all of the tested microorganisms, with minimum inhibitory concentration (MIC) values in the range of 0.32–4.86 mg/ml. The tested microbes \textit{Staphylococcus aureus}, \textit{Streptococcus pneumoniae} and \textit{Streptococcus pneumoniae} were highly susceptible to extract BE and antimicrobial activity of extract BE is better than that of extract EE against \textit{Staphylococcus aureus} and \textit{Streptococcus pneumoniae}. Both the extracts were found less susceptible against tested fungi.

KEYWORDS: Antimicrobial; Extracts; \textit{Lonicera hypoglauca} Miq.
INTRODUCTION

The plants of the genus *Lonicera hypoglauca* Miq (Caprifoliaceae) wildly grows and is also cultivated in southern area of China, especially in Hunan province. Flos Lonicera of *Lonicera hypoglauca* Miq is a common Chinese medicinal herb which has a long history of indigenous use in China. Leaves and flower of *Lonicera hypoglauca* Miq possess antibacterial activity in Chinese medicine, Flos Lonicera of *Lonicera hypoglauca* Miq Jinyinhua and *Lonicera japonica* Thunb both called Jinyinhua that is well-known Chinese traditional medicine for the treatment of diverse diseases (Yin *et. al.*, 2007, Su, 2009), especially for the treatment of sore throat infection and respiratory tract infection and intestinal tract infection diseases. In china, the leaves and flowers are boiled and administered to patient, especially Flos Lonicera (flower). It has attracted many interests.

Both *Lonicera hypoglauca* Miq. and *Lonicera japonica* Thunb are widely used as Jinyinhua in traditional Chinese medicine. Although they have similar geographic distribution, obviously various characteristics are observed (Pu *et. al.*, 2002). Studies of the phytochemistry and bioactivity of Jinyinhua have mostly focused on *L. japonica* (Japanese honeysuckle) that has been reported to possess properties like anti-inflammatory, antiangiogenic and anti-nociceptive activities (Xu *et. al.*, 2007, Yoo *et. al.*, 2008).

Studies have been also reported on the phytochemistry of *Lonicera hypoglauca* Miq. Ziguglycoside, scopoletin, daucosterol, β-sitosterol have been isolated from ethyl acetate, and macranthoidin A, macranthoidin B, chlorogenic acid have been isolated from n-butanol extracts of rattan of *Lonicera hypoglauca* Miq (He *et. al.*, 2006). Hexadecanoic acid, Docosane, Linoleic acid have also been reported from the essential oil of this plant (Guo *et. al.*, 2005). However, the bioactivity of *Lonicera hypoglauca* Miq. has barely been studied.

The micro-organisms which are often associated with throat, respiratory tract and intestinal tract infectious diseases belong to the genus bacillus and coccus. These organisms are present in water, soil, sewage and in the gastrointestinal tract of animals, included humans (Murray *et. al.*, 1998). As part of our contribution to phytochemical and biological survey and to validation of traditional uses of this medicinal plant we report herein the study on Flos Lonicera of *Lonicera hypoglauca* Miq antimicrobial activity.

In particular, the purpose of this study was to investigate the plant for the potential antimicrobial activity against selected bacterial strains, which may be involved in sore throat diseases and soft tissue infections, especially respiratory tract infection. Further bioassay-guided extractions were carried out in ethyl acetate extract and n-butanol, in order to obtain the most active extract with the final aim to identify the chemical classes responsible for the biological activity.

Materials & Methoda

Experimental

Chemicals and reagents

HPLC-grade ethyl acetate and n-butanol were purchased from Changsha Sheng Fan reagents Co., LTD, (Changsha, China); Norfloxacin and Fluconazole were purchased from Shanghai research born biochemical reagents Co., LTD, China; water was deionized by filtering through a Direct-Q system (Millipore, Bedford, MA, USA).
Plant material

Flos Lonicera (flower) of *Lonicera hypoglauca* Miq was collected in the month of June 2010 from HuNan, China. The botanical identification of the plant was done at Food and biology Department, Science & Technology University, Chang Sha, HuNan, china. A voucher specimen (no.FLHM01) has also been deposited at Food and biology Department, Science & Technology University, Chang Sha. Flos Lonicera (flower) was dried in the dark, in a ventilate room at 25–30°C, then grounded and the powder stored at −20°C.

Extraction

Flos Lonicera (flower) of *Lonicera hypoglauca* Miq was dried and coarsely powdered by grinding. The powdered material (200 g) was extracted with 2 l of ethyl acetate and n-butanol separately. Each filtrate was concentrated under reduced pressure at 50°C. The extracts were further dried at room temperature under reduced pressure. The yield of the extracts of ethyl acetate (EE) and n-butanol (BE) were 1.90 g (w/w) and 3.40 g (w/w), respectively.

Antimicrobial activity

Micro-organisms

The micro-organisms which are often associated with throat, respiratory tract and intestinal tract infectious diseases belong to the genus bacillus and coccus. The following strains were used for testing the antimicrobial activity of the crude extracts: *Staphylococcus aureus* (26003), *alpha hemolytic streptococcus* (32129), *beta hemolytic streptococcus* (53214), *Streptococcus pneumoniae* (32010), *Corynebacterium diphtheriae* (38101) (Gram-positive bacteria), *Shigella flexneri* (51236), *Salmonellaty phimurium* (53185), *Escherichia coli* (44104), *Pseudomonas aeruginosa* (10223), Bacillus subtilis (63501) (Gram-negative bacteria) were obtained from the health ministry identified microbes, Beijin, China. *Candida albicans* (ATCC 11006), *Candida parapsilosis* (ATCC22019), *Candida tropicalis* (ATCC01463), *Trichophyton mentagrophytes* (ATCC28185), *Cryptococcus neoformans* (ATCC32609) (Fungi) were obtained from Beijing university fungi and fungal disease research center, China.

