

Antibacterial Activity of Clerodendron Infortunatum and Scoparia Dulcis - A Comparative Study

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Abstract: *In vitro* antibacterial activity of ethnolic extract of leaves of *Clerodendron infortunatum* and whole herb of *Scoparia dulcis* were compared in the present study by agar-well diffusion method using two human pathogenic bacterial strains. The activity was measured by determining zone of exhibition. The zone of inhibition values were compared with the standard Gentamycin (20 mg/ml). *Streptococcus mutans* and *Pseudomonas aeruginosa* are the organisms used. The study conclude that ethnolics extract of *Scoparia dulcis* exhibited more antibacterial activity than ethnolic extract of *Clerodendron infortunatum*.

Keywords: *Clerodendron infortunatum*, *Scoparia dulcis*, Agar-well diffusion, Antibacterial activity.

I. Introduction:

Medicinal plants are natural resources yielding valuable herbal products which are often used in the treatment of various ailments. For this purpose the use of plant extracts in traditional medicine has been going on from ancient time. Herbalism and folk medicine, both ancient and modern, have been the source of much useful therapy. During the last twenty years renewed interest has emerged to help developing safer antimicrobial drugs from the natural sources, presumably due to the increasing development of drug resistance to human pathogenic organisms, as well as the appearance of undesirable side effects of certain antibiotics and the emergence of previously uncommon infections.

C. infortunatum is a flowering shrub and is so named because of its rather ugly leaf. The stem is erect, 0.5-4 high, with no branches and produce circular leaves with 6 inch diameter. Leaves are simple, opposite; both surfaces sparsely villous-pubescent elliptic, broadly elliptics, ovate or elongate ovate, 3.5-20 cm wide, 6-25 cm long, dentate, inflorescence in terminal, peduncled, few-flowered cyme; flowers white with purplish pink or dull purple throat, pubescent. Fruit berry, globose, turned bluish-black or black when ripe, enclosed in the red accrescent fruiting-calyx. The stem is hollow and the leaves are 6-8 inch (15-20 cm) long, borne in whorls of four on very short petioles. The inflorescence is huge consisting of many tubular snow white flowers in a terminal cluster up to 2 ft (0.6m) long. The tubes of the flowers are about 4 inch (10 cm) long and droop downward, and the expanded corollas are about 2 inch (5 cm) across. The fruits are attractive dark metallic blue drupes, about a half inch in diameter, fruit usually with 4 dry nutlets and the seeds may be with or without endosperm. It flowers from April to August [1]

The major constituents are sterols, sugars, flavonoids and saponins. Novel crystalline compounds such as clerodolone, clerodone, clerodol and a sterol designated clerosterol have been isolated from the root. Seven sugars namely raffinose, lactose, maltose, sucrose, galactose, glucose and fructose were identified [2]. Fumaric acid, caffeic acid esters, B-stiosterol and B-sitosterol glucoside were isolated from the flowers. [3] Apigenin, acacetin and a new flavone glycoside, characterised as the methyl ester of acacetin 7-O-glucuronide are isolated from the flowers. [4] Saponin is one of the major compounds of the leaf. [5] 24 beta-ethylsterols, clerosterol and 22-dihydroclerosterol, 24-methyl-sterols (24-methylcholestanol, 24-methylcholesterol, 24-methyl-22-dehydrochlesterol, and 24-methylathosterol) and 24 beta-ethyl-22-dehydrochlestanol are found in the seeds. [6] Scutellarin and hispidulin-7-O-glucuronide are present in the leaf. [7] Poriferasterol and stigmasterol are the components of the aerial parts. [8]

In Ayurvedic and Siddha traditional medicines, the leaves and roots of *C. Infortunatum* are used as herbal remedy for alopecia, asthma, cough, diarrhoea, rheumatism, fever and skin diseases. It is also known to have hepato-protective and antimicrobial activities.

