



E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2015; 3(6): 17-21  
Received: 06-12-2014  
Accepted: 21-12-2014

#### Wankupar Wankhar

Department of Physiology, Dr  
ALM PG IBMS, University of  
Madras, Taramani campus,  
Chennai-600 113, Tamilnadu,  
India.

#### Sakthivel Srinivasan

Department of Physiology, Dr  
ALM PG IBMS, University of  
Madras, Taramani campus,  
Chennai-600 113, Tamilnadu,  
India.

#### Ravindran Rajan

Department of Physiology, Dr  
ALM PG IBMS, University of  
Madras, Taramani campus,  
Chennai-600 113, Tamilnadu,  
India.

#### Sheeladevi Rathinasamy

Department of Physiology, Dr  
ALM PG IBMS, University of  
Madras, Taramani campus,  
Chennai-600 113, Tamilnadu,  
India.

#### Correspondence:

#### Sheeladevi Rathinasamy

Department of Physiology, Dr  
ALM PG IBMS, University of  
Madras, Taramani campus,  
Chennai-600 113, Tamilnadu,  
India.

## Phytochemicals screening and antimicrobial efficacy of *Scoparia dulcis* Linn (Scrophulariaceae) against clinical isolates

Wankupar Wankhar, Sakthivel Srinivasan, Ravindran Rajan and Sheeladevi Rathinasamy

#### Abstract

Herbal plants represent a reservoir of therapeutic agents and with the increase prevalence of multiple drug resistance strain against existing synthetic antibiotics there is a necessity for an alternative cure. In recent years, secondary plant metabolites with previously unknown pharmacological activities have gained importance and often studied for its therapeutic efficacy. Phytochemicals screening of *Scoparia dulcis* extracts reveals the presents of alkaloids, flavonoids, phenols, terpenoids, tannins and saponins. The antimicrobial activity of the plant were tested by broth dilution method ranging from 8mg/mL to 256mg/mL and the minimum inhibitory concentration (MIC) were determined when there is positive or negative growth of the tested microorganism on the nutrient agar plates. In this particular study methanol extract displayed a better MIC when compared to aqueous against both gram positive, gram negative bacteria and fungus and this could have attributed to its potent extraction capacity. Thus, justifying its efficacy as a potential broad spectrum antimicrobial agent.

**Keywords:** *Scoparia dulcis*, phytochemical, antimicrobial, (MIC) minimum inhibitory concentration.

#### 1. Introduction

Herbal remedy has the art of healing centered on beliefs and experiences, indigenously handed down through generations, referred as complementary or alternative medicine. Though the traditional Indian system of medicine has a long history of use, yet they lack adequate scientific documentation, particularly in light of modern scientific knowledge<sup>[1]</sup> and with more than 80% of the world's population relying on traditional medicine for their primary healthcare (WHO), it is therefore essential to evaluate plants for medicinal value and its chemotherapeutics. It is widely accepted fact that plants produce a diverse range of potential chemotherapeutics agents, besides these active components of herbal remedies have the advantage due to the synergic effect of these active chemotherapeutics agents. With increasing prevalence of multidrug resistant strains and the recent appearance of strains with reduced susceptibility to antibiotics, an overwhelm spectra of untreatable bacterial infections arises. This adds urgency to the search of new infection combating strategies and effective therapeutic agents<sup>[3]</sup> which is relatively safer and cheaper than the synthetic alternative.

In recent years plants and plant secondary metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated. *S. dulcis* Linn (Scrophulariaceae) is an important ethnomedicinal plant, commonly called as sweet broom weed is a perennial herb, widely distributed in tropical and subtropical regions. Traditionally the plant has been used as one of remedies for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids, hepatitis, analgesic, antipyretic, antiviral, emmenagogue, antibacterial, antifungal, antiviral, antiherpetic, antiinflammatory, cytotoxic, antiseptic, emollient, febrifuge, anti diarrhoeal and anti-spasmodic activities, hepatoprotective<sup>[4-7]</sup> neuroprotective and anticholinergic<sup>[8]</sup>. Thus, in continuation with the strategy of developing newer and safer therapy to fight multiple drug resistance microbes, we evaluate the phytochemical constituents of *S. dulcis* and its efficacy as anti-microbial agent.

