

## Original Research Article

### Efficacy of Ethanolic extract of *Solanum incanum* fruit extract for its antimicrobial activity

T.Indhumathi<sup>1\*</sup> and S.Mohandass<sup>2</sup>

<sup>1</sup>PG and Research Department of Biochemistry, Dr.N.G.P Arts and Science College, Coimbatore, Tamilnadu, India

<sup>2</sup>Principal, Rathinam Arts and Science College, Coimbatore, Tamilnadu, India

\*Corresponding author

#### A B S T R A C T

#### Keywords

Antibacterial activity, *Solanum incanum*, Minimum Inhibitory Concentration (MIC).

Plants have been an important source of medicine for thousands of years. Drugs may be obtained from various parts of the plant. So, an extensive study is required to detect the medical properties of the plant. Several medicinal plants have been tried against pathogenic microorganisms. The present study is to evaluate antibacterial activity of *Solanum incanum* fruit. The ethanolic extract of *Solanum incanum* fruit was used for analysis of minimum inhibitory concentration and zone of inhibition against gram positive and gram negative organisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Vibrio cholera*. Kirby-Bauer procedure was used for analysis and the cultures are grown in Muller-Hinton agar. From this study it was concluded that the MIC of *Solanum incanum* fruit against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Vibrio cholera* were found to be 500µg/ml and that for *bacillus subtilis* was found to be 250µg/ml and the zone of inhibition shown by the ethanol extract ranged from 10-26mm, at a concentration of 100µg/disc.

#### Introduction

*Solanum* species are the most potent plants against pathogenic microorganisms. *Solanum incanum* (L) is one of the important traditional medicinal plant belongs to Solanaceae family. Antibacterial activity of *Solanum incanum* was studied (John Britto and Senthilkumar 2001; Pavitra *et al.*, 2012) and presence of analysis of phytochemicals were also studied (Pavitra *et al.*, 2012). Other solanum species, *Solanum torvum* (leaf, stem and roots) showed

antibacterial and antifungal activity (Bari *et al.*, 2010) and antibacterial activity of *Solanum surattense* whole plant extract (Patil Suhas *et al.*, 2009) and leaf extract (Sheeba, 2010) were studied. Analysis, presence of phytochemicals and potent antibacterial activity of leaf, root and seed extracts were studied in *Solanum nigrum* (Sridhar *et al.*, 2011).

## Materials and Methods

### Collection of *Solanum incanum* fruit

*Solanum incanum* was collected from Sathyamangalam hills, Coimbatore, Tamilnadu. The plant was identified by Dr.G.V.S.Murthy, Scientist'F' & Head of Office, Botanical survey of India, Southern Regional Centre TNAU Campus, Coimbatore-03 with the number BSI/SRC/5/2/2012-13/Tech312. The fruits were collected from the plant and it was washed with water thoroughly to free from debris. The fruits were sliced and shade dried for 20 days. The dried fruit was ground coarsely and stored for further use.

### Preparation of ethanolic extract

The fruit was washed thoroughly and shade dried at room temperature. The dried fruit was subjected to size reduction to a coarse powder by using a dry grinder and passed through a sieve. Weighed 20 gm of powder in 100 ml of ethanol in a conical flask and kept in incubation shaker for two days and the extract was filtered through the filter paper and dried. This extract was used for the present studies.

### Qualitative phytochemical analysis

Identification of phytochemicals in the rhizome extracts are found by using the following tests. Chemical tests were carried out on the extracts of *Solanum incanum* using standard procedures, to identify the constituents as described below,

#### Alkaloids

##### Dragendorff's test

8 g of  $\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$  was dissolved in 20 ml of  $\text{HNO}_3$  and 2.72 g of potassium

iodide in 50 ml of water. These were mixed and allowed to stand when  $\text{KNO}_3$  crystals out. The supernatant was decanted off and made up to 100 ml with distilled water. The alkaloids were regenerated from the precipitate by treating with  $\text{Na}_2\text{CO}_3$  followed by extraction of the liberated base with ether. To 0.5 ml extract of *Solanum incanum* was added to 2.0 ml of HCL. To this acidic medium, 1.0 ml of reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloid.

##### Wagner's reagent (Iodine-potassium iodide solution)

1.2 g of iodide and 2.0 g of sulphuric acid and the solution was diluted to 100 ml, 10 ml extract of *solanum incanum* extract was acidified by adding 1.5% v/v HCL and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloid.

