Antimicrobial assay of methanolic crude of *Lonicera lanceolata*

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The objective of this study was to collect information about medicinal plant *Lonicera lanceolata*. The antimicrobial assay of four fractions (n-hexane, chloroform, ethyl acetate and ethanol) obtained from *Lonicera lanceolata* were screened against two human Gram-positive (*Staphylococcus aureus, Micrococcus luteus*) and four Gram-negative pathogen (*Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella pneumoniae*). The antimicrobial and antifungal activities were performed by Agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by Agar well dilution method and Viable cell count method respectively. Ethyl acetate fraction exhibited maximum antibacterial activity while the other fractions that is, hexane, chloroform, ethanol also showed standard antibacterial activities. MIC and MBC were determined for ethyl acetate fraction. MICs ranged from 0.312 to >10 µg/ml and MBCs from 0.260 to 15 µg/ml. The ethyl acetate and chloroform fractions showed excellent antifungal activity while other fractions have least antifungal activity.

**Key words:** *Lonicera lanceolata*, antimicrobial activity, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

*Lonicera lanceolata* belong to a genus *Lonicera* usually found in Bhutan, Nepal, China and mountains area of Pakistan. The genus *Lonicera* belong to the family Caprifoliaceae, which have about 12 genera and 450 species (Mabberley et al., 1997), found usually in moderate region of Northern Hemisphere. Most of the plants of genus *Lonicera* are used against variety of diseases like acute fever, headache, respiratory infections (Houghton et al., 1993), antioxidant (Ali et al., 2013), cytoprotective (Chang et al., 1992), hepatoprotective (Ya-Ping et al., 1992; Shi et al., 1999), antiviral (Chang et al., 1995), antitumor (Wang et al., 2009; Yip et al., 2006) and anti-inflammatory activities (Yoo et al., 2008). Microbial assay is a principal way of deterioration of foods and is usually responsible for the loss of quality and safety of foods. The pathogenic
microorganisms in foods are increasing with the increase in occurrence of food-borne diseases. The Staphylococcus aureus is mainly involved for post-operative wound infections, toxic shock syndrome, endocarditic, osteomyelitis and food poisoning (Mylotte et al., 1987). Listeria monocytogenes is usually responsible for the harsh food-borne illness, listeriosis, which has been one of the rising zoonotic diseases during the last two decades (Farber et al., 2000). The Eschenichia coli is found in human intestines and is responsible for urinary tract infection, coleocystitis or septicaemia (Singh et al., 2000).

Many compounds were isolated previously from genus Lonicera including iridoids, bisiridoids, sulphur containing monoterpenoids, alkaloidal glycosides, triterpenoids, saponins, coumarin glycosides and flavones glycosides (Machida et al., 1995; Bailleul et al., 1981; Souzu et al., 1969; Souzu et al., 1970) which have good activities against pathogens.

The aim of this study is to investigate the antimicrobial activity of the four fractions obtained from the crude of L. lanceolata. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were also carried out.

MATERIALS AND METHODS

Plant material

The whole plant material of L. lanceolata was collected from Hazara division, in May 2012. The plant was identified by Professor Manzoor Ahmad Plant Taxonomist on the basis of its morphology in Government Degree College Abbotabad, Pakistan, where a voucher specimen was deposited in herbarium (Accession No. D-056).

Preparation of crude extracts

Ninety grams of each powdered plant material were extracted with 80% methanol by maceration for 2 days with repeated stirring and the resulting liquid was filtered with filter paper (Whatman No. 3 filter paper, Whatman Ltd., England). This process was repeated several times. The solvent was evaporated by Rota-vapor (BU’CHI Rota-vaporR-205, Switzerland) at about 50°C. The gummy filtrate was placed in an oven at 50°C for about 2 days to evaporate the water. The dehydrated mass was change into powdered, packed into a glass vial and stored in a desiccator using silica gel (leven et al., 1979).

Preparation of fractions

Extraction of about 90 g of L. lanceolata was carried out with n-hexane, chloroform, ethyl acetate and ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure and the fractions were then transferred to vacuum oven at 50°C for about one day to remove any residual solvent. The resulting semisolid mass of each fraction was stored in a desiccator using silica gel (leven et al., 1979).

