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Antimicrobial Potential of Petroleum Ether Extract and Active Column Fractions of the *Solanum incanum* Leaves

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Solanum incanum **belongs to Solanaceae family and are extensively used in the treatment of stomachache, colic, headache, pneumonia, rheumatism and other pathological conditions. The therapeutic activity of the *solanum incanum* has been attributed to their content present in it. Phytoconstituents analysis showed the presence of Saponins, Tannins, Alkaloids and Flavonoids. Anti microbial activity of extracts from *solanum incanum* leaf has been evaluated against both gram positive and gram negative microorganisms such as *E.coli, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Bacillus subtilis, and Bacillus aureus*. Petroleum ether extract showed excellent antibacterial activity against *E. coli* (18 mm), *P. aeruginosa* (16 mm) and *B. Subtilis* (15 mm) and the MIC and MBC of *Bacillus subtilis* was found to be 7.81 µg/ml and 3.91 µg/ml respectively.**

Key words: Antimicrobial activity, *Solanum incanum*, Phytoconstituents, Solvent extracts.

Traditional cures and plant-based remedies remain the main solution to health problems in many developing countries[1]. Medicinal plants usefulness was estimated that over 80% of developing countries populations have resorted to traditional medicine. This need to use traditional medicine can be explained for the poverty of our populations, the lack of medical facilities and doctors, the religion and above all microbe resistance in relation to the modern medicine[2].

Solanum is the biggest and the most complex genus of the Solanaceae family[3]. The family Solanaceae is composed of about 90 genera and between 2,000 to 3,000 species. Examples of food plants in the *Solanaceae* are potato (*S. tuberosum*), aubergine (*S. melongena),* jasmine nightshade (*S. jasminoides*) and naranjilla (*S. psuedocapsicum*)**.** *Solanum incanum L* (Linnaeus) belongs to Solanaceae family commonly known as the Thorn Apple and Bitter Apple. Many of the medicinal uses of *Solanum incanum* L are based on its analgesic properties[4]. It is effective in throat disorders like sore throat, angina, stomachache, colic, headache, painful menstruation, benign tumours[5] as well as liver pain, pain caused by onchocerciasis, pneumonia, pleurisy, and rheumatism.

It is also useful in the treatment of skin problems, including skin infections, burn and in the treatment of cough, cold and as an expectorant[6]. In Southern Africa, the plant has been found to be effective in the treatment of a variety of external benign tumors in veterinary practice[7]. The fruit is a berry or a capsule and it is used for the treatment of toothache and chest complaint[8]. Though the *S. incanum* is extensively used for pain and fever management much of the study has centered on its anti-microbial[7] and anti-tumor[9, 10].

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MATERIALS AND METHODS

Plant material

Different parts of *Solanum incanum L.* including root, stem, leaves and flowers, were collected from a locality in Tenkasi (Tamilnadu). The sample was authenticated by Botanical Survey of India (BSI), Ministry of environmental & forests, Tamilnadu agriculture university campus, Coimbatore and voucher No-BSI/SRC/5/23/2010-11/Tech/1605. After collection, the fresh leaves of the plant were air-dried at room temperature. Dried leaves were made into powder and used for the extraction, isolation of active constituents as per standard methodology.

Microorganisms

The common pathogenic six microorganisms were used in these studies. In this three gram negative microorganisms were used such as *E. coli* (NCIM 2256), *P. aeruginosa* (NCIM 2037), *Serratia marcescens* (NCIM 2078) and three gram positive microorganisms such as *Micrococcus luteus* (NCIM 2871), *Bacillus subtilis* (NCIM 2710) and *Staphylococcus aureus* (NCIM 2794). All the test strains were collected from National Collection of Industrial Microorganisms (NCIM).

