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*Research Article*

# EVALUATION OF ANTI-MICROBIAL ACTIVITY OF *IPOMOEARENIFORMIS* METHANOLIC EXTRACT

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**ABSTRACT**

The present study was conducted to investigate anti-microbial activity of *Ipomoea reniformis* methanolic extract against strains of gram positive and gram negative bacteria. Tulsi plant is known to possess therapeutic potentials and have been used, by traditional medicinal practitioners, in cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose and kidney, epileptic, seizures, purgative and anti-microbial.The extract was tested for its antimicrobial activity against Gram-positive bacteria like *Bacillus subtilis* and Gram-negative bacteria like *Escherichia coli.* Inhibition of microbial growth was investigated using agar well diffusion method. HPTLC analysis of the extract was carried out for the presence of Scopoletin.

**Keywords:** Antimicrobial, Scopoletin, *Ipomoea reniformis*, Well diffusion method, HPTLC.

## INTRODUCTION

The anti-biotic era started in the 1950s, and from then onwards the use of plant anti-microbial declined[1]. Although, it was not the case so far as the traditional healing systems that heavily rely on the medicines from the natural sources, especially plants, are concerned. The emergence and spread of microbial resistance is growing each day, thereby necessitating the development of new anti-microbial of natural or synthetic origin[2]. As far as the sources are concerned, apart from the microbial sources, plants appear to be valuable anti-microbial resources. Plants can produce a large number of secondary metabolites that may exceed a hundred thousand molecules[3], all of these don't have anti-microbial potential, but some of them can produce significant activity against the human pathogens. One of the species that emerged from such an inventory is *Ipomoea reniformis*(Family:Convolvulaceae)[4]. *Ipomoea reniformis* chois (Convolvulaceae) is a perennial, much branched herb (creeper). It is found widely distributed all over the India, specially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa [1-7, 9] . *Ipomoea reniformis* is also known as *Merremia emarginata* Hallier f. In India it is known by various names in different regions viz., Mooshakarni in Sanskrit, Underkani in Bombay, Indurkani in Bengal, Underakani in Gujarat, Toinnuatali in Telugu, Chukakani in Urdu, Goromusha in Persian, Mushkani in Hindi, Paerattae-kirae in Tamil, Yellikkadukirai in Madras [2, 5,7, 8,10] . It is adulterated with *Centella asiatica* [5]. It is reported to have many important medicinal properties. In the Indigenous system of Medicine, *Ipomoea reniformis* has been claimed to be useful for cough, headache, neuralgia, rheumatism, diuretic, inflammation, troubles of nose, fever due to enlargement of liver and also in kidney diseases. Powder of leaves is used as a snuff during epileptic seizures, Juice acts as purgative and the root is having diuretic, laxative, and applied in the disease of the eyes and gums. The whole plant decoction is mainly responsible

for its medicinal uses [7, 8, 9, 10, 11]. Scopoletin has been isolated from the methanolic extract of whole herb of *Ipomoea reniformis* and has been quantified.Scopoletin has been used as an important bioactive marker and quantified in whole herb of *Ipomoea reniformis*. Scopoletin belongs to the class of Coumarin (6-methoxy-7-hydroxy coumarin) type of compounds and is reported in at least 27 families [12] including Convolvulaceae members like *Convolvulus pluricaulis, Evolvulus alsinoides, Clitoria ternatea, and Canscora decussata* [12]. Scopoletin is a fluorescent compound with $\lambda_{max}$ 230, 254, 260, 298, 346nm [13]. Scopoletin is reported to possess anti-inflammatory, immunomodulatory, anti-microbial and anti-oxidant activity [14-16]. The present investigation reports anti-microbial effect of methanolic extract of whole herb of *Ipomoea reniformis*. Although, *Ipomoea reniformis* growing all over the India, but little work is done on the chemical examination of it. The aim of this study was the antimicrobial activities of methanolic extract of *Ipomoea reniformis* against range spoilage bacteria, evaluating zone of inhibition.

## MATERIAL AND METHODS

### Plant Material

The whole herb of *Ipomoea reniformis* was collected from the botanical garden of the Shivam Pharmaceutical Studies & Research Centre, Valasan. The drug was authenticated at the Pharmacognosy department of the shivam pharmacy college, Valasan. A Voucher specimen (MKB/Ir-55/SPSRC-2013) has been deposited to Shivam Pharmacy College, valsan.

### Preparation of the Extracts

The shade dried, powdered whole herb (250gm) of *Ipomoea reniformis* were defatted by extracting with petroleum ether (60-80°C), followed by extraction with methanol using Soxhlet's extractor. The methanolic extract was then concentrated using rotary flash evaporator to a syrupy consistency. The residual solvent was removed by drying the extract in vacuum oven (yield - 25.5gm).

