Phytochemical and Antimicrobial Study of an Antidiabetic Plant: *Scoparia dulcis* L.

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ABSTRACT The antimicrobial and antifungal effects of different concentrations of chloroform/methanol fractions of *Scoparia dulcis* were investigated. The isolated fractions were tested against different bacteria like *Salmonella typhii*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Proteus vulgaris* and fungal strains such as *Alternaria macrospora*, *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*. The isolated fractions exhibited significant antimicrobial and antifungal activity against all the tested organisms compared with respective reference drugs. The isolated fractions of *S. dulcis* showed properties like antimicrobial and antifungal activities that will enable researchers in turn to look for application-oriented principles.

KEY WORDS: • antimicrobial activity • diabetes • herbal medicine • Scoparia dulcis

INTRODUCTION

*S. dulcis* L. (Scrophulariaceae), a folk medicine plant known as sweet broomweed, has been used as a remedy for diabetes mellitus1 and for hypertension.2 From Indian *S. dulcis*, an antidiabetic compound named amellin was isolated and characterized by Nath3 in 1943. *S. dulcis* is a perennial herb widely distributed in tropical and subtropical regions. In these regions, the fresh or dried plant has traditionally been used as one of the remedies for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids, and hepatitis and as an analgesic and antipyretic.4,5 Several biologically active substances from *S. dulcis*, including scoparic acid A, scoparic acid B,6 scopadulcic acid A and B, scopadulciol,7 and scopadulin,6 have been isolated that have been identified as contributing to the observed medicinal effect of the plant.

In our laboratory, we have previously reported the anti-hyperglycemic, antioxidant, antihyperlipidemic, and anti-apoptotic effects of crude extract of *S. dulcis in vivo* as well as *in vitro*.8–13 In continuation of the chemical investigation of active components isolated from *S. dulcis*, we attempted to isolate a phytochemical of biological importance from Indian *S. dulcis* and study its antibacterial and antifungal activity.

MATERIALS AND METHODS

Plant material

Whole plants of *S. dulcis* L. (40–60 cm in height) were collected from Neyveli, Cuddalore District, Tamilnadu, India in September 2001. The plant was identified and authenticated at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (number 3412) was deposited in the Department of Botany, Annamalai University.

Extraction and separation

Dried whole plants of *S. dulcis* were ground, and the coarse powder (3 kg) was extracted with 80% ethanol. The extract was concentrated in a vacuum drier, and the residue containing water was freeze-dried to give a brown powder (800 g). The residue obtained from the chloroform extract of powder was purified by using silica gel column chromatography and eluted in chloroform/methanol (95:5 vol/vol). This yielded an amorphous dark-brown compound of 1.1 g, which was melted at 198°C, and it was further identified by using the preliminary tests for terpenoids. Further chloroform/methanol (85:15 vol/vol) treatment yielded a low fraction of alkaloid (0.375%) and a high fraction of alkaloid (0.063%) obtained by silica gel column chromatography (Table 1).

Antimicrobial screening

The isolated fractions were tested for antimicrobial activity by the agar disc diffusion method.14,15
Preparation of culture medium and inoculation

Thirty-eight grams of Mueller-Hinton agar was weighed and mixed with 1,000 mL of sterile distilled water. It was sterilized by autoclaving at 120°C for 20 minutes. Under aseptic conditions, in the laminar flow hood 15 mL of agar medium was dispensed into presterilized Petri dishes to yield a uniform depth of 4 mm. They were then covered and allowed to cool at room temperature until the culture medium hardened. The inoculation of the bacterial culture on the agar surface was done by the spread plating technique.

Disc application and incubation

Discs 6 mm in diameter were prepared from Whatman No. 1 filter paper (catalog number 100397, Whatman International Ltd., Maidstone, UK). They were sterilized by autoclaving and subsequently dried at 80°C for 1 hour. The sterile discs were impregnated in the test compounds to be tested, which were dissolved in chloroform to a final concentration of 1%, 2%, and 4% and dried for 3–5 minutes. After drying, the discs with the compounds were placed on the Mueller-Hinton agar surface with flamed forceps and gently pressed down to ensure contact with the agar surface. A streptomycin antibiotic disc was used as the positive control and chloroform as a negative control. The discs were spaced far enough to avoid both reflections waves from the edges of the Petri dishes and overlapping rings of inhibition. Finally, the Petri dishes were incubated for 18 hours at 37°C, depending on the optimal growth, in an inverted position. After 18 hours, the diameter (in mm) of the inhibition zone around each disc was measured. Streptomycin was used as the standard. Antibacterial activities were indicated by a clear zone of growth inhibition.