Scoparia dulcis L, commonly known as sweet broom weed is a perennial herb widely distributed in tropical and subtropical regions. In these regions, fresh or dried *S. dulcis* plants have been traditionally used as remedies for stomach troubles, hypertension (9), diabetes, bronchitis (10) and as analgesic and antipyretic agents (11). In view of its high reputation and wide acceptance in ethnomedicine this plant has attracted not only wide publicity but also intensified research efforts by researchers (12). More recently, a number of the speculated medical values of *S. dulcis* have been validated by scientific research. These include hypoglycemic activity (13), antitumour promoting activity (14), antiviral activity (15), hyperlipidemic activity (16), antioxidant and analgesic activity (17). A significant analgesic activity was also demonstrated along

with the antihyperalgesic activity for *S. dulcis* decoction(18). Later it was investigated the antibacterial and antifungal activity of *S. dulcis*(19) by another author. Pure diterpene extracted from *S. dulcis* was reported to show cytotoxicity towards six human stomach cancer cell lines. Phytochemical screening of the herb revealed that it is rich in flavonoids and terpenes and the pharmacological actions of *S. dulcis* are believed to be due to the presence of these phytochemicals. The main chemicals include scopadulcic acids A and B, scopadiol, scopadulciol, scopadulin, scoparic acids A-C and betulinic acid. *S. dulcis* also contains coumarins, phenols, saponins, tannins, aminoacids, alkaloids, carbohydrates, glycosides. Some of these compounds are seems to be active against certain bacteria. This may leads to the traditional use as medicinal plants.

II. Materials and Methods:

Plant Material:-

- a) The plant *C. infortunatum* Linn. was collected from the campus of Government Medical College, Kottayam District of Kerala in February 2014 and identified at Jawaharlal Nehru Tropical Botanical Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram, Kerala as specimen No: TBGT No. 30757.
- b) The plant *S. dulcis* was collected from Nagarcoil District of Tamil Nadu in August 2013 and identified at JNTBGRI Palode, Thiruvananthapuram, Kerala as specimen No. TBGT No.26594

Preparation of Extract :-

- a) The leaves of *C. Infortunatum* was dried under shade and made in to coarse powder. Then the powdered plant material was extracted with 95% ethanol by maceration method. The extract was collected and solvent completely removed by distillation under reduced pressure. A semisolid to solid mass was obtained.
- b) The whole plant of *S. dulcis* was dried under shade and made in to coarse powder. Then the powdered material was extracted with 95% ethanol by maceration method and the solvent was completely removed by spray dried method.

Antimicrobial activity

Agar-well diffusion method

Principle

The antimicrobial constituents present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetre.

Reagents

1. Muller Hinton Agar Medium (1L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured on to 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 pressure (121°C) for 15 minutes.

