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In vitro growth inhibition of pathogenic bacteria by *Solanum seaforthianum L.*

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ABSTRACT

Acetone, petroleum ether, chloroform, ethyl acetate, methanol and water extract from leaves and stem of Solanum seaforthianum were investigated for the antibacterial activities. Growth inhibition was determined using disc diffusion method against nine bacterial species. Among various solvent extracts tested, methanolic stem extract exhibited high degree of inhibition followed by ethyl acetate and aqueous extracts. The petroleum ether, acetone and chloroform extracts showed low degree of inhibition. The results from this study thus showed that methanol extracts of Solanum seaforthianum have potentially growth inhibitory effects on pathogenic bacteria.

Key words: Antibacterial activity, disc diffusion method, high degree of inhibition, Solanum seaforthianum.

INTRODUCTION

Despite tremendous advancement in drug formulation, infectious diseases caused by bacteria are still a major challenge to researchers. Their impact is particularly large in developing countries [1]. This is largely due to indiscriminate use of antibiotics, which has lead to the decimation of sensitive organisms from the group with the consequent increase in the number of resistant organisms. [2]. Drug resistance to time honored antibiotics possess a serious threat to public and clinicians [3] [4]. Since most of the rampant killer diseases are of microbial origin and account for high proportion of mortality in all over the world. Therefore, there is an urgent need for the discovery of alternate, safer and more effective anti bacterials in order to control the life threatening pathogens. The screening of medicinal plant extracts for antibacterial activity has shown that higher plants represent a potential source of novel antibiotics [5], [6].

Solanaceae is a large plant family consists of two thousand and three hundred species nearly half of which belong to a single genus, *Solanum.* This genus comprises of a number of species which are widely known for the presence of variety of biological principles medical significance. *Solanum seaforthianum* is known as kattu kodi in Tamil is characterized by cluster of four to seven leaves and climb to a height of 20ft. Ethno medicinal information from tribal people of Sirumalai hills of Western Ghats of Tamil Nadu revealed that extract of this plants used by Paliyan tribe as a remedy for skin diseases and for the treatment of boils. There is however, no report on the antibacterial activity of *Solanum seaforthianum* in the literature. Yet, this plant is known for its possession of various medicinal alkaloids and flavonoids [7]. Hence, this study was aimed at investigating the inhibitory effect of *Solanum seaforthianum* by preliminary bioassay screening.

MATERIALS AND METHODS

Plant collectio
Plant materials were collected in fresh condition from Sirumalai hills of Dindigul district of Tamil Nadu and identified after critical examination and the plants were deposited in the Herbarium of the Department of Botany, St. Joseph's College, Tiruchirappalli, South India.

Plant powder preparation
The healthy plant samples (free from insect damaged, fungus-infected) dried in the laboratory at room temperature for 5-8 days or until they broke easily by hand. Once completely dry, plant parts were ground to a fine powder using an electronic blender. Plants were stored in a closed container at room temperature until required.

Test organisms
Test bacteria
Nine bacterial species were tested. The bacteria used in this study were collected from K.A.P. Vishwanatham medical college Trichy. The bacteria include *Staphylococcus aureus, E.coli, Klebsiella pneumoniae, Proteus vulgaris, Proteus mirabilis, Salmonella typhi, Pseudomonas aeruginosa, Serratia marcescens, and Vibrio cholerae.*

Maintenance of Bacterial Cultures
The test bacteria were maintained in Nutrient Agar slants. The cultures were sub cultured and the cultured strains were allowed to grow two days and they were stored at 5° C for future studies.

Assay for antibacterial testing
Antibacterial activity of the above mentioned four different solvent and aqueous extracts were assayed separately using disc diffusion method [8]. Petri plates containing 10 ml of Muller Hinton Agar medium were inoculated with 108 CFC/ml of each test bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10µl of the 3 mg/ml plant extracts (30µg/disc) placed on the surface of the medium. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. A standard disc containing chloramphenicol antibiotic drug (30µg/disc) was used as a positive control and they were incubated for 24 h. The assessment of antibacterial activity was based on the measurement of diameter of inhibition zone formed around the disc.

