Chapter 6

Studies on the antibacterial activity of *Swertia petiolata* and *Swertia tetragona*

6.1. Introduction

Traditional medicines hold a great promise as a source of easily available effective therapy for skin diseases to the people, particularly in tropical developing counties, including India. These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis, leprosy etc (Zahra et al., 2000, Cordova et al., 2002 and Harrison & Dorothy. 2003).

Nowadays using antibiotics to subside infection produces adverse toxicity to host organs, tissues and cells. The toxicity produced by the antimicrobial agents can be cured or prevented or antagonized with herbs (Lin and Song, 1989). Herbal molecules are safe, will overcome the resistance produced by the pathogens since they are in combined form or in pooled form of more than one molecule in the protoplasm of the plant cell. Some herbs have antibacterial and antifungal properties which will be useful to clinical use (Kalemba and Kunicka, 2003). Some *in vitro* studies have been conducted that herbal oral liquids can be given to clinical drug resistant strains and different serotype strains of infection (Lu *et al.*, 2002).

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms (Harbottle *et al.*, 2006). The past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coats *et al.*, 2002). For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against multi drug resistant microbe strains (Braga *et al.*, 2005). Many plants have been found to cure...
urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Brantner, 1994; Somchit et al, 2003). According to the WHO, medicinal plants would be the best source for obtaining variety of drugs (Santos et al. 1995). These evidences contribute to support and quantify the importance of screening natural products. The aim of the present study was to investigate the antibacterial activity of the two Swertia species against some common gram positive and grand negative pathogens.

Swertia species, as discussed in chapter-2, are used in several countries for treatment of a variety of diseases including the bacterial infections (Pant et al., 2000; Menkovic et al., 2002; Basnet et al., 1994). About 108 xanthones, 10 flavinoids, 5 alkaloids, 16 irridoids and seco-irridoid glycosides, some lignans, lactones and several other compounds have been identified in these species. Among these compounds, swertianolin, norswertianolin,swerchirin, amarogentin and swertiamarin have been described to possess antimicrobial activity (Ramesh et al., 2002; Leslie and Chungath, 1987; Sedoaway et al., 1989; Ikram and Haq, 1980). For example, Swertia purpurascens has been found to possess antibacterial activity, Swertia chirata has been reported to be effective against both Gram-positive and Gram-negative bacteria (although the activity was shown to be more towards Gram-positive bacteria), and Swertia corymbosa has been observed to inhibit the growth of Staphylococcus aureus and Salmonella typhi. Swertia petiolata is locally also used in ophthalmic infections and relief of pain. Traditionally its dried rhizome is mixed with milk and poured into affected eyes for pain relief. A herbal antiseptic and antifungal veterinary ointment “Melicon V” is prepared from Swertia chirata. The antimicrobial activity has been attributed to xanthones and their D-glucosides found in these species. Structures of some of these compounds reported for antibacterial activity have been shown in Figure 6.1.

![Figure 6.1: Showing structures of swerchirin, swertiamarin, α-mangostin and gartanine](image)

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Despite a substantial volume of data available to support the antibacterial activity of *Swertia* species, the *Swertia* species found in the Kashmir valley (*Swertia petiolata* and *Swertia tetragona*) have not been investigated for the antibacterial effect. In this study, we tested the antibacterial activity of *Swertia petiolata* and *Swertia tetragona* in selected Gram positive and Gram-negative bacteria.

### 6.2. Preparation of extract

Rhizomes of *Swertia petiolata* and the whole plant of *Swertia tetragona* were shade dried, and powdered. The powdered herbs were first soxhlet extracted with petroleum-ether (60:80) for 3 days and subsequently using chloroform and hydro-methanol (20-80). The extracts were dried under reduced pressure using a rotary flash evaporator. The percent yield was 5.8, 3.5 and 15.3% in case of *Swertia petiolata* for the above mentioned extracts respectively and 4.4, 3.2, and 14.2 in case of *Swertia tetragona*. The extracts were stored in airtight containers in refrigerator.

### 6.3. Bacterial culture

The bacterial cultures were grown on nutrient agar. The agar plates were earlier sterilized in an autoclave and then stored at 4°C in sterile conditions. *Bacillus subtilis*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* were cultured in appropriate broths at 30°C overnight.

### 6.4. Antibacterial assay

The extracts prepared from *Swertia* were dissolved in dimethyl formamide:water (0.5 ml :4.5 ml), which was earlier evaluated and ascertained not to interfere with the assay. The filter paper discs (Whatman No.1 filter paper discs φ, 6mm), were dipped in respective extracts (concentration of each extract 3mg/ml) and dried. Soaked and dried discs were placed on the prepared Petri plates (previously inoculated agar) and incubated at 37°C for 24 hours. Clear inhibition zones around the discs indicated antibacterial activity. The assay was carried out in
triplicate. The strength of activity was classified as strong for inhibition zone diameters (inhibition diameter) ≥ 15.0 mm, moderate for diameters ranging from 10-14.5 mm and weak for diameters < 10 mm. For comparative studies cefutaxim and amoxicillin in a concentration of 10 μg/ml were used as standard antibacterial agents. Detailed method of analysis has been given in chapter-3.