Microorganisms

Six bacterial species, two human Gram-positive and four Gram-negative that is, E. coli ATCC 25922, Klebsiella pneumoniae ATCC 70603, Pseudomonon saeruginos (clinical strain/PIMS), Enterobacter cloacae (clinical strain/PIMS), S. aureus (MRSA, clinical strain/PIMS) and Microoccus luteus (clinical strain/PIMS) were used in antimicrobial test. Strains were obtained from Microbiology Research Laboratory School of Life Science Beijing University of Chemical Technology (BUCT), China and their identification and characterization were carried out. These cultures were retained on agar slants at 4°C for antimicrobial screening. Microorganisms were incubated overnight at 37°C in Mueller-Hinton Broth (Oxoid) at pH 7.4. Ofloxacin (10 μg) and Ampicillin (10 μg) (Oxoid) were used as reference antibiotics (Table 1).

Anti-microbial screening

Screening for antibacterial activity by Agar well diffusion method

The antibacterial assay was carried out by means of Agar well diffusion method (Hadacek et al., 2000). All bacterial strains were first grown-up in nutrient broth at 37°C for 22-24 h in incubator till turbidity that became comparable to McFarland 0.5 turbidity standard was obtained. The inocula of the relevant bacteria were streaked on to the Muller Hinton Agar (oxoid) plates using a disinfected swab in order to confirm a uniform thick lawn of growth following incubation. Wells of 6 mm in diameter were produced on to nutrient agar plates by using a germ-free cork borer. The wells were filled with the given test agents (100 μl) and the plates were then allowed to keep on for 1:30 h at 25°C. At last, the plates were incubated at 37°C for 22-24 h and the resulting diameters of zones of inhibition were measured.

Determination of minimum inhibitory concentration (MIC)

Agar dilution method

Minimum inhibitory concentration of the crude extracts was found out by agar dilution method (EUCAST Definitive Document., 2000; Mukherjee, 2002; Anon., 2000). The sterilized Muller Hinton Agar (oxoid) was retained to cool to 50°C and approximately 20 ml of this was added to clean test tubes which contained 1 ml of dissimilar concentration of crude extract. This mixture was thoroughly mixed and poured into pre-labelled disinfected Petri Dishes. Petri dishes having only growth media were prepared in the same way so as to serve for comparison with Petri plate including crude extract. The concentrations of the extracts used in this test ranged from 0.312 μg/ml to 2000 μg/ml. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standards were inoculated on to the chain of agar plates using standard loop. The plates were then incubated at 37°C for 22-24 h. The lowest possible concentration which inhibited the growth of the respective organisms was taken as MIC. All tests were done in triplicate.

Determination of minimum bactericidal concentration (MBC)

Viable cell count method

Minimum bactericidal concentration of the L. lanceolata was calculated by the viable cell count method (Toda et al., 1989; Anon., 2003), and the results were written as number of viable cells as a percentage of the control.
Table 1. Zone of inhibition of reference antibiotics.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Ec</th>
<th>Kp</th>
<th>Ps</th>
<th>Ent</th>
<th>Mi</th>
<th>Sta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>15.1(±0.02)</td>
<td>14(±0.01)</td>
<td>11.9(±0.01)</td>
<td>13(±0)</td>
<td>10(±0.11)</td>
<td>9.6(±0.05)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>14.1(±0.05)</td>
<td>12.3(±0.5)</td>
<td>11(±0)</td>
<td>14(±0.11)</td>
<td>13(±0.05)</td>
<td>14(±0.05)</td>
</tr>
</tbody>
</table>

Ec: *Escherichia coli*; Kp: *Klebsiella pneumoniae*; Ps: *Pseudomonas aeruginosa*; Ent: *Enterobacter cloacae*; Mi: *Micrococcus luteus*; Sta: *Staphylococcus aureus* (methycillin resistant); mm: millimeter.