#### 2. Materials and Method

##### 2.1 Collection and Identification

The plant *S. dulcis* collected from the university campus. Dr. ALM PG IBMS (University of Madras) and was authenticated by Dr. D Aravind, Department of Medicinal Botany.

Voucher specimens have been deposited in the Herbarium of National institute of Siddha, Reg no NIS/MB/62/2012. The collected plant parts were separated from undesirable materials and dried in shade. The leaf was ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until the analysis commence.

## 2.2 Extraction Procedure

150 grams of fresh whole *S. dulcis* plants (leaves) were extracted with 500 mL of sterile distilled water and methanol using the Soxhlet apparatus. The extracts were then filtered with Whatman No 1 filter paper and then freeze dried, stored at 4 °C for further investigation. The extraction efficiency was quantified by determining the weight of each of the extracts (Table-1) and the percentage yield was calculated.

## 2.3 Quantitative analysis on phytochemical constituents

The extracts obtained were then subjected to qualitative chemical tests in order to detect the presences various chemical constituents. Major secondary metabolites classes such as flavonoids, phenol, terpenoids, saponins, tannins, steroids, glycosides, alkaloids, coumarins and vitamin-C were screened according to the reference method of [9-11].

**2.3.1 Test for alkaloids:** Methanolic and aqueous extract was warmed with 2% H<sub>2</sub>SO<sub>4</sub> for two minutes. It is filtered and few drops of Mayer's reagents were added, formation of a yellow cream precipitate indicates the presence of Alkaloids.

**2.3.2 Test for flavonoids:** Extracts have to be treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

**2.3.3 Test for phenolic:** The extract was dissolved in 5 mL of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green color indicated the presence of phenolic compounds.

**2.3.4 Test for terpenoids:** The extract was mixed with 2 mL of chloroform and concentrate H<sub>2</sub>SO<sub>4</sub> (3 mL) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive results of the presence of terpenoids.

**2.3.5 Test for tannins:** A small quantity of the extract is boiled with 5 mL of 45% solution ethanol for 5 minutes. Each of the mixture is cooled and filtered. 1 mL each of filtrate is diluted with distilled water and added with two drops of ferric chloride. A transient greenish to black color indicates the presence of Tannins.

**2.3.6 Test for steroids:** 2 ml of acetic anhydride was added to extract 2 mL of H<sub>2</sub>SO<sub>4</sub>. The color changed from violet to blue or green in some samples indicated the presence of steroids.

**2.3.7 Test for saponins:** A small quantity of different extract was diluted with 4 mL of distilled water. The mixture was shaken vigorously and then observed in standing for stable froth.

**2.3.8 Detection of glycosides:** Extracts have to be treated with 1 mL glacial acetic acid + FeCl<sub>3</sub> + concentrated sulphuric acid.

Formation of green – blue colour indicates the presence of cardiac glycosides

**2.3.9 Tests for coumarins:** Take a drop of ammonia on a filter paper; to this add a drop of aqueous extract of leaves. Fluorescence was not observed.

**2.3.10 Test for Vitamin-C:** Test solution was treated with Dinitrophenyl hydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin-C.

## 2.4 Antimicrobial Screening

### 2.4.1 Organisms Used

Gram positive organisms - *S. aureus* & ATCC *S. aureus*, Gram negative organisms - *Escherichia coli*, *Salmonella typhi*, *Pseudomonas spp*, *Klebsiella spp*, ATCC *E. coli* and Fungus- *Candida albicans* that are isolated from patient samples provided by the department of microbiology in coordination with ICH hospital, Egmore, Chennai, India. The samples were subcultured on Mac Conkey agar and incubated for 24 hours at 37 °C.

### 2.4.2 Preparation of Inoculum

The inoculums was prepared from overnight colonies and emulsifying it by diluting with sterile broth to match 0.5 McFarland standard which can be used for visual comparison and adjust the suspension to a density equivalent to approximately (1x10<sup>6</sup> to 5x10<sup>6</sup> CFU/mL) using spectrophotometer (Nano Drop).

