

## NOTES

### Phytochemical analysis and antimicrobial evaluation of chloroform extracts of stem and roots of *Scoparia dulcis* L.

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Natural compounds derived from medicinal plants serve as safe alternatives than conventional drugs in the treatment of various ailments, particularly microbial infections marred by antibiotic resistance. In this context, we screened the common Liquorice weed, *Scoparia dulcis* L. for phytochemicals and antimicrobial potential. Phytochemical and HPTLC analysis of chloroform extracts of stem and roots of *S. dulcis* showed the presence of diversity of secondary metabolites. The extracts of the plant were assayed for antimicrobial activity *in vitro* against selected pathogenic bacteria and fungi. The results have shown the plant extracts to possess antibacterial and antifungal activities. The highest antibacterial activity exhibited by chloroform extract of the stem was against *Staphylococcus aureus* and roots against *Escherichia coli*. The minimum inhibitory concentrations of stem and roots chloroform extracts (MIC, 150, 75 mg/mL of each) showed highest antibacterial and antifungal activities against *E. coli* and *Aspergillus niger*.

**Keywords:** Antibacterial, Antifungal, Liquorice weed, Sweet broom weed

Plants have been proving to be the best source of therapeutic drugs and are widely used in treating infections caused by microorganisms<sup>1,2</sup>. Plant-derived drugs are safe and more dependable than their expensive, synthetic counterparts, which often have adverse side effects<sup>3</sup>. Therefore, pharmaceutical industries have focused on extracting traditional uses of medicinal plants in their drug discovery<sup>4</sup> functions. Stem-bark decoction of *Albizia gummifera* for venereal diseases<sup>5</sup>; aqueous leaf extract of *Glyphaea brevis* for hepatitis and intestinal diseases<sup>6</sup>; oil extracts of the roots, seeds and stem barks of *Monodora myristica* to cure malaria, helminthiasis, scabies and dysenteric syndromes<sup>7</sup>; Leaf extracts of *Ruta graveolens* for ulcers, reproductive disorders and

parasitic infections<sup>8</sup>; rhizome and root extracts of *Zingiber officinale* and *Hertia cheirifolia* as antioxidant, anticancer and antibacterial agents<sup>9,10</sup>; and extracts of *Boswellia ovalifoliolata* to treat inflammation, arthritis, amoebic dysentery, other fungal skin infections<sup>11</sup> are some examples.

*Scoparia dulcis* L. (Fig. 1), commonly called Liquorice weed or Sweet broom weed, is an important annual medicinal herb from Scrophulariaceae, that grows to a half meter in height, and is widely distributed in many tropical countries around the world. Indigenous tribes in Ecuador brew a tea of the entire plant to reduce swellings, aches and pains<sup>12</sup>. The whole plant is used as antiemetic for headaches<sup>13</sup> and belly pain among infants, and as an antiseptic wash for wounds, hypertension, menstrual disorders<sup>14</sup>, and is also used to treat diabetes, and haemorrhoids among adults. Chloroform extracts of stem and roots of *S. dulcis* have been chosen in this study to screen phytochemicals, understand their characterization by TLC and HPTLC analysis, and evaluate potential antimicrobial properties against resistant Gram-positive, Gram-negative bacteria and fungi.

### Materials and Methods

#### Chemicals

Nutrient agar (NA) and potato dextrose agar (PDA) were purchased from Hi-Media, Mumbai, India. All the other chemicals used were of analytical grade. Milli-Q water was used for TLC and HPTLC analysis.



Fig. 1 —*Scoparia dulcis* plant

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[Suppl. data available in NOPR at <http://nopr.niscair.res.in>]

**Bacterial and fungal test pathogens**

*Escherichia coli* (NCIM No. 2067), *Pseudomonas aeruginosa* (NCIM No. 2036), *Bacillus subtilis* (NCIM No. 2724), *Staphylococcus aureus* (NCIM No. 2079) and fungal strains, *Aspergillus niger* (NCIM No. 1055), and *Candida albicans* (NCIM No. 3471) were procured from National Collection of Industrial Microorganisms (NCIM), Pune, India. These cultures were grown on appropriate medium at 37°C for overnight incubation and maintained at 4°C in a refrigerator.