RESULTS AND DISCUSSION

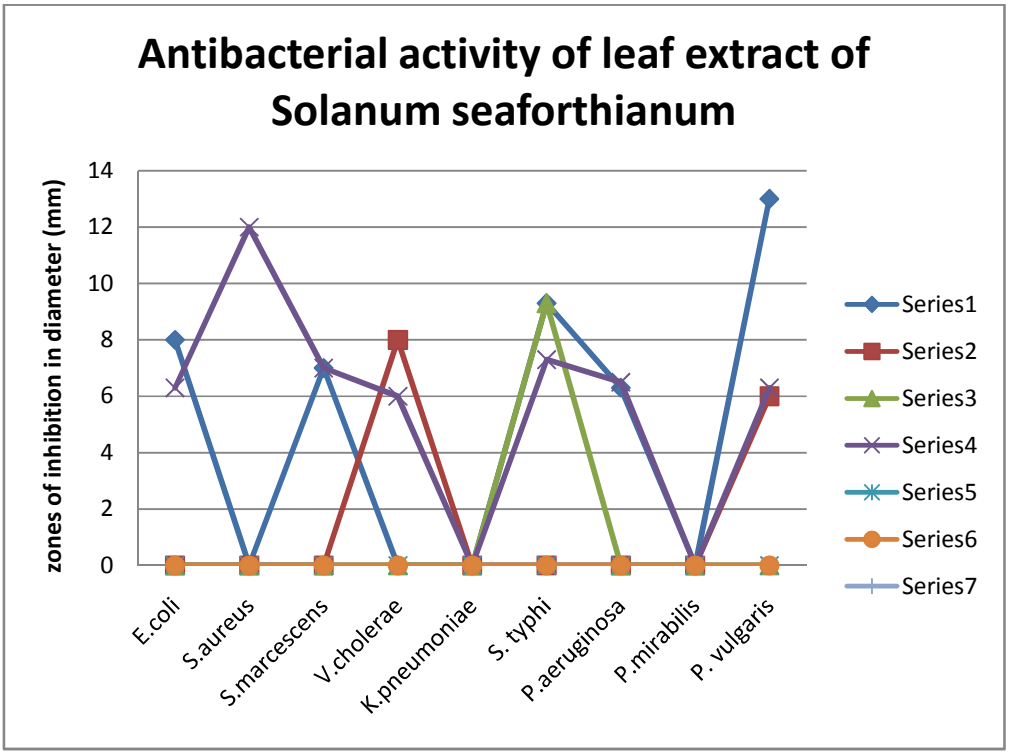
The evaluation of the activity of the aqueous, methanol, ethylacetate, chloroform and acetone extracts of different parts of *Solanum seaforthianum* (leaf and Stem) against both gram-positive and gram-negative bacteria by using the disc diffusion method is given in Tables1&2. The *in vitro* results were observed in terms of inhibition zone around each disc caused by diffusion of antibacterial properties from the plant extract impregnated disc into the surrounding medium. As can be seen from Tables 1and 2, among various solvent extracts tested methanolic stem extracts exhibited high degree of inhibition followed by ethylacetate and aqueous extracts. The petroleum ether and chloroform extract showed low degree of inhibition against all the test bacteria. In addition, the inhibition zones formed by standard antibiotic disc (chloramphenicol 30 mcg/disc) and those filter paper discs injected with methanol, ethylacetate, chloroform and acetone (negative controls) are also listed in Tables 1 & 2. The stem extracts exhibited high degree of inhibition than the other parts used. The diameter of inhibition zones were noted in the stem extracts (Table 2), the methanol extract showed significant antibacterial activity against the test bacteria. The zones of inhibition were higher in the case of *Vibrio cholerae Serratia marcescens E.coli, Pseudomonas aeruginosa* and *Klebsilla pneumoniae.* Moderate inhibition observed against in ethyl acetate, chloroform aqueous and petroleum ether stem extracts whereas low degree of inhibition zones was noted in acetone extracts with *Proteus mirabilis*, other organisms were found to resistant. It was surprising to note that *Staphylococcus aureus* and *Salmonella typhi*, the multi drug resistant bacteria [9] even to well known antibiotics were found to be sensitive to the methanolic stem extract. Similarly *Pseudomonas aeruginosa* a very resistant bacterium was also susceptible to the leaf extract. The results of the antibacterial screening of leaf are shown in Table 1 where some of the extracts showed complete absence of inhibition zones (petroleum ethe and aqueous extracts) against *Proteus mirabilis, Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa and E. coli.*

Table 1 Anti bacterial activity of leaf extracts of *Solanum seaforthianum* on pathogenic bacteria by (Disc diffusion method
Inhibition zone diameter in mm (Mean ± SD)

Test Bacteria	Methanol extract		Choloroform		Acetone		Ethyl acetate		Pet. Ether		Aqueous		Positive Control Chloramphenicol (30mcg/disc)
	Experimentall 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	
E.coli	8.0±2.8	-	-	-	-	-	6.3±0.5	-	-		-	-	2.0 ± 0.00
S.aureus	-	-	-	-	-	-	12.0±8.4	-	-		-	-	1.8 ± 0.00
S.marcescens	7.0±1.4	-	-	-	-	-	7.0±1.0	-	-		-	-	2.5 ± 0.00
V.cholerae	-	-	8.0±1.4	-	-	-	6.0±0.0	-	-		-	-	1.7 ± 0.00
K.pneumoniae	-	-	-	-	-	-	-	-	-		-	-	2.0 ± 0.00
S. typhi	9.3±3.0	-	-	-	9.3±4.9	-	7.3±1.1	-	-		-	-	1.5 ± 0.00
P.aeruginosa	6.3±0.57	-	-	-	-	-	6.5±0.7	-	-		-	-	2.2 ± 0.00
P.mirabilis	-	-	-	-	-	-	-	-	-		-	-	-
P. vulgaris	13.0±9.8	-	6.0±0.0	-	-	-	6.3±0.7	-	-		-	-	2.0 ± 0.00