### 2.4.3 Minimum Inhibitory Concentration (MIC)

A current definition of the minimum inhibitory concentration (MIC) is the lowest concentration which resulted in maintenance or reduction of inoculums viability [12]. Serial tube dilution technique was used to determine MIC of the extracts against gram-positive, gram-negative bacteria and fungus. Various concentrations of aqueous and methanol extract from 8 mg/ml, 16 mg/ mL, 32 mg/mL, 64 mg/mL, 128 mg/mL and 256 mg/mL were respectively prepared aseptically with nutrient broth [13]. The extract was filtered through syringe filter unit (0.2 µm). To each of these dilutions, a loopful of inoculums (clinical isolates) were inoculated and adjusted to 0.5 McFarland standards in all the tubes and incubated at 37 °C for 24 hrs. After incubation, a loopful of the inoculums from each tube was inoculated onto nutrient agar plates. The tests were continued out in duplicates. Kanamycin was used as negative control and broth along with isolate alone was used as positive control.

## 3. Result and Discussion

The result for percentage yield of extraction was quantified by determining the weight of each of the extracts and the percentage yield (Table-1) was calculated for both the extract. The Antimicrobial efficacy of the plant was evaluated by determined as the MIC of the extract. The lowest concentration which did not show any growth of the tested microorganism when a loopful of the inoculums from each tube was inoculated onto nutrient agar plates is determined as the MIC of *S. dulcis* extracts at their respective concentration (Table-3). Various workers have shown that Gram positive bacteria are more susceptible towards plants extracts when compared to Gram negative bacteria [14, 15]. These differences may be attributed to the fact that the cell wall in Gram positive

bacteria is of a single layer, whereas the Gram negative cell wall is multilayered [16]. However, this current study our plant extracts displayed antimicrobial activity against both Gram negative and positive bacteria with broth dilution method. To support our finding Zulfiker *et al.* [17] reported antimicrobial activity of *S. dulcis* against a wide range of human pathogenic microorganisms, including Gram-positive, Gram-negative bacteria. This is in agreement with Latha *et al.* [18] who also reported that chloroform/methanol fractions of *S. dulcis* exhibited potent activity in controlling the growth against human pathogenic fungi and bacteria this is followed by Jonathan [19] who also reported antimicrobial activity of the same plant. This demonstration of antimicrobial activity of *S. dulcis* against wide range of pathogenic organism may be the indicative presence of broad spectrum therapeutic agents necessary to fight resistance bacterial strains. Given that natural source derived compounds do not enhance the “antibiotic resistance” a common phenomenon encountered with the long term use of synthetic antibiotics [20] extraction of these bioactive compound plays a crucial role in determining the therapeutic efficacy of any plant.

Several biologically active substances from *S. dulcis* have been isolated that have been identified and contributing to its observed medicinal effect. Aromatic and medicinal plants are

known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth [21]. The result of the preliminary phytochemical screening was carried out on aqueous and methanolic extracts revealed the presence of a wide range of phytoconstituents (Table-2) including Alkaloids, Flavonoids, phenolic, terpenoids, tannins, saponins supporting the reason for its wide range of biological activities which may be responsible for the antimicrobial activity. This is in agreement with Zulfiker *et al.* [22] who previously reported that phytochemical screening of ethanolic extracts of *S. dulcis* which revealed the presence of flavonoid, alkaloid, tannin, carbohydrate, glycoside. Thus, the present investigation clearly indicate the aqueous extract showed milder antimicrobial activity against the gram positive, gram negative as well as fungus when compared to methanol extract, which certainly indicates that stronger extraction capacity of methanol could have shaped greater active constituents and these observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent. However, both exhibited antibacterial and antifungal activities of the plants and this could be of considerable interest for the development of new drugs from natural resources.

**Table 1:** (Weight of dry extracts in grams /Initial dry plant extracts) × 100

S. No	Plant extract of <i>S. dulcis</i>	Weight of dry extracts in grams	Initial dry plant extracts in grams	Extraction Yield % after freeze drying
1.	Aqueous	18.6	150	12.4%
2.	Methanol	20.4	150	13.6%

**Table 2:** Qualitative analysis of the phytochemicals of *S. dulcis* extracts

Biochemicals	Aqueous	Methanol
Alkaloids - Mayer's reagent	+	+
Flavonoids - Alkaline Reagent test	+	+
Coumarins - Ammonia test	-	-
Terpenoids - Salkowski test	+	+
Tannins - Ferric Chloride test	+	+
Saponins- Frothing test	+	+
Steroids - Liberman Burchard test	-	-
Glycosides - Keller-Killiani test	-	-
Vitamin-C - DNPH test	-	-

‘+’ Presence : ‘-’ Absence.