**Collection of plant material**

The medicinal plant was collected during the month of July 2016 from Devarapalli, Visakhapatnam district, Andhra Pradesh. The plant was taxonomically identified and authenticated by Prof. M Venkaiah, Department of Botany, Andhra University, Visakhapatnam and its certification number is 22002. The plant material was washed thoroughly with tap water and then rinsed with distilled water. Plant parts including roots, stem and leaves were separated and shade-dried at room temperature. The dried plant parts were finely powdered using an electric grinder and subjected for solvent extraction.

**Preparation of *Scoparia dulcis* stem and root extracts**

About 30 g of the ground dry stem and roots material of *Scoparia dulcis* was used for the extraction. Extraction was carried out by stepwise solvent extraction procedure by increasing polarity of solvents using *n*-hexane, chloroform and methanol. Briefly, 200 mL refluxing solvent was taken in a round-bottom flask, refluxed and distilled. This procedure was repeated 6-7 times. At the completion of the extraction process, the plant extracts were concentrated in a Buchi Rotavapor R-200 under reduced pressure, transferred into appropriately-labeled vials and allowed to stand at room temperature (25±2°C). The concentrated samples were stored at 4°C until further use.

**Phytochemical screening**

The prepared chloroform extracts of stem and root of the plant were screened for various phytochemical components, such as alkaloids, flavonoids, phenolics, steroids, terpenoids, saponins, carbohydrates and phytosterols by standard phytochemical screening methods<sup>15</sup>.

**TLC analysis**

In TLC (Thin layer chromatography), separation depends on the relative affinity of compounds in

stationary and mobile phases. Silica gel G was coated on glass plates and allowed for air dry followed by activation of the plates at 110°C for 10 min. One per cent solution of extracts were prepared and spotted onto the TLC plates. Mobile phases were used in different ratios of different solvent polarities. Three different types of solvent systems used in the TLC chamber are ethyl acetate and *n*-hexane (9:1), solvent system-I, ethyl acetate and chloroform (3:1), solvent system-II and chloroform and methanol (19:1), solvent system-III. After the run, plates were air dried and sprayed with different spraying reagents. The movement of the active compound was expressed by its retention factor ( $R_f$ ) values, calculated as:

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

**HPTLC analysis**

About 2.5 mg of roots and stem extracts were dissolved in 5 mL of chloroform and the solution was centrifuged at 3000 rpm for 5 min and used as test solution for HPTLC (High Performance Thin Layer Chromatography) analysis. Applied concentration of both chloroform extracts of stem and roots of plant was 0.5 mg/mL and standard alkaloid; boldine was 1 mg/mL. The samples (2 µL) were spotted in the form of bands of width 5 mm with a Camag microlitre syringe on precoated silica gel glass plate 60F-254 (10×10 cm) of 250 µm thickness (E-Merck, Darmstadt, Germany) using a Camag Linomat IV (Switzerland).

The ethyl acetate: *n*-hexane (9:1) system was employed as mobile phase for separation of secondary metabolites. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The plate was placed in photodocumentation chamber and captured the images under UV light at 254 nm<sup>16</sup>, 366 nm and in daylight. The scanning of the plate was done at 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag).

**Antimicrobial activity**

Antimicrobial activity and minimum inhibitory concentration (MIC) of chloroform extracts of both stem and roots of *Scoparia dulcis* were studied using agar well diffusion assay<sup>17</sup> against Gram positive and Gram negative bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus* and fungal strains, *Aspergillus niger* and *Candida albicans*<sup>17</sup>. To study the antibacterial activity, working solution was prepared

by dissolving both stem and roots extracts in 1.0 mL of DMSO at a final concentrations of 400 and 200 mg/mL, respectively. Similarly, a working solution of stem and root extracts were prepared at a final concentration of 200 and 100 mg/mL, respectively, to study the antifungal activity. The working solutions were centrifuged at 3000 rpm for 15 min and the supernatant was collected and stored at 4°C for further use. Antibiotics ampicillin and nystatin (1.0 mg/mL) were used as standard.

The agar well diffusion medium was prepared by pouring 20 mL of molten Mueller-Hinton agar in Petri dishes and were allowed to solidify. Wells of 6 mm diameter were punctured in agar medium using sterile iron cork borer. A volume of 100 µL of microbial cultures ( $0.5 \times 10^8$  cells/mL) were seeded into warm molten Mueller-Hinton agar. Precisely, 40 µL each ampicillin and nystatin, DMSO, chloroform and working solutions of stem and roots extracts were loaded into separate wells.