Preparation of test sample

The EE and BE extracts were dissolved in 10% N-N dimethyl formamide (DMF) which is reported to be non-toxic to microorganisms at this percentage (Pujol et. al., 1990). Norfloxacin and Fluconcazole (Shanghai research born biochemical reagents Co., LTD, China) were used as positive reference standards for bacterial and fungal strains, respectively.

Preparation of inocula

The inocula of microbial strains were prepared from 18 h old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (~10⁴ for bacteria and ~10³ for fungi colony forming unit (CFU) per milliliter) (McFarland, 1987).

Evaluation of minimum inhibitory concentration (MIC)

Tube-dilution method was used to determine the minimum inhibitory concentration (MIC) of the EE and BE extracts against the microorganisms under study. The EE and BE extracts were dissolved in 10% N-N dimethyl formamide (DMF). The final concentrations of EE and BE extracts for bacteria were 4.0 and 6.0 mg/ml, respectively. The final concentration 5.0 mg/ml was used for EE and BE extracts against fungi. Serial two-fold dilutions were prepared from the stock.
solution to give concentration ranging from 4.00–0.006 to 6.0–0.008 mg/ml for EE and BE extracts, respectively against bacterial strains. The concentration range (5.0–0.008 mg/ml) of EE and BE extracts was evaluated against fungal strains. Norfloxacin and Fluconazole were dissolved in sterile distilled water and two-fold dilutions were prepared (1.0–0.002 mg/ml). One ml of each concentration was mixed with 1.0 ml of sterile peptone water (10^4 CFU/ml for bacteria and 10^3 CFU/ml for fungal concentration, obtained from a McFarland turbidity standard no. 0.5). Solvent control was prepared with DMF (10%) and blank control was prepared from virgin media. Tubes were incubated for 24 and 48 h at 37°C for bacteria and fungi, respectively. Assay was performed in replicates and the mean value of three experiments was recorded (n = 3) with standard deviation. MIC was determined as the lowest concentration that inhibits the visible microbial growth (Murthy et al., 2006; Kuta, 2008).

Table 1: MIC values (mg/ml) of the extracts of Lonicera hypoglauca Miq

<table>
<thead>
<tr>
<th>Microbial strains</th>
<th>MIC mean ± standard deviation</th>
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<tr>
<td></td>
<td>EE</td>
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<tr>
<td><strong>Gram-positive</strong></td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
<td>0.46 ± 0.14</td>
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<tr>
<td><em>alpha hemolytic streptococcus</em></td>
<td>0.87 ± 0.26</td>
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<tr>
<td><em>beta hemolytic streptococcus</em></td>
<td>0.98 ± 0.33</td>
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<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>0.53 ± 0.17</td>
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<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>0.76 ± 0.26</td>
</tr>
<tr>
<td><strong>Gram-negative</strong></td>
<td></td>
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<tr>
<td><em>Shigella flexneri</em></td>
<td>1.32 ± 0.38</td>
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<tr>
<td><em>Salmonella typhimurium</em></td>
<td>1.53 ± 0.53</td>
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<tr>
<td><em>Escherichia coli</em></td>
<td>1.23 ± 0.44</td>
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<td><em>Pseudomonas aeruginosa</em></td>
<td>1.88 ± 0.67</td>
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<tr>
<td><em>Bacillus subtilis</em></td>
<td>1.42 ± 0.41</td>
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<tr>
<td><strong>Fungi</strong></td>
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<tr>
<td><em>Candida albicans</em></td>
<td>2.69 ± 0.86</td>
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<tr>
<td><em>Candida parapsilosis</em></td>
<td>2.85 ± 0.93</td>
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<tr>
<td><em>Candida tropicalis</em></td>
<td>3.05 ± 1.03</td>
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<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>4.15 ± 1.42</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>4.86 ± 1.65</td>
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Note: Nor, Flu, standard antibiotics (Norfloxacin for bacteria, fluconazole for fungi).

Values are mean ± standard deviation of three experiments in replicate.

RESULTS AND DISCUSSION

The antimicrobial activity expressed as mg/ml, of the two extracts of the flower of Lonicera hypoglauca Miq against various strains of bacteria and fungi are summarized in Table 1. The organisms *Staphylococcus aureus* and *Streptococcus pneumoniae* were found to be most susceptible to the extract BE and EE in bacteria strains with MIC values of 0.32 ± 0.11,
The extract BE was effective against Candida albicans with MIC value of 1.96 ± 0.69. The MIC values more than 2.0 mg/ml were observed against Candida albicans and Candida parapsilosis for extract EE, and Candida parapsilosis for extract BE. The MIC values of more than 3.0 mg/ml were observed against Candida tropicalis, Trichophyton mentagrophytes and Cryptococcus neoformans. for extract BE, and Candida tropicalis for extract EE, while MIC values of the extract EE were found more than 4.0 mg/ml against Trichophyton mentagrophytes and Cryptococcus neoformans. Both the extracts were found to be less effective than the standard antibiotics used in the present study and their efficacy was least against fungi (Table 1). The experiment results suggested that the extracts have antimicrobial activities. The benefit of local application of the flower of Lonicera hypoglauca Miq as the treatment of sore throat and respiratory tract infection in Chinese could be attributed to their antimicrobial activity as observed in this study.

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