Antifungal studies

Antifungal activities of all the isolated fractions were screened for in vitro growth inhibitory activity against Candida albicans, Fusarium oxysporum, Alternaria macrospora, and Aspergillus niger by using the disc diffusion method.14,15 The fungi were cultured in potato dextrose agar medium, which was prepared by using potato (200 g), dextrose (15 g), and agar (20 g) dissolved in 1 L of distilled water, poured in the sterilized Petri plates, and allowed to solidify. The plates were inoculated with a spore suspension of C. albicans, F. oxysporum, A. macrospora, and A. niger (106 spores/cm³ of medium). The compounds to be tested were dissolved in chloroform to a final concentration of 1%, 2%, and 4% and soaked in filter paper discs (Whatman no. 4, 5 mm in diameter). After drying, the discs with the compounds were placed on the surface of the potato dextrose agar medium with flamed forceps and gently pressed down to ensure contact with the agar surface. After 4 days, the inhibition zone that had appeared around the discs in each

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Chloroform/methanol (95:5)</th>
<th>Chloroform/methanol (85:15)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>Terpenes</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>First alkaloid</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Second alkaloid</td>
<td>–</td>
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</tbody>
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The following scale was used: absent, –; low concentration, +; high concentration, ++; very high concentration, +++.

### Table 1. Phytochemical Screening of S. dulcis

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Chloroform/methanol (95:5)</th>
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<tr>
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<td>Second alkaloid</td>
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<thead>
<tr>
<th>Bacterium</th>
<th>Chloroform/methanol (95:5)</th>
<th>Chloroform/methanol (85:15)</th>
<th>Chloroform/methanol (85:15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>S. typhi</td>
<td>6</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>S. aureus</td>
<td>5</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>E. coli</td>
<td>11</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>6.5</td>
<td>9.5</td>
<td>11</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>7</td>
<td>8.5</td>
<td>9.5</td>
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Data are the means of three replicates. The zone of inhibition is given in mm. Std., Str., standard streptomycin; Std. Amp., standard ampicillin.
plate was measured. To avoid the activity of the solvent that was used in the test solutions, a solvent-only treated plate was maintained. An untreated control plate was also maintained in order to calculate the percent inhibition. Carbendazim was used as the standard.

RESULTS

Antibacterial activity

The antibacterial activities of the isolated compounds are presented in Table 2. The compounds were screened for activity against Salmonella typhi, Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, and Proteus vulgaris at 1%, 2%, and 4% concentrations by the agar disc diffusion method. Results were compared with the standard drugs (streptomycin and ampicillin). The compounds showed an appreciable zone of activity at 4% concentration against the tested pathogens.

Antifungal activity

The antifungal activities of the compounds isolated were studied using the agar disc diffusion method (Table 3). The compounds were tested against A. macrospora, C. albicans, A. niger, and F. oxysporum at 1%, 2%, and 4% concentrations. The compounds exhibited significant activity against all the fungi tested. All the fractions of S. dulcis showed good activity against the tested organisms. Results were compared with the standard drug carbendazim.

DISCUSSION

The discovery of natural products with promising biological activity has demonstrated the significance of medicinal plants in nature, and attempts to conserve and cultivate these plants for the production of medicinal compounds by alternate methods are underway.

The bulk bioactive compounds of S. dulcis are terpenoids such as scoparic acid A, B, and C, scopadulcic acid A and B, and scopadulciol. Among them, scopadulcic acid B and scopadulciol were found to be unique biomolecules with inhibitory effects on replication of herpes simplex virus type 1, the gastric proton pump, and bone resorption stimulated by parathyroid hormone. In addition, a line of evidence reported that scopadulcic acid B showed antitumor-promoting activity.

In the present study, initial antimicrobial screening of the crude extracts from S. dulcis showed varying degrees of antimicrobial activity against human pathogenic bacteria such as S. typhi, S. aureus, E. coli, B. subtilis, P. aeruginosa, and P. vulgaris and plant pathogenic fungi such as A. macrospora, C. albicans, A. niger, and F. oxysporum. Antimicrobial activity of the compounds may be of four types: (1) they hamper cell wall synthesis; (2) they inhibit microbial protein and nucleic acid synthesis; (3) they disrupt microbial membrane structure and function; and (4) they block metabolic pathways through inhibition of key enzymes. In the present study, chloroform/methanol fractions of S. dulcis showed the highest zones of inhibition (4%) against the bacteria. In the 5% chloroform/methanol fraction, the isolated terpenoids with a cyclic structure containing one or more functional groups may be responsible for antimicrobial activity. This second alkaloid possesses a higher level of antimicrobial activity when compared with the first alkaloid that was obtained in the 2% chloroform/methanol fraction. The activity increases with an increase in concentration of the compounds in all the cases. The variation in the effectiveness of the different compounds against different microorganisms depends upon the impermeability of the microbial cells or the difference in the ribosome of the microbial cells. Although the compounds are active, they did not reach the effectiveness of the conventional bactericides streptomycin and ampicillin and the fungicide carbendazim.

Since the chloroform/methanol fractions of S. dulcis exhibited potent activity in controlling the growth of microorganisms, they should be further analyzed to isolate and characterize the active compounds using various techniques like chromatography and spectroscopy.

ACKNOWLEDGMENTS

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