**Table 3:** lowest concentrations which did not show any growth of the tested microorganism after macroscopic evaluation was determined as the MIC of aqueous (AE) and methanol extract (ME) of *Scoparia dulcis* Linn at the respective concentration. “+” Indicates growth and “-” No growth

S.no	STRAIN	8mg/mL		16mg/ mL		32mg/mL		64mg/mL		128mg/mL		256mg/mL	
		AE	ME	AE	ME	AE	ME	AE	ME	AE	ME	AE	ME
1.	<i>E.coli-1</i>	+	+	+	+	-	-	-	-	-	-	-	-
2.	<i>E.coli-2</i>	+	+	+	+	+	+	+	-	-	-	-	-
3.	<i>E.coli-3</i>	+	+	+	+	+	-	+	-	-	-	-	-
4.	<i>E.coli-4</i>	+	+	+	+	+	-	-	-	-	-	-	-

5.	<i>E.coli-5</i>	+	+	+	+	+	+	-	-	-	-	-	-
6.	<i>E.coli-6</i>	+	+	+	+	+	+	-	-	-	-	-	-
7.	ATCC <i>E.coli</i> 25922	+	+	+	+	+	+	+	-	-	-	-	-
8.	<i>Klebsiella spp -1</i>	+	+	+	+	+	+	-	-	-	-	-	-
9.	<i>Klebsiella spp-2</i>	+	+	+	+	+	+	+	-	-	-	-	-
10.	<i>Staphylococcus spp-1</i>	+	+	+	+	-	-	-	-	-	-	-	-
11.	<i>Staphylococcus spp-2</i>	+	+	+	+	-	-	-	-	-	-	-	-
12.	<i>Staphylococcus spp-3</i>	+	+	+	+	+	+	+	-	-	-	-	-
13.	<i>Staphylococcus spp IE-1</i>	+	+	+	+	+	-	+	-	+	-	-	-
14.	<i>Staphylococcus spp IE-2</i>	+	+	+	-	+	-	+	-	+	-	-	-
15.	<i>Staphylococcus spp IE-3</i>	+	+	+	-	+	-	+	-	+	-	-	-
16.	ATCC <i>Staphylococcus</i> 25923	+	+	+	+	-	+	-	-	-	-	-	-
17.	<i>Pseudomonas spp-1</i>	+	+	+	-	+	-	-	-	-	-	-	-
18.	<i>Pseudomonas spp-2</i>	+	+	+	+	+	-	-	-	-	-	-	-
19.	<i>Salmonella typhi-1</i>	+	+	+	+	+	-	+	-	+	-	-	-
20.	<i>Salmonella typhi-2</i>	+	+	+	-	+	-	+	-	+	-	-	-
21.	<i>Salmonella typhi-3</i>	+	+	+	+	+	-	+	-	+	-	-	-
22.	<i>Candida albicans</i>	+	+	+	+	-	-	-	-	-	-	-	-

#### 4. Conclusion

Thus summarizing these results, it is evident that methanol proved superior when compared to aqueous extract in this particular study. However, both exhibited antimicrobial activity and this may have occurred due to the synergic effect of their chemical constituents. Further, in depth toxicity and dosage may reveal its efficacy as an herbal drug.

#### 5. Conflict of Interest

We declare that we have no conflict of interest.

#### 6. Acknowledgement

The author is grateful for the valuable suggestion offered by Mr. Sathyanarayan, Miss. Punitavathy and Miss. Lavanya of Microbiology department. The financial assistance provided by the UGC (UPE-Phase II) from University of Madras is gratefully acknowledged.