### Microorganisms

The following bacterial strains were used in the antimicrobial tests. Gram positive bacteria *Bacillus subtilis* (ATCC 6633). Gram negative bacteria were *Escherichia coli*. All microbial strains were obtained from the Bio-science department Vallabh Vidyanagar. In vitro antimicrobial activity was determined by using nutrient agar (Himedia Laboratories Pvt. Ltd., Mumbai). Each medium was autoclaved at 121° C, 15 psi for 15 min before inoculation. The bacteria used in the tests were obtained from 24 h cultures.

### Antimicrobial Activity

Antimicrobial activity of methanolic extract was determined using agar well diffusion method. About 15ml of sterilized selective agar based mediums were added aseptically to sterile plates to prepare a basal layer. The plates were incubated at 37° C ±0.5° C for 24 hrs. The basal layer was seeded the next day with 7ml of sterilized selective agar based medium containing 1ml of suspension of standard inoculums. The plates were allowed to set. Each Petri dish was divided into four sectors, and in each sector a bore of 6mm diameter was made using sterilized borer in the solidified medium. Using sterilized dropping pipettes, each bore in different sector was carefully loaded with 75µl of Methanolic extract of *Ipomoea reniformis* and allowed to diffuse at room temperature for 2 hrs. The plates were then incubated at 37°C for 24 hrs for bacteria. The zone of inhibition of growth of microorganisms around the well was measured in mm, with the help of a scale.

### HPTLC analysis of the Methanolic extract

### Selection of chromatographic condition

Proper selection of HPTLC method depends upon the nature of sample (ionic, ionizable or neutral molecule), molecular weight and solubility. To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase composition and solvent ratio were studied. The resulting chromatograms were recorded and chromatographic retention factor and resolution were calculated. The conditions that gave best resolution and retention factor were selected for estimation.

### Selection of detecting wavelength

The sensitivity of HPTLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drugs that are to be detected.

In the present study standard solution of 200-1000ng/spot of pure Scopoletin was prepared in methanol. Both standard and sample solution were then scanned in the UV region of 200-400nm and the overlain spectrums were recorded.

**Reagents and materials**

Stationary phase: Precoated HPTLC aluminum sheet (MERCK) silica gel $60F_{254}$ (10cm x10cm)
Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)
Reference standard: Scopoletin (Sigma Aldrich, USA, Dutta enterprise, Anand)
Test sample: Methanolic extract of *Ipomoea reniformis* (MEIR)

**Instrument**

A Camag TLC system comprising of

- Camag Linomat V, a sample applicator as bands using the spray on technique, Camag Switzerland
- Camag Hamilton 100µl HPTLC syringe
- Camag twin through chamber (20 x 10 $cm^2$)
- UV cabinet ( Chromatogram inspection under short wave and long wave UV light)
- Camag TLC scanner III (chromatogram evaluation by classical densitometry)
- Camag WINCATS software (version 4.06)
- Camag reprostar (image acquisition, documentation by conventional photography)
- Analytical Balance
  Model: CP124S
  Make: Sartorious GOTTINGEN AG, Germany ISO 9001 certified
  Maximum: 120g
- Sonicator:
  Model: Fast Clean Ultrasonic Cleaner
  Make: Enertech electronics Pvt. Ltd.

**Chromatographic conditions**

Stationary phase: Pre coated HPTLC aluminum sheet (MERCK) Silica gel 60 $F_{254}$ (10 cm X 10 cm)
Mobile phase: Toluene: Ethyl acetate: Formic acid (5:4:1)
Spotting volume for standard: 2µl, 4µl, 6µl, 8µl, 10µl
Spotting volume for test: 8µl
Chamber saturation time: 45 mins.
Chromatographic technique: Ascending
Temperature: 37ºC
Wavelength: 278nm and 366nm

**Preparation of standard solution**

10mg of Scopoletin was dissolved in 10 ml of methanol (1mg/ml=1000ng/µl). From this stock solution standard solutions of 20-100µg/ml was prepared by transferring aliquots (0.2 to 1ml) of stock solution to 10 ml volumetric flask and adjusting the volume to 10 ml with methanol. The concentrations of Scopoletin were 200, 400, 600, 800 and 1000ng/spot.

**Calibration curve for Scopoletin**

2 µl to 10 µl of each of the standard solution containing 200 to 1000 ng/spot Scopoletin respectively were applied on a HPTLC plate. The plate was developed in a mobile phase, Toluene: Ethyl acetate: Formic acid (5:4:1) and scanned at 278nm and 366nm. Calibration curve of Scopoletin was prepared by plotting peak area vs. concentration of Scopoletin applied.