#### MIC assay

The MIC of the chloroform extracts of stem and roots were determined against all microbial cultures at final concentration of 150 and 75 mg/mL against bacteria and fungi, respectively. All the experiments were performed in triplicate and the plates were incubated at 37°C for 24-48 h. Diameters of the zones of inhibition of bacteria and fungi were measured using a meter ruler, and the mean value for each organism was recorded and expressed in millimeters.

#### Statistical analysis

Results were expressed as mean  $\pm$  S.D of triplicate ( $n=3$ ) values. The data was subjected to one-way analysis of variance (ANOVA) to determine the significance of individual differences at  $P < 0.05$  level. Significant means were compared by the Duncan's multiple range test and all statistical analysis were carried out using SPSS statistical software package (SPSS, version 20 Chicago, USA).

### Results and Discussion

Phytochemical analysis of stem extract (chloroform) showed the presence of alkaloids, steroids, cardiac glycosides and phenols but the absence of terpenoids and flavonoids. Phytochemical analysis of root extract (chloroform) showed the presence of alkaloids, terpenoids, cardiac glycosides and phenols but the absence of steroids and flavonoids, as given in Table 1.

In TLC, three spots were visible (Fig. 2) with solvent system-I for stem extract, with  $R_f$  values 0.20, 0.48, 0.86 and four spots were observed for roots extract, with  $R_f$  values 0.06, 0.23, 0.50, 0.86. Two spots were visible with solvent system-II for stem extract, with  $R_f$  values 0.19, 3.61 and only one spot was observed for roots extract, with  $R_f$  value 0.66. One spot was visible with solvent system-III for stem extract, with  $R_f$  value 0.91 and for roots extract, with  $R_f$  value 0.88 as shown in Fig. 2.

In HPTLC, only one spot was observed for standard and root extract, with  $R_f$  values 0.21 and 0.38 and two spots for stem extract with  $R_f$  values 0.39, 0.46 shown in Fig. 3 and its graphical representation shown in suppl. Fig. 1 (All supplementary figs are available at <http://nopr.niscair.res.in>).

The results of antimicrobial assay showed the significant activity of the chloroform extracts of stem and root against all the microorganisms studied. The zone of inhibition indicated the strength of antimicrobial activity (Suppl. Fig. 2 A & B). The diameter of inhibition zones for each of the samples was compared with standard antibiotics. DMSO and chloroform did not show any inhibitory effect. The

Table 1 — Phytochemical analysis of chloroform extracts of stem and roots of *Scoparia dulcis*

Metabolite	Stem extract	Roots extract
Alkaloids	+++	+++
Flavonoids	--	--
Carbohydrates	+++	+++
Reducing disaccharides	+++	+++
Cardiac glycosides	+++	+++
Steroids	+++	--
Terpenoids	--	+++
Phenolic compounds	++	++
Phytosterols	+++	--

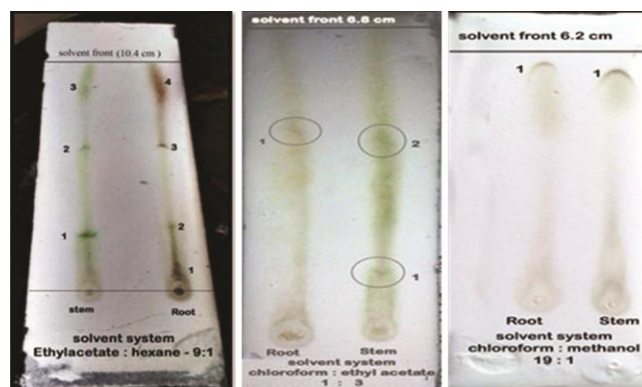


Fig. 2 — TLC studies of chloroform extracts of stem and roots of *Scoparia dulcis*