Extraction

About 900 g of dry sample powder was weighed and extracted in soxhlet extractor with 1500 ml of Petroleum ether, The extract was collected after filtration using Whatman No.1 filter paper and concentrated by rotary evaporator, which were used for further Phytochemical analysis[11,]

Column chromatography

10 g of the petroleum ether extract of *Solanum incanum* were chromatographed over silica gel column (100 - 200 mesh). The admixture was packed on a silica gel column and eluted start with 100% Hexane, then added increase with solvent polarity Chloroform, Ethyl acetate, Ethanol and methanol in the ratio of 90:10, 80:20 70:30, 50:50, 30:70, 20:80, 10:90. Based on TLC profile, the elutes were pooled into the same fractions. Further Column packed and elutes fraction (120-125) with Ethyl acetate: Ethanol (90:10) gave a colorless compound and further purification of the isolated compound was performed with Acetone and Methanol.

Thin Layer Chromatography

The TLC development was set at Twin through chamber were examined in various solvent systems. The optimal solvent for the separation was determined. The silica gel 60 F254 pre-coated aluminium plate, of 0.2 mm thickness, using Toluene: Ethyl acetate (5:5) as the developing solvent system. Visualization was carried out by dipping the plate in vanillin sulfuric acid (1%) and heat on 105° C when the color of the spot appeared distinctly.

The formula for calculating the Rf values is

Rf value = Distance moved by the molecule (location of the spot)/ Distance moved by the mobile phase (solvent front)

Antibacterial Activity

Agar disc diffusion assay

The antibacterial activity of the *Solanum incanum* leaf extracts was determined by the disc diffusion method. Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 µl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. Filter paper discs (Whatman No. 1, 6 mm diameter) were placed on the inoculated agar surface. A 20 µl of the extracts (500 µg/ml, 250 µg/ml and 125 µg/ml) and column fractions were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotic gentamicin (10 µg) was placed as control. Plates were incubated at 37°C for 24 h. The antibacterial activity was determined by measuring the inhibition zone. All the analysis was performed in triplicate.

Determination of minimum inhibitory concentration

A minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18-24 h. The extracts that showed antibacterial activity were subjected to the serial broth dilution technique to determine their minimum inhibitory concentration. Briefly, the stock solutions of the extracts were subjected to two-fold serial dilution in the Muller-Hinton broth to obtain concentrations from 500 µg/ml to 7.81 µg/ml. Standard antibiotic gentamicin was placed as

control. A 10 µl of 10^7 (CFU) bacterial cultures were added to the tubes and were incubated at 37° C for 18 h. MIC was determined by visual observation. The minimum concentration of the extracts that showed no detectable growth was taken as the minimum inhibitory concentration[12].

Determination of minimum bactericidal concentration

A minimum bactericidal concentration (MBC) is the lowest concentration of an antibiotic required to kill a microorganism. The MBC was determined by sub-culturing 10 µl of the test dilutions from MIC tubes on to fresh Mueller-Hinton agar plates. Plates were incubated for 18-24 h. The highest dilution that yielded no single bacterial colony on the plates was recorded as MBC.

RESULTS AND DISCUSSION

Column chromatography

The dried powdered sample of *solanum incanum L*. was subjected to petroleum ether extraction. About 10 g of the extract was subjected to column chromatography on silica gel. The selection of solvents in a systematic order proves the effect of polarity on the extraction and the extracted phytochemicals. About 135 fractions with different R_f values were separated during column chromatography and they were tabulated in Table 1.

Table 1. R_f values of petroleum ether extraction fractions of *Solanum incanum L*

Fractions	R_f values
1-8	-
9-14	0.25, 0.36
15-20	0.22, 0.25, 0.31
21-26	0.18, 0.25, 0.32
27-35	0.31, 0.45, 0.46, 0.66, 0.67
36-42	0.25, 0.42, 0.45, 0.66, 0.68
43-49	0.13, 0.14, 0.15, 0.33, 0.35
50-56	0.56, 0.57, 0.58, 0.59, 0.66
57-63	0.57, 0.58, 0.60, 0.71, 0.88, 0.90
64-69	0.32, 0.44, 0.50, 0.54, 0.80
70-75	0.19, 0.25, 0.44
76-81	0.25, 0.31, 0.38, 0.42
82-87	0.22, 0.32, 0.40, 0.44
88-94	0.20, 0.25, 0.31, 0.76
95-100	0.21, 0.25, 0.32
101-105	0.25, 0.32, 0.36
106-110	0.20, 0.24, 0.35
111-115	0.60
116-120	0.42
121-125	0.62, 0.69
126-130	0.60, 0.72
131-135	0.45, 0.61

