Chapter XI

Antimicrobial Screening
ANTIMICROBIAL SCREENING

Present investigation describes the antimicrobial activity of leaf extracts of **Clerodendrum phlomidis**, **Lycium babarum** and **Sida cordifolia** against two bacteria (one gram positive and one gram negative) and one fungal pathogen.

**Materials and Methods**

Freshly collected leaves of the selected plant species were used in the present investigation collected from study area.

**Test Organisms**

All the test organisms were clinical isolates obtained from different patients diagnosed as having bacterial or fungal infections were procured from Department of Microbiology and Immunology, Sardar Patel Medical College, Bikaner, India. The Micro-organisms used for screening were **Staphylococcus aureus** (Gram positive), **Escherichia coli** (Gram negative) and a fungal pathogen, **Candida albicans**. The bacterial cultures of **S. aureus** and **E. coli** were maintained on nutrient broth (10% peptone, 0.5% labanco and 0.5% NaCl, pH adjusted to 7.5) whereas **C. albicans** on Sabouraud liquid medium (1% peptone, 4% glucose, adjusted to pH 5.8). These micro-organisms were allowed to grow at 35-37°C. The inoculum which was used for screening studies was prepared by adjusting the concentration of microorganisms in the medium using a spectronic-20 colorimeter (Bausch and Lomb) set at 630 nm. Forty per cent transmittance was used in case of **S. aureus** and **E. coli** and 65% transmittance in case of **C. albicans**.
The Reference Antibiotic Discs

The antibiotics known to be effective against each of the test microorganisms in their established doses were used as references for comparison of the antimicrobial activity of the test samples. These were chloramphenicol (30 μg) for *S. aureus* and *E. coli*, pencillin (10 units) for *S. aureus*, streptomycin (10 μg) for *E. coli* and mycostatin (100 units) for *C. albicans*.

Testing for Antimicrobial Activity

Petri plates were rinsed with sterile distilled water, dried rapped in tinfoils and sterilized in an oven at 100°C for 18 hour. Each of the sterilized petri plates was then preceded with 10 ml of the growth medium (Khanna and Staba, 1968; Khanna *et al.*, 1971), 4.0 ml of the inoculum in case of bacteria (*S. aureus* and *E. coli*) and 6.5 ml of the inoculum in case of *C. albicans*. Each of the mixtures was thoroughly shaken to ensure uniform distribution of the inoculum. In total 110 petri plates were employed out of which 90 were used as test petri plates (5 replica for each micro-organisms, 15 for each plant species) and 20 as control petri plates (5 replica for each micro-organisms, common for all the three plant species).

Paper discs measuring 6 mm diameter which absorbed about 0.1 ml of the test samples, (extract in ethyl ether and 50% ethanol) were employed for the screening purpose. Thus each of the test petri plates contained paper disc (s) of the reference antibiotic (s) of desired dose, paper disc of the test sample
in 50% ethanol, paper disc of the test sample in ethyl ether.

Petri plate containing the paper discs (6 mm diameter) dipped in ethyl ether and 50% ethanol were run as controls.

All the test and the control petri plates were kept at 5°C for 45-55 minutes so as to allow the diffusion of the substances and then were incubated at 35-37°C for 18 hours.

The inhibition zones formed by the test samples were measured and compared with those of the standard reference antibiotic discs as given below:

**Results and Discussion**

Antimicrobial screening of ethyl ether and alcoholic (50% ethanol) extracts of leaves of three plant species i.e. *Crotalaria burhia, Euphorbia caducifolia* and *Leptadenia pyrotechnica* showed positive reactions against all the three test organisms.

Maximum antimicrobial activity was exhibited by the leaves extracts (alcoholic extract) of *Euphorbia caducifolia* against *Escherichia coli*, whereas leaves extracts of *Leptadenia pyrotechnica* showed maximum antifungal activity against *Candida albicans* (Table- 11.1).

A number of plants have been screened for their antimicrobial activity. The antimicrobial principles and their distribution have extensively been reviewed by Skinner (1955) followed by Nickell (1959) who surveyed 174 plants belonging to 157 families of vascular plants. Su Lee *et al.* (1972) have screened aquatic plants from Minnesota for their antimicrobial property and
have found many plants active against selective microorganisms.

In vitro antimicrobial activity of *Acacia arabica* was reported by Chandel *et al.* (1993). Antifungal activities from leaves of *Acacia nilotica* against *Pythium apheridermatum* have been reported by Khan *et al.* (1996). The aerial parts of *Pulicaria crispa*, growing wild in Saudi Arabia, a volatile oil were extracted. The activity of the oil on several microorganisms (*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris* and *Candida albicans*) was investigated (Hassan *et al.*, 1989).

Harsh (1982) and Kapoor (1991) observed antimicrobial activity of plant parts and tissue cultures of some medicinal arid zone plants of western Rajasthan.