6.3. Results

The zones of inhibition after 24 h incubation were measured. Data have been shown in tables 6.1 and 6.2. The hydro-alcoholic extract prepared from *Swertia petiolata* and *Swertia tetragona* showed strong antibacterial activity against all the strains of Gram-positive and Gram-negative bacteria. Petroleum ether and chloroform extracts showed only marginal activity (Table 6.1 and 6.2). The antimicrobial activity was stronger against Gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aeruginosa* (Fig 6.1, 6.3, and 6.6) and lesser against Gram-negative bacteria, *E. coli* and *Pseudomonas aeruginosa* (Fig 6.2, 6.4 and 6.5). However, in comparison to amoxycillin and cefutaxim, the antimicrobial activity of the two extracts was found to be lower (Table 6.1 & 6.2).
Table 6.1: Antimicrobial activity of various fractions prepared from the rhizome of *S. petiolata*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Petroleum ether (3mg/ml)</th>
<th>Chloroform (3mg/ml)</th>
<th>Hydromethanol (3mg/ml)</th>
<th>Amoxicillin (10µg/ml)</th>
<th>Cefotaxim. (10µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

The experiment was performed in triplicate, and the diameter of the zone of inhibition was measured in mm; Diameter >4 mm = +, 5-10 mm = ++, 10 - 15 mm = +++, > 15 mm = ++++.
Table 6.2: Antimicrobial activity of various fractions prepared from the whole plant of *S. tetragona*

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Petroleum ether (3 mg/ml)</th>
<th>Chloroform (3 mg/ml)</th>
<th>Hydro-methanol (3 mg/ml)</th>
<th>Amoxicillin (10 μg/ml)</th>
<th>Cefutaxim (10 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

The experiment was performed in triplicate, and the diameter of the zone of inhibition was measured in mm; Diameter >4 mm = +, 5-10 mm = ++, 10-15 mm = ++++, > 15 mm = ++++.
Fig 6.1: Effect of hydro-alcoholic extract of *S. petiolata* on *Staphylococcus aureus* (i) compared with amoxicillin (ii)

Plate 1

Plate 2

Fig 6.2: Plate 1: The effect of *S. petiolata* (chloroform extract) on *Pseudomonas aeruginosa* (i) compared with amoxycillin (ii); Plate 2: The effect of (hydro-alcoholic extract of) *S. petiolata* on *E. coli* (i) compared with amoxicillin (ii)
Fig 6.3: Effect of *S. Petiolata* (hydro-alcoholic extract) on *Bacillus subtilis* (i), compared with cefutaxime (ii).

Plate 1: Effect of *S. tetragona* (petroleum ether extract) on *Pseudomonas aeruginosa* (i) compared with cefutaxime on the same strain of bacilli. Plate 2: Effect of (hydro-alcoholic extract) of *S. tetragona* on *E. Coli* (i) compared with the petroleum ether extract of *S. tetragona* (ii), and cefutaxime (iii).
Fig 6.5: Effect of (i) cefutaxim, (ii) S. tetragona (hydro-alcoholic extract), and (iii) S. tetragona (chloroform extract) on Pseudomonad aeruginosa

Fig 6.6: Plate 1: Effect of S. tetragona (hydro-alcoholic extract) on Bacillus subtilis (i) and chloroform extract of the same drug (ii); Plate 2: Effect of S. tetragona (hydro-alcoholic extract) on Bacillus subtilis (i) in comparison to the petroleum-ether extract (ii), and amoxicillin (iii).
6.4: Discussion

Both the species of Swertia show antimicrobial activity. However hydro-alcoholic extracts exhibited a strong antibacterial activity as compared to petroleum-ether and chloroform extracts, which show only marginal activity against all the gram positive and gram negative strains tested. Moreover, the activity as assessed by the area of zone of inhibition was much more in case of gram positive bacteria as compared to gram negative bacteria. This is in accordance with the earlier studies performed on the other species of Swertia. *S. purpurascens* showed positive activity against selected test microorganisms (Ikram and Haq, 1980). *S. chirata* extracts were found to be effective against Gram-positive and Gram-negative bacteria; the activity being more pronounced against the former type of organisms. In another study, the aqueous, MeOH, CHCl₃, and hexane extracts of *S. corymbosa* were tested in vitro for their antimicrobial properties. Maximum inhibitory activity was observed against Staphylococcus aureus and Salmonella typhi (Ramesh et al., 2002). Several xanthones and their D-glucosides also showed antimicrobial activities (Leslie and Chungath, 1987), and swertiaframarin, isolated from *S. japonica*, exhibited antibacterial activity against Staphylococcus aureus [Sedawy et al., 1989].

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists (Kafaru, 1994). Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent (Betoni et al, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties (Lewis, 2006). A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity (Lee et al, 2007). There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004; Islam et al, 2008; De-Boer, 2005).
It has been proposed that the mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells (Walsh et al, 2003). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Aboaba, 2001).

The xanthones and their D-glucosides, swertianolin, norswertianolin, swerchirin, amarogentin and swertiamarin in earlier studies (Ramesh et al, 2000) have been shown to possess the antimicrobial activity. Thus it can be assumed that the activity exhibited by these two species can be either due to one of these compounds or due to all these constituents which are soluble in water and alcohol acting in synergism.

6.5. Conclusion

It can be therefore concluded that these two Swertia species used in the present study possess strong antimicrobial activity. The activity against gram positive bacteria is more profound than gram negative bacteria, and the antibacterial principles are soluble in hydro-alcohol, because the other two fractions did not show any significant antimicrobial activity. The antibacterial principles in these extracts exert their activity, possibly by causing leakage to plasma membranes.

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