Antibacterial potential of *Skemmia laureola* methanolic extract

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In the present research, an attempt was made to explore the therapeutic potential of *Skimmia laureola*. The crude methanolic extract of *Skimmia laureola* leaves was evaluated for its antibacterial activity against gram positive (*Staphylococcus aureus*) and gram negative (*Agrobacterium tumefaciens*) bacteria. *Skimmia leureola* extract at different concentrations (60 mg/ml, 90 mg/ml, 120 mg/ml and 150 mg/ml) exhibited significant inhibitory effect against both *Staphylococcus aureus* and *Agrobacterium tumefaciens*. Comparing the plant extract results with standard antibiotic, lincomycine and ceftrixone, both standards have greater zone of inhibition against the gram positive and gram negative bacteria. In present antimicrobial investigation, it was found that gram positive (*Staphylococcus aureus*) were more sensitive than gram negative bacteria (*Agrobacterium tumaefaciens*) for *Skimmia laureola*, lincomycine and ceftrixone.

**Keywords:** *Skimmia laureola*, antibacterial action, *Staphylococcus aureus*, *Agrobacterium tumefaciens*

**INTRODUCTION**

Medicinal plants are best source to obtain different drugs. About 80\% of people in industrialized countries use traditional medicines, which are derived from medicinal plants (Ahmad et al., 2011). In developing countries, more than 40\% of the people are put to death due to infection of the microorganism, besides this is also spoilage of food materials due its pathogenicity (Marino et al., 2001). In the modern antibiotic formation the plants is used as raw material and the antibiotic also depends upon the medicinal plants and from many years the antibiotic depends exclusively on leaves, flowers and bark of the plants, but in some cases the roots are also used. In some plants the carbon copies of chemicals is also identified in the plants. The world health organization also accepted the importance of the plants in medicines. According to WHO the medicinal plant is that plant in which one or more organs contain any substance which can be used for therapeutic purposes or which is useful for the synthesis of drugs (Junaid et al., 2006). Previous studies have concluded the valuable aspects of plant derived drugs as
excellent resource of antibiotics, antioxidants and anti-inflammatory agents (Mathur et al., 2011). The present work was designed with aim to investigate the antibacterial activity of *Skimmia laureola* plant and its comparison with antibiotics.

**MATERIALS AND METHODS**

**Plant material and extraction**

Leaves of *Skimmia laureola* were collected from Thandiani (Abbottabad) and identified by a taxonomist Dr. Manzoor Hussain, Department of Botany, Govt. Postgraduate College Abbottabad, KPK, Pakistan. After cleaning of adulterant material, the leaves rinsed with distilled water and kept under shade till drying and then weighed. Extraction from leaves was taken by simple maceration process. The leaves were taken and grinded up to powder form by kitchen blender, and then deepen in the methanol. This poorly homogenized mixture was kept for 4 weeks under the shade at room temperature (25°C±2°C”) in extraction bottle. After 4 weeks maximum amount of methanol was separated from the mixture, filtrate was filtered twice, first using the ordinary filter paper and then Watt’s man No. 4 filter paper. The remaining methanol was then completely evaporated by vaparotator (rotary) at a temperature of 60°C and at a rotation of 80 rpm to obtain the *Skimmia laureola* crude extract.

**Preparation of samples**

150 mg of *Skimmia laureola* methanolic extract was dissolved in 1 ml of dimethyle sulfoxide (DMSO). This stock solution was used for further dilution with DMSO i.e. 120 mg/ml, 90 mg/ml and 60 mg/ml. Lincomycine 400 mg and ceftixione 500 mg was also diluted as i.e. 2 mg/ml in the DMSO for comparative study.

**Isolation of Staphylococcus aureus**

*Staphylococcus aureus* is gram positive bacteria and is normal flora of skin. Sterilized cotton swab was rubbed with neck and then spread on the nutrient agar media. The plates were incubated at 35°C for 24 hrs. All colonies were screened for gram positive bacteria *Staphylococcus aureus*.