#### Flavonoids

In a test tube containing 0.5 ml extract of *Solanum incanum*, 5-10 drops of dilute HCL and small piece of ZnCL or Magnesium, were added and the solution was boiled for few minutes. In the presence of flavanoids, reddish pink or dirty brown colour was produced (Peach and Tracy, 2006).

#### Saponins

In a test tube containing about 5 ml extract of *Solanum incanum*, a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 mins. A honey comb like froth formed showed the presence of saponins.

## Reducing Sugar

### (a) Fehling's test

Solution A: 34.65g of copper sulphate was dissolved in water and made up to 500ml.

Solution B: 125g of 1.0ml extract of *Solanum incanum* mixture of equal parts of Fehling's solution A and B were added.

The contents were boiled for a few minutes. Formation of red or brick red precipitate indicates the presence of carbohydrates (Peach and Tracy, 2006).

### (b) Benedict's test

73g of sodium citrate and 100g of sodium carbonate were dissolved in 500ml water. To this solution 17.3g of copper sulphate dissolved in 100ml of water was added leaves. To 0.5ml extract of *Solanum incanum*, 5.0ml of Benedict's reagent and boiled for 5 minute. Formation of a bluish green showed the presence of carbohydrates. (Peach and Tracy, 2006).

## Proteins

### Million's Test

One part of mercury was digested with 2 parts of HNO<sub>3</sub> and the resulting solution was diluted with 2 volumes of water.

To a small quantity extract of *Solanum incanum*, 5 to 6 drops of water million's reagent was added. A white precipitate which turns red on heating, which indicates the presence of proteins.

## Phenols

### (a) Ferric chloride Test

To 1ml of *Solanum incanum* extract, 2.0ml

of distilled water followed by drops of 10% aqueous FeCl<sub>3</sub> solution were added. Formation of blue or green indicates the presence of phenols.

### (b) Lead acetate Test

1.0ml of *Solanum incanum* extract was diluted to 5.0ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed which indicates the presence of phenols.

### (c) Liebermann's Test

A small quantity extract of *Solanum incanum* was dissolved in 0.5ml of 20% sulphuric acid solution, followed by the addition of a few drops of aqueous sodium nitrate solution. A red colour was obtained on dilution and it turned blue when made alkaline with aqueous sodium hydroxide solution.

## Glycosides

A small amount extract of *solanum incanum* was dissolved in 1ml of water and aqueous sodium hydroxide solution was added. Formation of a yellow colour indicates the presence of glycosides.

## Terpenoids

5ml of *solanum incanum* extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown colouration of the interface formed shows the presence of terpenoids.

## Anthraquinone

### Borntrager's test

About 0.5 g of the extract was taken into a dry test tube and 5 mL of chloroform was

added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink violet or red color in the ammoniacal layer (lower layer) indicates the presence of anthraquinone.

### Test For Resins

Two grams of the ethanolic extract was dissolved in 10ml of acetic anhydride. A drop of concentrated sulphuric acid was added. Appearance of purple color, which rapidly changed to violet, was indicative of the presence of resins. Same procedure was repeated using the aqueous extract of the plant material.

### Test for steroid

0.5 ml methanolic fruit extracts were evaporated and dissolved in 2ml chloroform. 2ml of conc. H<sub>2</sub>SO<sub>4</sub> was introduced carefully by the side wall of the test tube. Formation of red colour ring confirmed the presence of steroid.

### Antimicrobial Activity

The crude ethanol extract and their fractions were tested for their antibacterial activity against five Gram positive and Gram negative bacteria by disc diffusion method. Five pathogenic bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Vibrio cholerae*.

### Minimum inhibitory concentration (MIC)

MIC of crude ethanol extract and their fractions were determined by serial dilution technique against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Vibrio cholerae*.

### Preparation of test drug:

Serial 2-fold dilutions of the test antimicrobial agent were made in 1ml of Muller Hinton Broth. Series of 10-15 dilutions to final concentrations of 100-1.56µg/ml are prepared.

### Preparation of inoculum:

Overnight culture are grown at 37°C Kirby-Bauer procedure and diluted to Muller Hinton Broth. This overnight culture was diluted to 10<sup>-2</sup>.