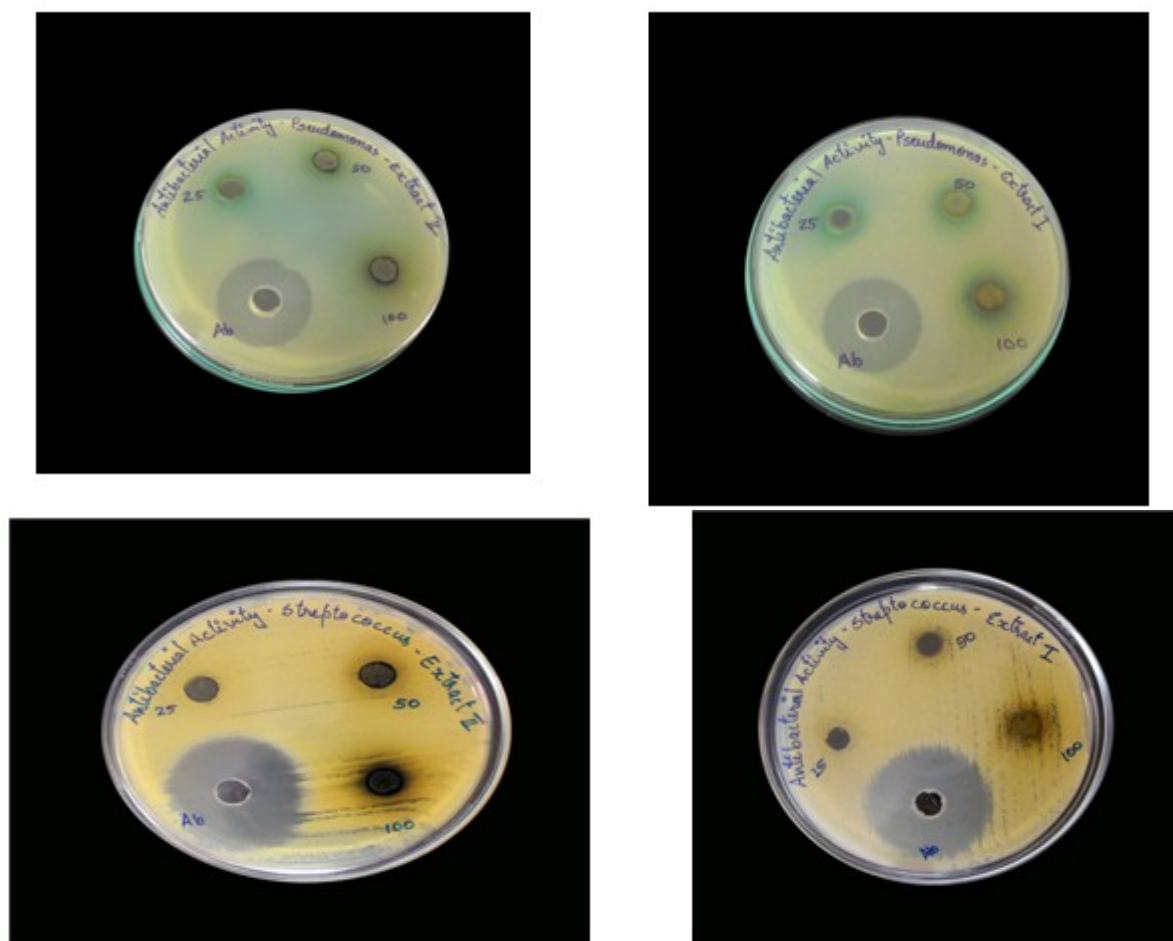
3. Gentamycin (standard antibacterial agent, concentration : 20 mg / ml)

Procedure

Petriplates containing 20ml Muller Hinton Medium were seeded with 24hr culture of bacterial strains of *Streptococcus mutans* and *Pseudomonas aeruginosa*. Wells of approximately 10mm was bored using a well cutter and 25µl, 50µl and 100 µl of samples were added to the well. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Gentamycin was used as a positive control.

Reference: National Committee for Clinical Laboratory Standards. (1993a). Performance Standards for Antimicrobial Disk Susceptibility Tests-Fifth Edition: Approved Standard M2-A5. NCCLS, Villanova, PA

III. Result



Extract	Product	Organisms
I	C.infortunatum	S. mutans and P. aeruginosa
II	S. dulcis	S. mutans and P. aeruginosa

Organism: Streptococcus mutans

Sample	Volume of Sample (µl)	Zone of inhibition (mm)
Gentamycin		33
EXTRACT 1		
(C. infortunatum)	25	Nil
	50	Nil
	100	Nil
EXTRACT 2		
(S. dulcis)	25	Nil
	50	Nil
	100	11