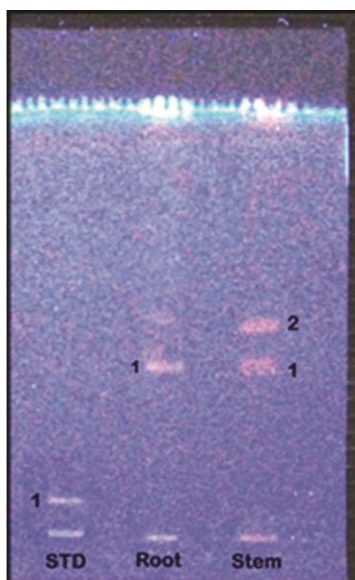


Fig. 3 — HPTLC profile showing the presence of alkaloids in chloroform extracts of roots and stem

chloroform extract of the stem at 400 mg/mL concentration (suppl. Fig. 2A) showed highest antibacterial activity in the following order against *Staphylococcus aureus* (15 mm), *Bacillus subtilis* ( $12.6 \pm 0.5$  mm), *Escherichia coli* (12 mm) and *Pseudomonas aeruginosa* ( $11.6 \pm 0.5$  mm). The chloroform extract of the stem (200 mg/mL) showed highest antifungal activity against *Aspergillus niger* ( $10.6 \pm 1.15$  mm) than *Candida albicans* with inhibition zone of  $10.3 \pm 0.5$  mm and its graphical representation is depicted in (Fig. 4A). The chloroform extract of the roots (400 mg/mL) (suppl. Fig. 2B) showed highest antibacterial activity in the following order against *E. coli* ( $12.6 \pm 0.5$  mm), *S. aureus* (12 mm), *P. aeruginosa* ( $10.6 \pm 0.5$  mm) and *B. subtilis* ( $10.3 \pm 0.5$  mm). Highest antifungal activity was observed against *C. albicans* ( $14 \pm 1.0$  mm) with the chloroform extract of the root (200 mg/mL) than *A. niger* ( $11.6 \pm 1.5$  mm) and its graphical representation is depicted in (Fig. 4B).

The diameter of inhibition zones for root extracts, DMSO,  $\text{CHCl}_3$ , ampicillin and nystatin were measured [(suppl. Fig. 2B). Available online at NOPR]. All tests were conducted in triplicate and the mean values  $\pm$  SD are presented in Fig. 4B.

The MIC was determined as the minimum concentration of chloroform extracts of stem and root that inhibits the visible growth of bacterial and fungal strains. The optimized concentration of the stem chloroform extract for MIC (150 mg/mL) studies