Thin Layer Chromatography

Column fractions of *solanum incanum* were subjected to various qualitative phytochemical tests like carbohydrates, tannin, saponin, alkaloid, quinines, glycosides, terpenoids, phenols and coumarin as per standard procedure[13, 14]. It was evident that the fractions of 1 -20 showed positive results for alkaloid and saponin and the fractions of 21–35, 39-49 showed the positive result for

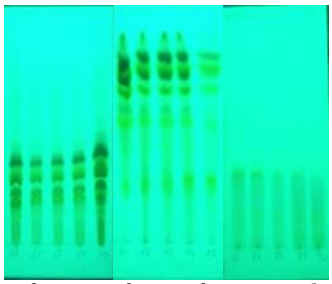

Fig. 1. Identification of spots fraction under UV 254 nm

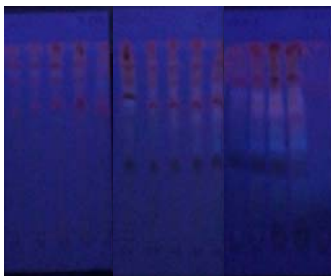

Fig. 2. Identification of spots fraction under UV 366 nm

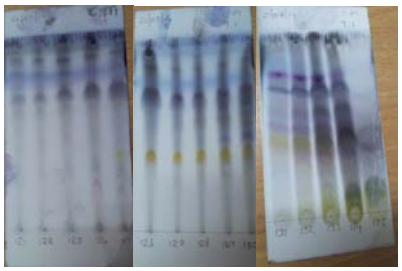

Fig. 3. Identification of spots fraction under visible light at 512 nm

alkaloid, coumarin. It was also apparent that the fractions of 50-69, 70-94 showed the positive result for alkaloids, terpenoids, coumarin but the fractions of 121 to 135 had tannins was shown in Table 2. Identification of spots fraction under UV 254 nm, 366 nm and in visible light at 512 nm was shown in Fig 1-3.

According to the phytochemical investigation of column fractions of petroleum ether extract, the three fractions B and E were selected for the antimicrobial investigation due to the presence of more active constituents such as flavonoids, terpenoids, tannins and alkaloids, coumarin than the other fractions. These bioactive components are naturally occurring in most plant materials, known to be bactericidal thus conferring the antimicrobial property to plants.

Antibacterial activity of petroleum ether extract and their column fractions (B and E) of *solanum incanum* leaves were analyzed by agar disc diffusion method. According to the results obtained in this analysis, zone of inhibition was produced in petroleum ether extract (500 µg/ml) against different microorganisms in the range of 12-18 mm, 250 µg/ml extract was shown the zone of inhibition 11-16 mm and 125 µg/ml extract produced 11-12 mm (Table 3). Column fraction B was exhibited more antibacterial activity against *B. subtilis*, *P. aeruginosa* and their zone of inhibition was found to be 17 mm, 15 mm

Table 2. Phytochemical analysis of column fractions of *Solanum incanum*

Phytochemical test	Inference of Column Fractions of *Solanum incanum*						
	1-20(A)	21-35(B)	39-49(C)	50-69(D)	70-94(E)	95-120(F)	121-135(G)
Carbohydrates	-	-	+	-	-	+	+
Tannins test	-	+	+	-	+	-	-
Saponin test	+	-	-	-	-	-	-
Alkaloid test	+	+	-	+	+	-	-
Quinones	-	-	-	-	-	-	-
Glycosides test	-	-	-	-	-	-	-
Cardiac glycosides test	-	-	-	-	-	+	-
Flavonoids	-	+	-		+	-	-
Terpenoids test	-	+	-	-	+	-	-
Triterpenoids	-	-	-	-	-	-	-
Phenols	-	-	-	-	-	+	-
Coumarins	-	+	+	-	+	-	-