**Isolation of Agrobacterium tumefaciens**

*Agrobacterium tumefaciens* is gram negative bacteria which causes crown gall diseases in the plants (plant cancer) was also isolated from the infected parts of *Rosa indica* family *Rosaceae*, number of infected plants parts were cut and reis with distilled water and then dropped into the sterilized water for 3 hrs, then transferred the culture from water to the carrot discs in a sterile dishes in filter paper and incubated at a temperature of 25°C for 3 weeks. Only those samples were taken which cause gall formation. The culture from gall was taken by wire loop and streaked on the yeep agar media and incubated at 25°C for 24hrs. The culture from the yeep agar media was transferred to yeep broth media and incubated at 25°C for 24hrs. Then by the spread plate technique 0.01 ml of culture broth was spread on yeep agar media. All the colonies were screened for Agrobacterium tumefaciens.

**Inoculums preparation for Staphylococcus aureus**

The bacterial strain from 24 hrs old culture were streaked on the same fresh culture media and incubated for 24 hrs. Then a loop bacterial culture was formed. The streak plate was transferred to nutrient broth and incubated for 24 hrs at 35°C at 120 rpm and then 0.01 ml of the broth culture was spread on the nutrient agar and observed the number of colony forming unit (CFU).

**Inoculums preparation for Agrobacterium tumefaciens**

The bacterial strain from 24 hours old culture was streaked on the same fresh culture media and incubated for 24 hrs. Then a loop bacterial culture was formed. The streak plate was transferred to nutrient broth and incubated in shaking incubator for 24 hrs, 25°C at 210 rpm and then 0.01 ml of broth culture was spread on the yeep extract medium and observed the CFU.

**Disc diffusion method**

The antimicrobial assay was performed by using the disc diffusion method. Actively growing cell of both the strains were spread by spread plate technique with the help of micropipette. Culture of 0.01 ml was spread on the nutrient agar medium plates. Subsequently, filter paper discs (6 mm diameter) saturated with plant crude extract/antibiotic was placed on the surface of each inoculated nutrient agar medium. After placing the discs the plates were incubated at 35°C for 24 hrs. Then the zone of inhibition around the discs was measured (Rodrigues et al., 2008).

**Agar well diffusion method**

The antibacterial activity was also performed by using agar well diffusion method. The agar plates were prepared by using nutrient agar (Merck). The microorganism culture was evenly spread on the surface of agar plates by sterile swab sticks. Four wells (5 mm in diameter) were made in each plate with the help of sterile cork borer. The plant extracts/antibiotic were added in each well and incubated at 35°C for 24 hrs. After incubation the plates were observed for the presence of bacterial growth and for the
Table 1. Antibacterial effect of different concentrations of Skimmia laureola extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc. mg/ml</th>
<th>Staphylococcus aureus</th>
<th>Agrobacterium tumefaciens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60 mg/ml</td>
<td>12 mm</td>
<td>10 mm</td>
</tr>
<tr>
<td>2</td>
<td>90 mg/ml</td>
<td>15 mm</td>
<td>13 mm</td>
</tr>
<tr>
<td>3</td>
<td>120 mg/ml</td>
<td>30 mm</td>
<td>16 mm</td>
</tr>
<tr>
<td>4</td>
<td>150 mg/ml</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Zone of inhibition (mm) after 24hrs.

measurement of zone of inhibition around the well. The size of zone of inhibition was measured in millimeters for antibacterial activity (Mathab et al., 2006).

Media Supplementation method

In media supplementation method, the pure crude extract of each plant was mixed in the sterilized yeep agar media. After sterilization the pure crude extracts 150 mg were mixed in yeep agar media. Then both the strains were spread from the culture with the help of cotton swab on the plates and incubated at 35C˚ for 24 hrs and the growth of microorganisms was measured.