Akhtar *et al.* (1997) reported the antimicrobial activity of plant decoctions against *Xanthomonas campestris* on detached citrus leaves. Antibacterial activity of plant diffusate against *Xanthomonas campestris* is also reported by Akhtar *et al.* (1997). Turkusay and Onogal (1998) studied the antifungal effects of leaf extracts of *A. sativa, X. strumarium, F. carica, N. tabacum* and *D. stramonium* against *Alternaria alternata, A. solani* and *Botrytis cinerea*.

The antimicrobial activity from twigs and leaves of four Chilean species of *Pseudognaphatium; P. robustum, P. heterotrichium, P. viravira* and *P. cheiranthifolium* were studied by Mendoza *et al.* (1997). Tereschuk *et al.* (1997) studied antimicrobial activity of the leaves of *Tagetes minuta*. 
Antimicrobial activities of extracellular secondary metabolites of local and international Fusarium species were screened by Mandeel et al. (1999). Antimicrobial activity and flavonoids from leaves and flowers of *Adhatoda vasica* Nees was observed by Ahmed-El-Sawi et al. (1999).

Khan et al. (2001) studied the antimicrobial activity from methanolic extracts of *Castanopsis acuminatissima* leaves, stem and root barks. Ethanolic extracts of 45 Indian medicinal plants traditionally used in medicine were studied for their antimicrobial activity against certain drug resistant bacteria and a yeast *Candida* (Ahmed and Beg-Arina, 2001). Kostava (2001) studied the antimicrobial activity and isolate the flavonoid contents from the bark, leaves and flowers of *Fraxinus ornus*.

Shahid (2002) studied the antimicrobial activity of leaves and flowers of *Acacia nilotica*, *Acacia senegal*, *Maytenus emarginata*, *Parkinsonia aculeata* and *Prosopis cineraria* against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Ranga (2002) studied the antimicrobial activity of leaves of *Pulicaria crispa* and *Xanthium strumarium* against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Sarda and Rao (2003) investigated that essential oil of *Lippia nodiflora* showed more antimicrobial activity against *E. coli*, *Streptococcus lactis*, *Bacillus subtilis* and *Lactobacillus bulgaricus*.

Bahaduriya and Kumar (2004) studied the antimicrobial activities of
aerial parts of *Lawsonia alba*, *Solunum dulcamara* and *Allium sativum* against *Escherichia coli*, *Enterobacter aerogens*, *Proteus mirabilis* and *Staphylococcus aureus* and found that extracts of *Lawsonia alba* shown maximum antimicrobial activity.

Mishra and Mishra (2004) evaluated that out of bark oil and leaf oil of *Cinnamomum zeylanicum*, bark oil was found to have more antimicrobial activity against *E. coli*, *Streptococcus aureus*, *Salmonella senftenberg* and *Vibrio cholerae*.

Khatri (2005) observed antimicrobial activities against some fungal and bacterial pathogens using leaf extracts of some medicinal plants like *Abutilon indicum* and *Solanum nigrum* collected from Hanumangarh district.

Rao (2007) has observed the antimicrobial activities against some fungal and bacterial pathogens from some arid zone plants.

Singh (2008) observed antimicrobial activities against some fungal and bacterial pathogens using leaf extracts of some medicinal plants like *Achyranthes aspera*, *Cocculus pendulus* and *Phyllanthus niruri* collected from Jhunjhunu district.

Kapoor *et al.* (2011) studied the antimicrobial activity of leaves extracts of some Tiliaceous plant species like *Corchorus depressus*, *Corchorus tridens* and *Grewia tenax* of arid region of Rajasthan.

Kapoor and Mishra (2013) studied the antimicrobial activity of some arid herbal Plants of Rajasthan.
Kapoor and Mishra (2013) studied the antimicrobial activity of leaves extracts of some Capparidaceous medicinal plants like *Capparis decidua, Cleome gynandra* and *Cleome viscosa* of North-west Rajasthan.

Kapoor and Pandita (2013) studied the antimicrobial activity of leaves extracts of some exotic tree species like *Colophospermum mopane, Holoptelea integrifolia, Kigelia pinnata* and *Putranjiva roxburghii* growing in Rajasthan desert.

Kapoor and Purohit (2013) studied the antimicrobial activity of leaves extracts of some Fabaceous plant species like *Clitoria ternatea, Sesbania bispinosa* and *Tephrosia purpurea* of Rajasthan desert.

Kapoor *et al.* (2013) studied the antimicrobial activity of leaves extracts of some medicinal plants like *Achyranthes aspera, Cocculus pendulus* and *Phyllanthus niruri* collected from Jhunjhunu district.

Kapoor and Bansal (2013) studied the antimicrobial activity of leaves extracts of some medicinal tree species like *Acacia tortilis, Prosopis cineraria, Salvadoria persica* and *Tecomella undulata* growing in Nagaur district of Rajasthan.

Kapoor *et al.* (2013) studied the antimicrobial activity of leaves extracts of some medicinal plants like *Crotalaria burhia, Euphorbia caducifolia* and *Leptadenia pyrotechnica* growing in Jaisalmer district of Rajasthan.