Table 2. Inhibition zones of *Lonicera lanceolata* plant extracts.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Ec</th>
<th>Kp</th>
<th>Ent</th>
<th>Ps</th>
<th>Mi</th>
<th>Sta</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-hexane</td>
<td>2(±0.4)</td>
<td>6(±0.3)</td>
<td>6(±0)</td>
<td>3(±0.3)</td>
<td>2(±0.7)</td>
<td>5(±0.4)</td>
</tr>
<tr>
<td>Chloroform</td>
<td>8(±0.4)</td>
<td>6(±0.6)</td>
<td>11(±0.4)</td>
<td>7(±0.4)</td>
<td>7(±0.5)</td>
<td>9(±0.2)</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>14(±0.3)</td>
<td>14(±0.4)</td>
<td>12(±0.2)</td>
<td>11(±0.2)</td>
<td>13(±0.5)</td>
<td>15(±0.5)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2(±0.2)</td>
<td>4(±0.1)</td>
<td>6(±0.2)</td>
<td>4(±0.6)</td>
<td>4(±0.3)</td>
<td>3(±0.5)</td>
</tr>
</tbody>
</table>

Ec: *Escherichia coli*; Kp: *Klebsiella pneumoniae*; Ps: *Pseudomonas aeruginosa*; Ent: *Enterobacter cloacae*; Mi: *Micrococcus luteus*; Sta: *Staphylococcus aureus* (methycillin resistant); mm: millimeter.

Screening for antifungal activity

These fractions were screened against *Aspergillus niger*. The required quantity of each fungal culture was placed in 2 ml of sabauraud dextrose broth. This suspension was constantly spread on Petri plates containing sabauraud dextrose agar media using germ-free swabs. Samples were applied into wells using same technique for bacteria and incubated at room temperature for 3 days. The plates were checked for the presence of zones of inhibition and the results were recorded. Itraconazole was used as a positive control.

RESULTS

Almost all fractions of *L. lanceolata* presented hopeful activity against both Gram positive and negative bacterial pathogens. Ethyl acetate fraction has high antibacterial assay as compared to other fractions which is cleared from Table 2 and Figure 1. Therefore it was further considered for determination of MIC and MBC, respectively. The MIC values ranged from 0.312 to > 10 µg/ml for all tested strains while the MBC values reported were so many times higher than MIC (Table 3), (Figure 2). The MBC value for *K. pneumoniae* was not detected.

In the similar way nearly same pattern of defencelessness was examined against fungal strain *A. niger*. The maximum antifungal activity was presented by chloroform and ethyl acetate fractions. Ethanol and n-hexane fractions have lowest antifungal activity which is represented in Table 4 and Figure 3.

DISCUSSION

The antimicrobial activities of four fractions obtained from the crude of *L. lanceolata* were tested against six bacteria species *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *S. aureus*, and *M. luteus*. Almost all fractions exhibited more or less antimicrobial activity against the test cultures. Ethyle acetate fraction exhibited best activity against these bacteria. Besides that, chloroform, n-hexane and ethanol fractions have lowest activity against these bacteria. Ethyle acetate and chloroform fractions obtained from the crude of *L. lanceolata* were primarily reported as active against the *A. niger*.

The MIC of the ethyl acetate fraction was taken as 0.312 µg/ml. It is important that the MIC value is too high to be taken in susceptible ranges (Paul et al., 2006). The MBC value of ethyl acetate fraction is many times higher than MIC. The antibacterial and antifungal assays were carried out by Agar well diffusion method. The MIC was carried out by using Agar well diffusion method while MBC was performed by viable cell count method. The MBC values for *K. pneumoniae* were not detected.

This investigation is probably the first to exhibit the antimicrobial assay of four fractions obtained from crude of *L. lanceolata*. As a comprehensive literature review to the best of our knowledge there is no information about the antimicrobial activity of these four fractions obtained from this plant.

Conclusion

It is clear from the present studies that four fractions were obtained from the crude of *L. lanceolata* which shows prominent antimicrobial activity. The ethyl acetate fraction showed highest antimicrobial activities. The MIC of ethyl acetate fraction was taken as 0.312 µg/ml. More
Figure 1. Inhibition zones of *Lonicera lanceolata* plant extracts. *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae*, *M. luteus*, *S. aureus*.

Table 3. MIC and MBC of *Lonicera lanceolata* ethyl acetate fraction.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (µg/ml)</th>
<th>MBC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>&gt;10</td>
<td>14</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>&gt;10</td>
<td>N.d</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>&gt;10</td>
<td>12</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td><em>M. luteus</em></td>
<td>0.625</td>
<td>1.877</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>0.312</td>
<td>1.260</td>
</tr>
</tbody>
</table>

N. d = not detected.

Figure 2. MIC and MBC of *Lonicera lanceolata* of ethyl acetate fraction.
investigations are mandatory to expose the hidden medicinal importance of plant *L. lanceolata*.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