Table 3. Zone of inhibition produced by the extracts of *Solanum incanum L*

Extract conc (µg/ml)	Zone of inhibition in mm					
	E.coli	*S. aureus*	*P.aeruginosa*	*B.subtilis*	*S. marcescens*	*M.luteus*
Pet ether 500 µg/ml	18	14	16	15	14	12
250 µg/ml	16	12	15	13	12	11
125 µg/ml	12	11	-	12	-	-
Fraction B500 µg/ml	14	13	15	17	16	14
250 µg/ml	10	12	11	15	8	9
125 µg/ml	9	-	-	12	-	-
Fraction E500 µg/ml	15	13	12	10	15	14
250 µg/ml	12	11	10	9	12	12
125 µg/ml	10	9	-	7	8	7
Gentamicin 10 µg/ml	22	20	21	21	23	20

- = No inhibition, *P. aeruginosa* = *Pseudomonas aeruginosa* (NCIM 2037), *S. aureus* = *Staphylococcus aureus* (NCIM 2794). *E. coli*= *Escherichia coli* (NCIM 2710), *B. Subtilis*=*Bacillus subtilis* (NCIM 2710), *S. marcescens* = *Serratia marcescens* (NCIM 2078), *M. luteus* = *Micrococcus luteus* (NCIM 2871).

Table 4. Minimum Inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *solanum incanum* L leaves

Extracts	MIC and MBC of different extracts of *solanum incanum* L (µg/ml) *E. coli*	*S. aureus*	*P. aeruginosa*	*B. subtilis*	*S. marcescens*	*M.luteus*
Pet. ether MIC	31.25	31.25	62.5	3.91	15.62	31.25
MBC	62.5	62.5	125	7.81	31.25	62.50
Fraction BMIC	62.5	250	-	62.5	15.62	15.62
MBC	125	125	-	125	31.25	31.25
Fraction EMIC	62.5	31.25	31.25	62.5	-	62.5
MBC	31.25	15.62	15.62	31.25	-	31.25

respectively. Column fraction E was produced the zone of inhibition 15 mm, 13 mm against *E. coli, B. subtilis* respectively. Overall antibacterial activity of *solanum incanum* extracts against all microorganisms was found to have better activity than the *S. marcescens* and *M. luteus*. The observed antibacterial activity is attributed to the presence of bioactive compounds in the extracts. The presence of these bioactive compounds in crude extracts is known to confer antibacterial activity against disease-causing microorganisms [15] and offer protection to plants themselves against pathogenic microbial infections[16].

The lowest value of MIC and MBC of petroleum ether extract against *Bacillus subtilis* was found to be 3.91 µg/ml and 7.81 µg/ml respectively, in case of B fraction was produced the highest value of MIC and MBC against *staphylococcus. aureus* such as 250 µg/ml and 125 µg/ml respectively (Table 4).This analysis was revealed the presence antimicrobial constituents in this plant.

Phytochemicals are secondary metabolites produced by all plants in which some has medicinal uses. The phytochemical screening revealed the extract richness in Tannins, Phlobatannin, Saponins, Flavonoids, Steroids and Alkaloids. Quantitative analysis of phenolics, alkaloids, saponins and flavonoids had revealed that Mentha spicata possessed maximum phenolic (18.41 %), Gmelinaarborea highest alkaloids (5.66 %) & flavonoids (22.80 %) and Trigonella foenum-graecum highest saponin (50.12 %) contents. Quantitative analysis of total polyphenols, tannins, proanthocyanidins and flavonoids in 20 Serbian and Chinese cultivars of Soybean (Glycine max L.) was performed.

CONCLUSION

The present analysis has revealed that the active constituents extracted from the *solanum incanum* L have high antimicrobial activity against both gram positive and gram negative microorganisms when compared with the standard drug such as gentamicin.The evidence of the present analysis also proved that the plant extracts would be an alternate antimicrobial activity than the chemical substances.

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