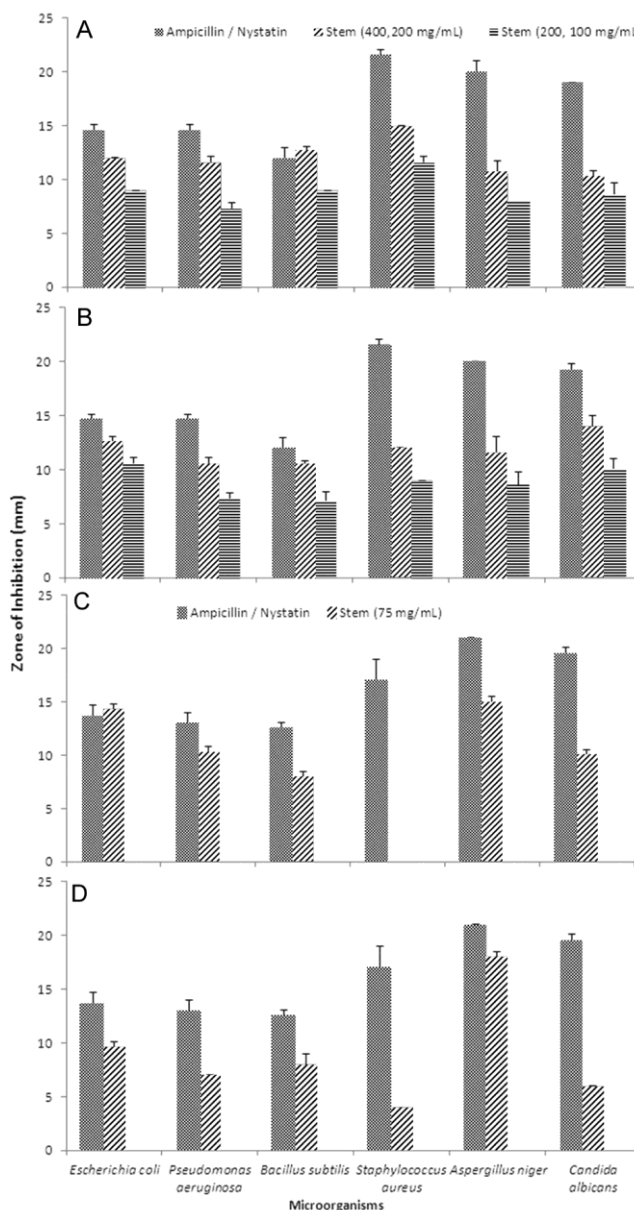


Fig. 4 — Antimicrobial activity of chloroform extract of stem and root of *Scoparia dulcis* against microorganisms. (A & B represents activity at different concentrations of stem and root; and (C & D) shows respective Minimum inhibitory concentration

(Suppl. Fig. 2C) revealed that the inhibition is in the following order of bacteria: *E. coli* ( $14.3 \pm 0.5$  mm), *P. aeruginosa* ( $10.3 \pm 0.5$  mm), *B. subtilis* ( $8.0 \pm 0.5$  mm); however, no inhibition zone was observed against *S. aureus*; and MIC (75 mg/mL) for fungi against *A. niger* ( $15.0 \pm 0.5$  mm) and *C. albicans* ( $10.0 \pm 0.5$  mm) (Suppl. Fig. 2C). Its graphical representation is depicted in (Fig. 4C). The optimized concentration of the chloroform extract of root for (150 mg/mL) (Suppl. Fig. 2D) revealed inhibition in the following

order for bacteria *E. coli* (9.6±0.5 mm), *B. subtilis* (8.0±1.0 mm), *P. aeruginosa* (7.0 mm), and *Staphylococcus aureus* (4.0 mm); MIC (75 mg/mL) for fungi against *A. niger* (18.0±0.5 mm), and *C. albicans* (6.0 mm) (Suppl. Fig. 2D). Its graphical representation is depicted in Fig. 4D.

The diameter of inhibition zones with chloroform extracts of stem and root (MIC), DMSO, CHCl<sub>3</sub>, ampicillin and nystatin were measured (Suppl. Fig. 2 C & D). All tests were conducted in triplicate and the mean values±SD were presented in Fig. 4 (C & D).

Preliminary phytochemical analysis in the present study showed the presence of a potential group of bioactive compounds. The observed antimicrobial activity could be attributed to the secondary metabolites present in the plant. Earlier workers have also reported antimicrobial activities of various plants<sup>8,10,11,18,19</sup>.

In this study, the presence of phytochemicals like alkaloids, steroids, cardiac glycosides, terpenoids and phenols in the chloroform extracts of stem and root as revealed by the results corroborates with the earlier reports<sup>20,21</sup>. In the HPTLC profile of alkaloids, the retardation factor of chloroform extracts of stem (0.46) and root (0.38) was more than the standard alkaloid (boldine) (0.21). Chloroform fractions exhibited potent activity in controlling the growth against human pathogenic fungi and bacteria as shown by Jonathy<sup>21</sup>. Dose-dependent activity was observed with respect to the increased diameter of the zone of inhibition as the concentration of plant extract increased from 200 mg to 400 mg/mL. DMSO and chloroform did not exhibit any activity. Gram positive bacteria (*Staphylococcus aureus*) are relatively more sensitive to non polar solvent extracts (*n*-hexane and chloroform) compared to polar solvent extracts (acetone, methanol and water). Each bacterium showed unique sensitivity towards different extracts which could be due to the difference in structure of each bacterium and the mechanism by which the extracts exhibit their antibacterial action. Many researchers have also shown that extracts of plants inhibit the growth of various microorganisms at different concentrations<sup>22-24</sup>.

## Conclusion

Preliminary and HPTLC screening of chloroform extracts of stem and root showed the presence of major secondary metabolites, such as alkaloids, steroids, terpenoids, cardiac glycosides and phenols.

The antimicrobial activity of chloroform extracts of stem and root exhibited dose-dependent moderate activity when compared with standard drugs (ampicillin and nystatin). The root extract showed more potency for susceptibility of tested microbes than the stem extract. MIC of fungi was more susceptible to the chloroform extracts of root and stem when compared with bacterial species. The antimicrobial activity could be due to the phytochemicals alkaloids and phenols present in the plant.

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