#### 7. References

- Shrivastava S, Leelavathi S. Preliminary Phytochemical evaluation of leaf extracts of *Catunaregam spinosa* Thumb. International Journal of Pharmaceutical Sciences Review and research 2010; 3(2):114-118.
- Parekh J, Chanda S. *In vitro* Antimicrobial activity of some Indian folklore medicinal plants. Journal of Cell and Tissue research 2006; 6(1):577-580.
- Freire SMF, Emim AJS, Lapa AJ, Souccar C, Torres LMB. Analgesic and anti-inflammatory properties of *Scoparia dulcis* L. extract and glutinol in rodents. Phytother Res 1993; 7:408-414.
- Hayashi T, Okamura K, Tamada Y, Iida A, Fujita T, Morita N. A new chemotype of *Scoparia dulcis*. Phytochemistry 1993; 33: 349-352.
- Hayashi T, Kawaski M, Miwa Y, Taga T, Morita N. Antiviral agents of plant origin III. Scopadulin, a novel tetracyclic diterpene from *Scoparia dulcis* L. Chemical and Pharmaceutical Bulletin 1990; 38: 945-947.
- Tsai JC, Peng WH, Chiu TH, Huang SC, Huang TH, Lai ZR. Hepatoprotective effect of *Scoparia dulcis* on carbon tetrachloride induced acute liver injury in mice. American Journal of Chinese Medicine 2010; 38:761-775.
- Ahmed M, Shikha HA, Sadhu SK, Rahman MT, Datta BK. Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*. Pharmazie 2001; 56(8):657-60.
- Harborne JB. Phytochemical methods guide to modern Technique of Plant analysis. Edn 3, Chapman and Hall, London, 1998.
- Sofowora A. Medicinal plants and Traditional medicine in Africa. John Wiley and Sons, New York, 1993, 2:6-56.
- Trease GE, Evans WC. A textbook of Pharmacognosy. Edn 3, Bacilluere Tinal Ltd, London, 1989.
- Carson CF, Hammer KA, Riley TV. Broth micro-dilution method for determination of susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (Tea tree oil). Microbios 1995; 82:181-185.
- Mahfuzul HMD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S. Foodborne Pathog Dis 2007; 4(4):481-488.
- Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. J Ethnopharmacol 1999; 68:267-274.
- Parekh J, Chanda S. *In vitro* antimicrobial activities of extract of *Launaea procumbens* Roxb. (Labiatae), *Vitis vinifera* (Vitaceae) and *Cyperus rotundus* (Cyperaceae). Afr J Biomed Res 2006; 9:89-93.
- Yao J, Moellering R. Antibacterial agents. In: Manual of Clinical Microbiology, Murray P, Baron E, Pfaller M, Tenover F, Tenover R (Eds), ASM, Washington DC, pp: 1995, 1281-1290.

16. Md. Zulfiker AH, Siddiqua M, Laizuman N, Md. Razibul H, Nizamuddin, Nahid H, Md. Sohel R. *In vitro* antibacterial, antifungal & cytotoxic activity of *Scoparia dulcis* L. Int J Pharm Pharm Sci 2011; 3(2):198-203.
17. Latha KM, Ramkumar L, Pari PN, Damodaran V, Rajeshkannan, Suresh T. Phytochemical and Antimicrobial Study of an Antidiabetic Plant, *Scoparia dulcis* L. J Med Food 2006; 9 (3):391–394.
18. Jonathan Y. Phytochemical Analysis and Antimicrobial Activity of *Scoparia Dulcis* and *Nymphaea Lotus*. Australian Journal of Basic and Applied Sciences 2009; 3(4):3975-3979.
19. Stojanović-Radić Z, Nešić M, Čomić L, Radulo N. Antimicrobial activity and cytotoxicity of commercial rosemary essential oil (*Rosmarinus officinalis* L.). Biologica Nyssana 2010; 1(1-2):83.
20. Elumalai EK, Ramachandran M, Thirumalai T, Vinothkumar P. Antibacterial activity of various leaf extracts of *Merremia emarginata*. Asian Pacific J of Trop Biomed 2011; 1(5): 406-408.
21. Zulfiker AHM, Mahbubur RM, Hossain KM, Hamid K, Mazumder MEH, Rana SM. *In vivo* analgesic activity of ethanolic extracts of two medicinal plants - *Scoparia dulcis* L. and *Ficus racemosa* Linn. Biology and Medicine 2010; 2(2):42-48.