Table 2 Anti bacterial activity of stem extracts of *Solanum seaforthianum* on pathogenic bacteria by (Disc diffusion method)
Inhibition zone diameter in mm (Mean ± SD)

Test Bacteria	Methanol extract		Choloroform		Acetone		Ethyl acetate		Pet. Ether		Aqueous		Positive Control Chloramphenicol (30mcg/disc)
	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	Experimental 30 µg / Disc	Negative Control	
E.coli	12.0±10.6	-	-	-	-	-	10.0±4.2	-	-		-	-	2.5± 0.00
S.aureus	-	-	-	-	-	-	7.3±0.5	-	-		6.3±0.57	-	-
S.marcescens	12.0±9.5	-	6.3±0.57	-	-	-	6.6±1.1	-	6.3±0.5		6.6±0.57	-	2.3±0.00
V.cholerae	14.6±9.0	-	6.3±0.57	-	-	-	6.0±0.0	-	-		-	-	2.5 ± 0.00
K.pneumoniae	11.0±7.0	-	-	-	-	-	7.6±1.1	-	-		-	-	1.6 ± 0.00
S. typhi	-	-	-	-	-	-	7.0±1.1	-	-		7.3±0.5	-	1.9 ± 0.00
P.aeruginosa	11.3±8.3	-	-	-	-	-	-	-	6.3±0.5		-	-	2.1 ± 0.00
P.mirabilis	-	-	6.3±0.57	-	6.5±0.7	-	7.6±0.5	-	7.0±0.0		-	-	-
P. vulgaris	-	-	-	-	-	-	-	-	-		7.3±1.1	-	2.1 ± 0.00


Antibacterial activity of leaf extract of Solanum seaforthianum
zones of inhibition in diameter (mm)
14
12
10
8
6
4
2
0
E.coli
S.aureus
S.marcescens
V.cholerae
K.pneumoniae
S. typhi
P.aeruginosa
P.mirabilis
P. vulgaris
Series1
Series2
Series3
Series4
Series5
Series6
Series7