Pouring of test solution, incubation and measurement the zone of inhibition

The test solution was poured by micropipette in the respective wells, and in case of disc diffusion, four concentrations of the extracts (150 mg/ml, 120 mg/ml, 90 mg/ml and 60 mg/ml) for positive control (lincomycine and ceftrixone) were applied to plates. The plates were incubated at 35C˚ for Staphylococcus and 25C˚ for Agrobacterium. After 24 hrs of incubation the diameter of zone of inhibition around the well and disc was measured. Antibacterial activities of all dilution of extracts were determined against the two strains of the bacteria.

RESULTS

The methanolic extract of Skimmia laureola leaves was tested against the Staphylococcus aureus and Agrobacterium tumefaciens. The extract of the concentration at 60 mg/ml showed, 12 mm and 10 mm zone of inhibition against Staphylococcus aureus and Agrobacterium tumefaciens respectively. The extract at the concentration of 90 mg/ml displayed 15 mm and 13 mm zone of inhibition against Staphylococcus aureus and Agrobacterium tumefaciens respectively. The extract at 120 mg/ml concentration showed the 30 mm against Staphylococcus aureus and 16 mm zone of inhibition against the Agrobacterium tumefaciens. At 150 mg/ml concentration, the Skimmia laureola extract fully inhibited the growth (media supplementation) as shown in Table 1. Lincomycine have 20 mm zone of inhibition against the gram positive bacteria, Staphylococcus aureus and 20 mm against the gram positive bacteria, Agrobacterium tumefaciens. Ceftrixone have the zone of inhibition 32 mm against the gram positive bacteria and 25 mm against the gram negative bacteria. By comparing the zone of inhibition of Skimmia laureola with the zone of inhibition of Standard antibiotics, it becomes cleared that the Skimmia laureola have also high activity against the gram positive bacteria and low activity against the gram negative bacteria, which is similar to the standard antibacterials (Figure 1).

DISCUSSION

The agar well diffusion method is commonly used for the screening of antimicrobial activities. In this study we have used the agar well diffusion method for the determination of antimicrobial activity of medicinal plants (Doughari and Manzara, 2008; Share et al., 2008). Disc diffusion method is also usually for antibacterial activity of bacteria and for the antibiotic comparison with extracts. In this study the disc diffusion method for determining the antimicrobial activity of methanolic extracts of a medicinal plant was observed. Similar technique of the disc diffusion method has been used by other researchers (Jonathan et al., 2000; Doughari and Okafor, 2008; Maksimovic et al., 2008). The media supplemented with extracts is also the best method for evaluation of antimicrobial activity of methanolic extracts of the plant material. The culture medium used in the study was the nutrient agar which is also used for culturing routine pathogens. This medium is also suitable for the growth of the pathogens used in this study i.e. Staphylococcus aureus. The yeep medium was also used in the study, was the culturing medium for the Agrobacterium tumefaciens. Skimmia laureola plant show good activity against gram positive (Staphylococcus aureus). The zone of inhibition of the activity on plant
extracts was also different among the gram negative and gram positive as well as the zone of inhibition the *Skimmia laureola* was 30 mm against the gram positive bacteria (*Staphylococcus aureus*). On the other side, gram negative bacteria (*Agrobacterium tumefaciens*) the zone of inhibition of *Skimmia laureola* was 10 mm. Therefore, it is clear that the gram positive bacteria are more sensitive than the gram negative bacteria. The antibiotic zone of inhibition was also differ among the gram positive and gram negative bacteria i.e. the zone of inhibition of lincomycin (400 mg) against the gram positive bacteria was 30 mm and of the ceftrixone (500 mg) was 32 mm, and against the gram negative bacteria lincomycin 20 mm and ceftrixone 25 mm zone of inhibition. From this comparison it becomes clear that the gram positive bacteria *Staphylococcus aureus* is more sensitive than the gram negative bacteria, *Agrobacterium tumefaciens*.

REFERENCES