1. The sterile tubes were labeled 1-8 and 8<sup>th</sup> tube was taken as control.
2. 1ml of Muller Hinton Broth was transferred to all tubes except 6<sup>th</sup> & 7<sup>th</sup>.
3. 0.5ml of broth was transferred to 6<sup>th</sup> & 7<sup>th</sup> tubes.
4. 1ml of drug solution was added to 1<sup>st</sup> tube and mixed well.
5. From the 1<sup>st</sup> tube transfer 1ml of solution to the 2<sup>nd</sup> tube and was repeated up to 6<sup>th</sup> tube.
6. From the 6<sup>th</sup> tube 0.5ml of solution was taken and transferred to 7<sup>th</sup> tube.
7. 0.01ml of culture was added to all the test tubes.
8. All the tubes were incubated at 37°C for 18-24hrs.
9. After incubation observe the turbidity or OD value by Spectrophotometric method.

### Zone of Inhibition

The standardized inoculums is inoculated in the plates prepared earlier (aseptically) by dipping a sterile swab in the inoculums removing the excess of inoculums by passing by pressing and rotating the swab firmly against the side of the culture tube above the level of the liquid and finally streaking the swab all over the surface of the medium 3 times rotating the plate through an angle of 60°C

after each application. Finally pass the swab round the edge of the agar surface. Leave the inoculums to dry at room temperature with the lid closed.

Each Petri dish is divided into 2 parts, in one part extract disc such as SIC (100µg) disc (discs are soaked overnight in extract solution) and one quadrant for Standard Ciprofloxacin 10µg, are placed in each quadrant with the help of sterile forceps. Then Petri dishes are placed in the refrigerator at 4° C or at room temperature for 1 hour for diffusion. Incubate at 37 ° C for 24 hours. Observe the zone of inhibition produced by different Antibiotics. Measure it using a scale and record the average of two diameters of each zone of inhibition.

## Results and Discussion

### Phytochemical Analysis

The plant possesses numerous biologically active compounds which could serve as potential source of vegetable drugs in herbal medicine (Chukwuma *et al.*, 2010). It was reported that most of the plants of Solanaceae contain alkaloids, tannins, steroids, saponins, as well as reducing sugars (Amadi *et al.*, 2010). Our results of the qualitative phytochemical tests also confirmed that point. The fruit extract was identified to have alkaloids, flavonoids, phenols, carbohydrates, tannins, triterpenoids, glycosides, steroids, resins and saponins, which is illustrated in table 1.

### Antimicrobial Activity

#### Minimum inhibitory concentration (MIC)

In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju A V, *et al.*,

2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin M F, *et al.* 1985).

Phytochemicals are extensively found at different levels in many medicinal plants. Numerous plants used in traditional medicine are effective in treating various ailments caused by oxidative stress, bacterial and/or viral infections. Research has shown that medicinal plants exhibit antioxidant (Nam and Kang, 2004; Katalinic *et al.*, 2006; Kiselova *et al.*, 2006), as well as antimicrobial (Chan *et al.*, 2008) activity.

MIC of crude ethanol extract of *Solanum incanum* fruit and their fractions were determined by serial dilution technique against some gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Vibrio cholera*. The results for Minimum inhibitory concentration (MIC) of *Solanumincanum* fruit against pathogenic bacteria was represented in the following table 2 and figure 1a, 1b, 1c, 1d and 1e.

From this study, it was concluded that Minimum inhibitory concentration (MIC) of *Solanumincanum* fruit against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *Vibrio cholera* were found to be 500µg/ml. But Minimum inhibitory concentration (MIC) of *Solanumincanum* fruit against *Bacillus subtilis* was found to be 250µg/ml.

#### Zone of Inhibition

In the disc diffusion method, concentration gradients of the drug in a nutrient medium was prepared and grow of the bacteria, seeded in the medium after an inoculation period was observed.

**Table.1** Phytochemical analysis in ethanolic extract of *Solanum incanum* fruit

Sl no.	Test	Ethanolic fruit extract of <i>Solanum incanum</i>
1.	Alkaloids (a)Mayer's test (b)Dragendroff's test (c)Wagner's test	+ + +
2.	Flavanoids (a)Shinoda test	+
3.	Saponins	+
4.	Phenols (a)Ferric chloride test (b)Lead acetate test	+ +
5.	Steroids (a)Libermann Buchard test (b)Salkowski test	+ +
6.	Glycosides	+
7.	Resins	+
8.	Triterpenoid (a)Libermann Buchard test (b)Salkowski test	+
9.	Tannins (a)Ferric chloride test	+
10.	Anthraquinones (a)Borntrager's test	-
11.	Carbohydrates (a)Molisch's test	+