Kapoor and Lakhera (2013) studied the antimicrobial activity of leaves extracts of some medicinal plant species like *Euphorbia hirta, Glinus lotoides*
and *Rhynchosia minima* collected from Jodhpur district of Rajasthan.

Kapoor *et al.* (2014) studied the antimicrobial activity of leaves extracts of some medicinal tree species like *Ailanthus excelsa*, *Pongamia pinnata* and *Salvadora oleoides* collected from Sikar district of Rajasthan.

Kapoor and Kumar (2014) recently studied the antimicrobial activity of leaves extracts of some medicinal tree species like *Butea monosperma*, *Cassia fistula* and *Madhuka indica* collected from Sirohi district of Rajasthan.

The present study indicates that these plant species have definitely some secondary products which are responsible for antibacterial and antifungal activity. The activity of all these test extracts against both bacterial and fungal pathogen thus show that arid zone plants are more resistant to bacterial and fungal attacks due to the presence of some biologically active substances.
Table- 11.1
Antimicrobial Screening of Leaf Extracts of Selected Plant Species and Standard Reference Antibiotics

<table>
<thead>
<tr>
<th>Plants</th>
<th>Leaves extracts</th>
<th>Test Organisms</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
<td>S. aureus</td>
<td>C. albicans</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/C^a</td>
<td>I/P^a</td>
<td>I/C^a</td>
<td>I/S^a</td>
</tr>
<tr>
<td>Clerodendrum</td>
<td>Ether extract</td>
<td>1.01</td>
<td>1.62</td>
<td>0.64</td>
<td>0.62</td>
</tr>
<tr>
<td>phlomidis</td>
<td>Alcoholic extract</td>
<td>0.44</td>
<td>0.66</td>
<td>0.62</td>
<td>0.66</td>
</tr>
<tr>
<td>Lycium</td>
<td>Ether extract</td>
<td>0.50</td>
<td>0.90</td>
<td>1.56</td>
<td>1.14</td>
</tr>
<tr>
<td>babarum</td>
<td>Alcoholic extract</td>
<td>1.00</td>
<td>1.86</td>
<td>0.85</td>
<td>0.59</td>
</tr>
<tr>
<td>Sida</td>
<td>Ether extract</td>
<td>1.10</td>
<td>0.88</td>
<td>0.91</td>
<td>0.86</td>
</tr>
<tr>
<td>cordifolia</td>
<td>Alcoholic extract</td>
<td>0.82</td>
<td>0.64</td>
<td>0.87</td>
<td>0.77</td>
</tr>
</tbody>
</table>

^a Ratio of diameters of the inhibition zone to extracts (10 μg) under observation (I) and diameter of inhibition zone due to standard reference antibiotics.

C= Chloramphenicol (30 μg) against S. aureus = 30 mm and E. coli = 32 mm.
P= Penicillin (10 units) against S. aureus = 32 mm.
S= Streptomycin (10 μg) against E. coli = 20 mm.
M= Mycostatin (100 units) against C. albicans = 32 mm.
Plate 11.1 : Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Clerodendrum phlomidis* against *Escherichia coli*

A = Alcoholic extract disc
E = Ethyl ether extract disc
C = Chloremphenicol (30 µg)
S = Streptomycin (10 µg)

Plate 11.2 : Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Clerodendrum phlomidis* against *Staphylococcus aureus*

A = Alcoholic extract disc
E = Ethyl ether extract disc
C = Chloremphenicol (30 µg)
P = Penicillin (10 Units)

Plate 11.3 : Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Clerodendrum phlomidis* against *Candida albicans*

A = Alcoholic extract disc
E = Ethyl ether extract disc
M = Mycostatin (100 units)
Plate 11.4: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Lycium babarum* against *Escherichia coli*

A = Alcoholic extract disc
E = Ethyl ether extract disc
C = Chloremphenicol (30 μg)
S = Streptomycin (10 μg)

Plate 11.5: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Lycium babarum* against *Staphylococcus aureus*

A = Alcoholic extract disc
E = Ethyl ether extract disc
C = Chloremphenicol (30 μg)
P = Penicillin (10 Units)

Plate 11.6: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Lycium babarum* against *Candida albicans*

A = Alcoholic extract disc
E = Ethyl ether extract disc
M = Mycostatin (100 units)
Plate 11.7: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Sida cordifolia* against *Escherichia coli*  
A = Alcoholic extract disc  
E = Ethyl ether extract disc  
C = Chloremphenicol (30 μg)  
S = Streptomycin (10 μg)

Plate 11.8: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Sida cordifolia* against *Staphylococcus aureus*  
A = Alcoholic extract disc  
E = Ethyl ether extract disc  
C = Chloremphenicol (30 μg)  
P = Penicillin (10 Units)

Plate 11.9: Test plate showing inhibition zones due to antimicrobial activity of leaves extracts of *Sida cordifolia* against *Candida albicans*  
A = Alcoholic extract disc  
E = Ethyl ether extract disc  
M = Mycostatin (100 units)