Organism: Pseudomonas aeruginosa

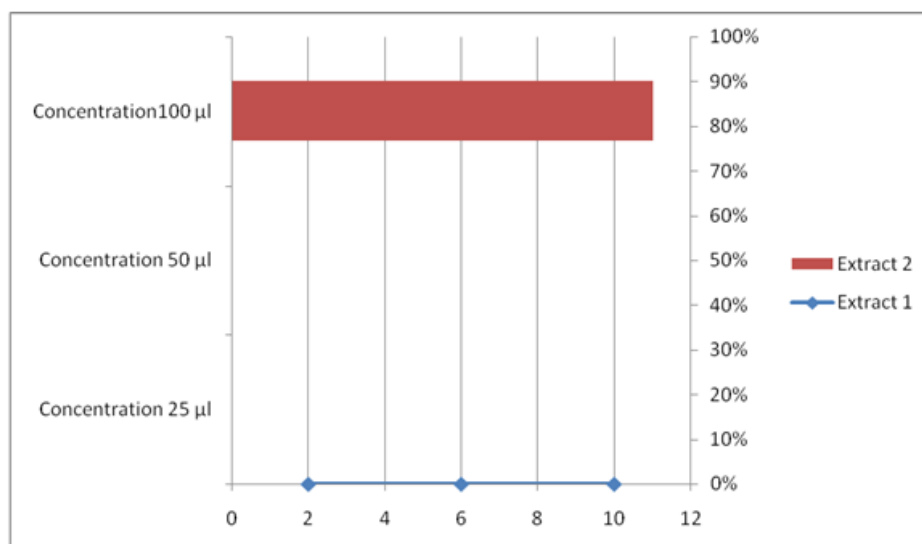
Sample	Volume of Sample (µl)	Zone of inhibition (mm)
Gentamycin		30
EXTRACT 1		
(C.infortunatum)	25	Nil

	50	Nil
	100	9
EXTRACT 2		
(S.dulcis)	25	Nil
	50	Nil
	100	10

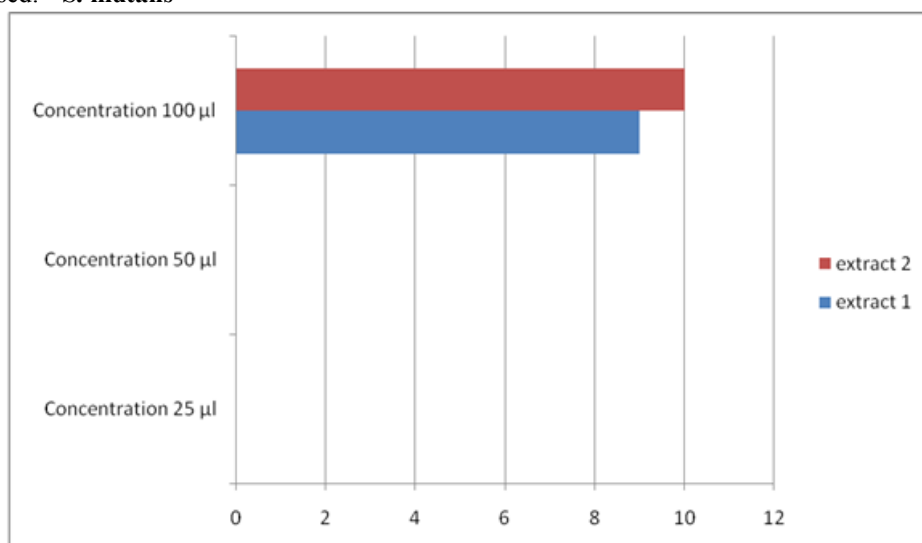
NOTE: sample concentration : 100mg/1ml

IV. Discussion:

The present study revealed that the ethnolic extract of both medical plants *Clerodendron infortunatum* and *Scoparia dulcis* has antibacterial activity, Antibacterial 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 path way through inhibition of key enzymes. In the present study the ethnolic extract of *Clerodundron infortunatum* showed average zone of inhibition of 9mm with *Paeroginosa* and nil zone of inhibition with *S. Mutans*. Ethnolic extract of *Scoparia dulcis* showed zone of inhibition of 10 mm with *P. aeroginosa* and 11 mm with *S. mutans*. Antibacterial activity against both gram positive (*S. mutans*) and gram negative (*P. aeroginosa*) bacteria may be indicative of the presence of broad spectrum antibiotic compound.



Organism used:- **S. mutans**



Organism used:- **P. aeroginosa**

V. Conclusion:

Based on the results it can be concluded that the ethnolic extract of *Scoparia dulcis* has potential antibacterial activity against both gram positive and gram negative organisms than the ethnolics extract of *Clerodendron infortunatum*. Hence crude extract of *Scoparia dulcis* may be utilized in the treatment of infectious diseases caused by the resistant organisms. The present study offers a scientific basis for traditional use of crude drugs. Further evaluation of the antibacterial properties of the plant extract against a more extensive panel of microbial agents is reasonable.

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