**RESULT AND DISCUSSION**

**Antimicrobial Activity**

The minimum inhibitory concentration (MIC) of the methanolic extract against different microorganisms is tabulated in Table 1. The studied concentration of the methanolic extract was 5 mg exhibited antimicrobial activity against the test microorganisms with zone sizes 4.5cm & 3.1 respectively. The minimum inhibitory concentration of the methanolic extract was found 70 & 49 mg respectively against the different test organisms. The photographs of the plates exhibiting antimicrobial activity against the different test microorganisms are shown in Figure1 & 2 respectively.

**HPTLC analysis**

HPTLC Chromatogram of standard scopoletin and *Ipomoea reniformis* extract shows the Peak of same $R_f$ value. (Figure 3).

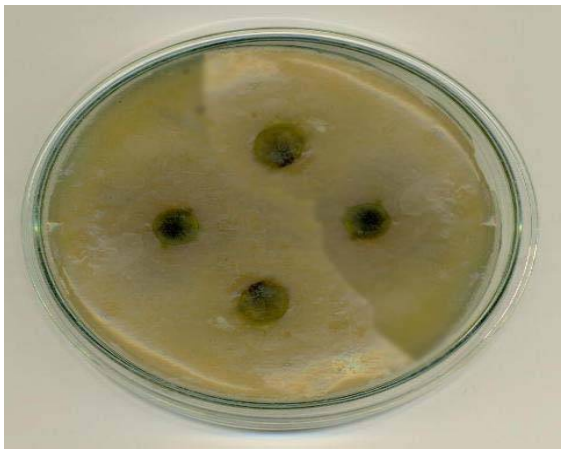

**Fig. 1: Effect methanolic Extract of *Ipomoea reniformis* on *E. coli***

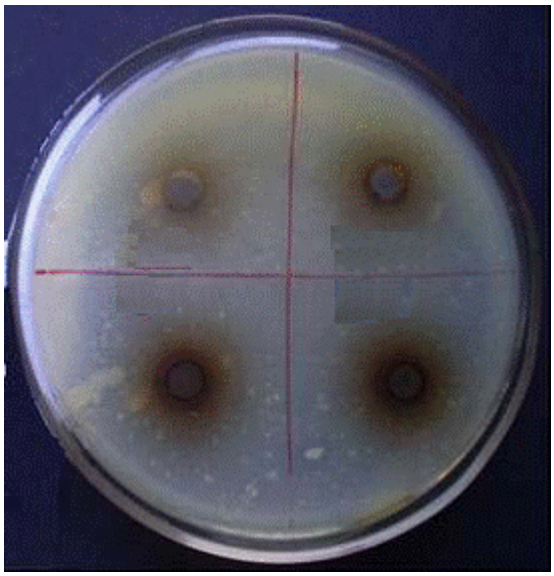

**Fig. 2: Effect methanolic Extract of *Ipomoea reniformis* on *B. subtilis***

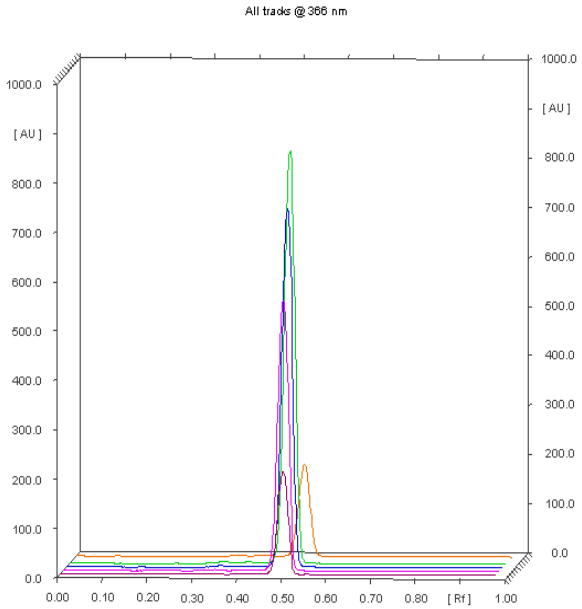


**Fig. 3: Calibration curve for standard Scopoletin (200-1000 ng/spot) and Isolated Scopoletin in Methanol**

**Table 1: Antimicrobial Activity of Methanolic extract of *Ocimum snactum* against tests organisms**

| Organism | Zone of Inhibition(cm) at MIC | Minimum inhibitory Concenteration (MIC) mg/ml. |
|---|---|---|
| Bacteria(Gram Positive) | | |
| *Bacillus subtilis* | 4.5 cm | 70 |
| Bacteria (Gram negative) | | |
| *Escherichia coli* | 3.1 cm | 49 |

## CONCLUSION

Scopoletin is the one of most prominent phytoconstituent present in the *Ipomoea reniformis* plant which may be responsible for anti-microbial activity.

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