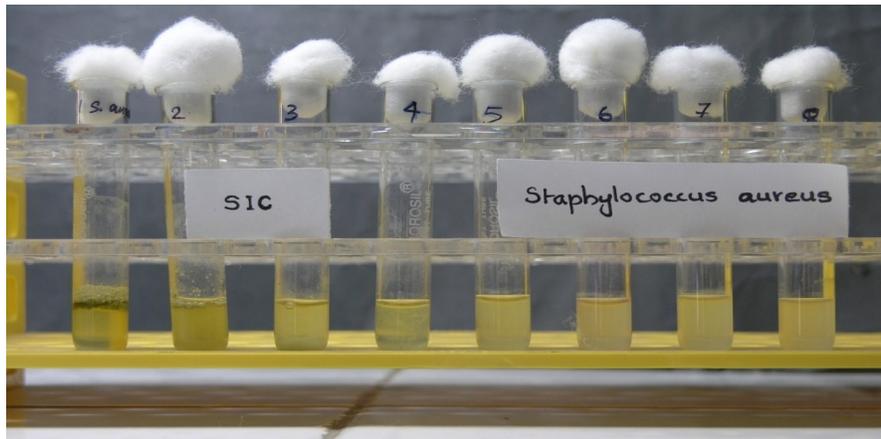
(+) presence

(-) absence

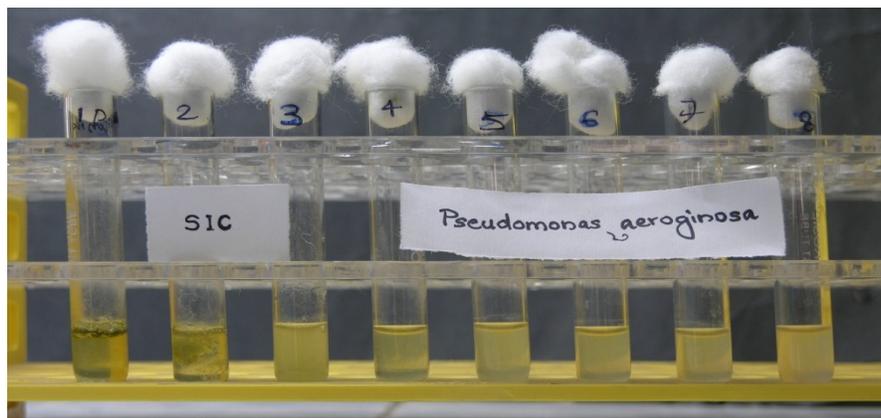
**Table.2** Minimum Inhibitory Concentration of *Solanum incanum* fruit extract against gram positive and gram negative bacteria at different concentration

S.NO	ORGANISMS	1000 µg/ml	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml	15.625 µg/ml
1.	<i>Staphylococcus aureus</i>	-	-	+	+	+	+	+
2.	<i>Bacillus subtilis</i>	-	-	-	+	+	+	+
3.	<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	+
4.	<i>Salmonella paratyphi</i>	-	-	+	+	+	+	+
5.	<i>Vibrio cholerae</i>	-	-	+	+	+	+	+

**Figure.1a** MIC of *Staphylococcus aureus*



**Figure.1b** MIC of *Pseudomonas aeruginosa*



**Figure 1c:** MIC of *Salmonella paratyphi*



**Figure.1d** MIC of *Vibrio cholerae*



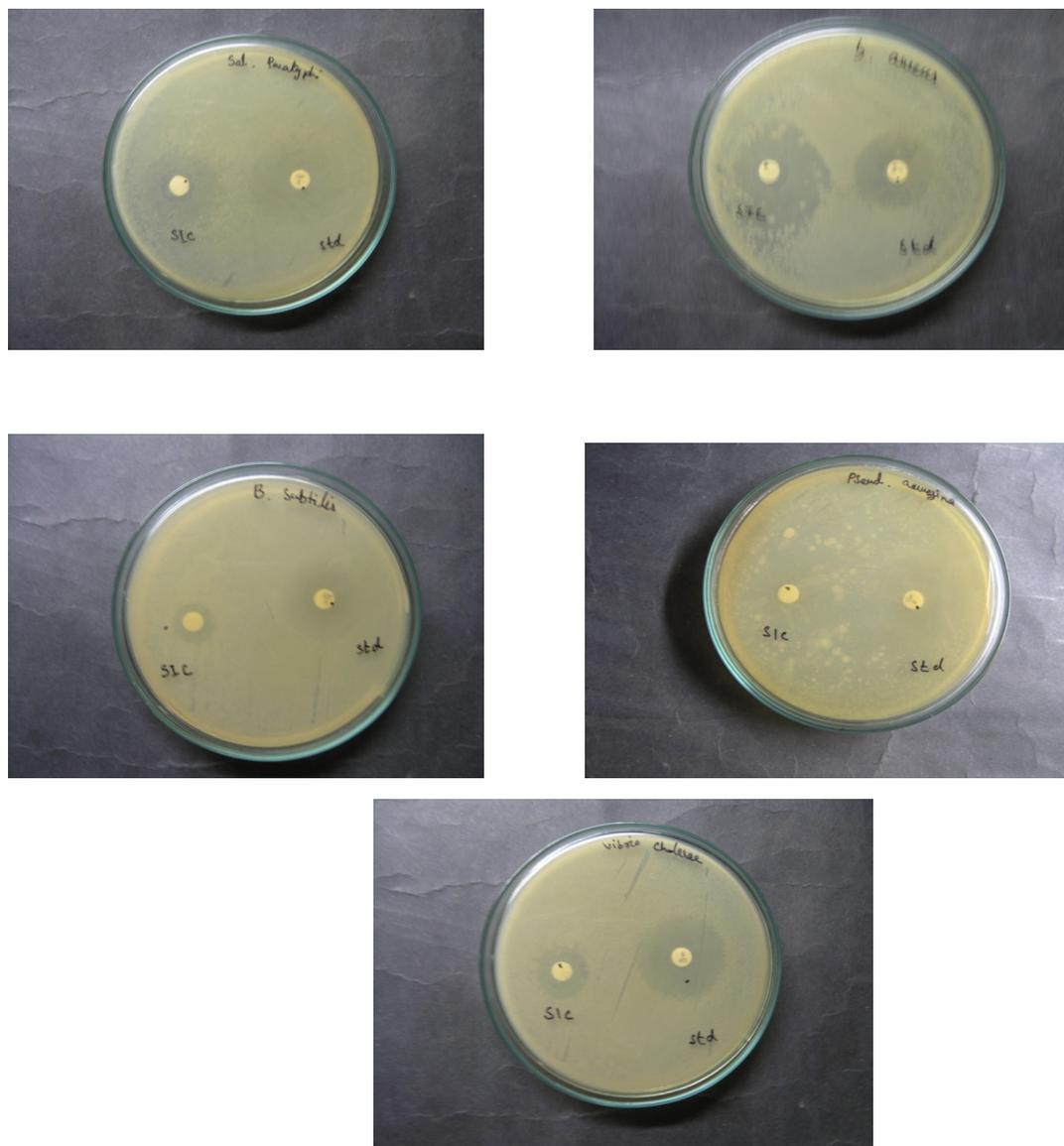
**Figure.1e** MIC of *Bacillus subtilis*



**Table.3** Zone of Inhibition of *Solanum incanum* fruit against human pathogens

S.NO	ORGANISMS	CIPROFLOXACIN(5µg/disc)(Standard )	SAMPLE(100µg/disc)
1.	<i>Staphylococcus aureus</i>	24mm	26mm
2.	<i>Bacillus subtilis</i>	25mm	12mm
3.	<i>Pseudomonas aeruginosa</i>	16mm	10mm
4.	<i>Salmonella paratyphi</i>	29mm	25mm
5.	<i>Vibrio cholerae</i>	28mm	17mm

**Figure.2** Zone of Inhibition of *solanum incanum* fruit against human pathogens



The clear zone of growth inhibition was noted around the disc due to diffusion of drug and growth of bacteria.

The diameter of the zone denotes the relative susceptibility of the test microorganism to a particular antimicrobial. The term susceptible implies that an infection caused by strain tested may be expected to respond favorably to the indicated antimicrobial agent for that type of infection and pathogen.

The results of antibacterial activity of crude ethanol extract of the fruit of *Solanum incanum* was presented in Table 3. The zone of inhibition produced by the crude ethanol extract was ranged from 10-26 mm, at a concentration of 100 µg/disc. The crude ethanol extract showed the highest antibacterial activity (26 mm) against *Staphylococcus aureus* and also showed good antibacterial activity (10-25 mm) against all pathogenic bacteria.

The figure 2 shows the maximum antibacterial activity of ethanolic extract of *Solanum incanum* fruit against *Staphylococcus aureus* and *Salmonella paratyphi* compared with other organisms such as *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Vibrio cholera*.

The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we

found in this study that the plant extracts by alcohol (ethanol) provided more consistent antibacterial activity compared to those extracted by water. The zone of inhibition produced by the crude ethanol extract was ranged from 10-26 mm, at a concentration of 100 µg/disc.

The antimicrobial activity of *Solanum incanum* fruit may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

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