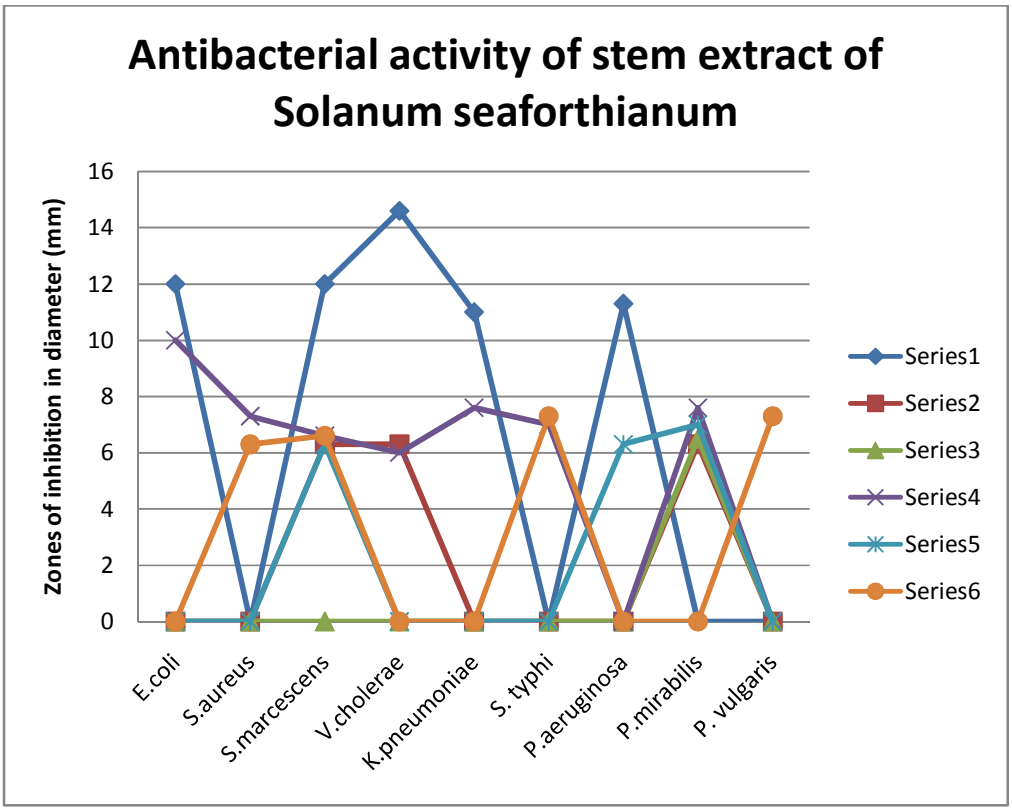
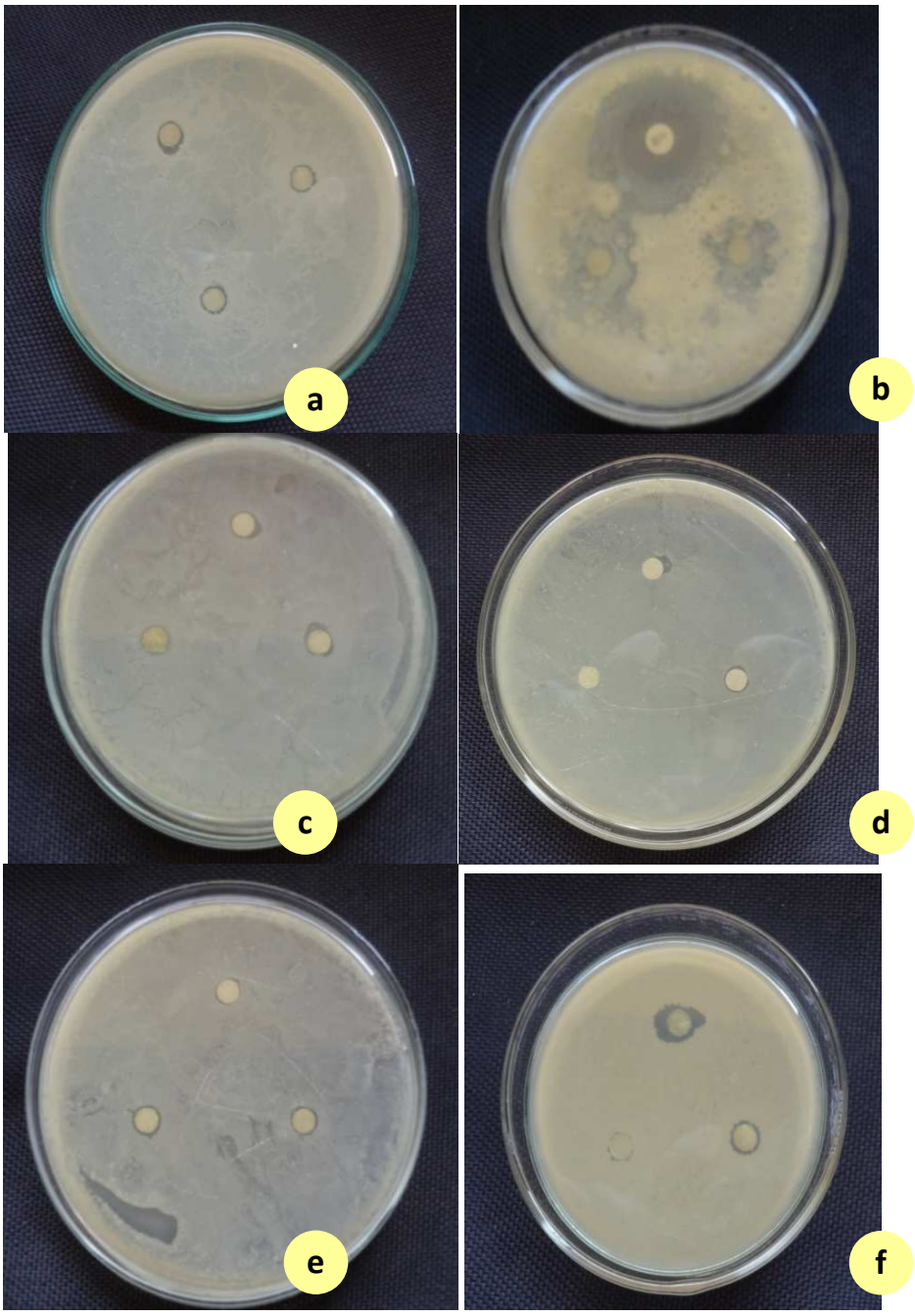

Antibacterial activity of stem extract of Solanum seaforthianum
Zones of inhibition in diameter (mm)
16
14
12
10
8
6
4
2
0
E.coli
S.aureus
S.marcescens
V.cholerae
K.pneumoniae
S. typhi
P.aeruginosa
P.mirabilis
P. vulgaris
Series1
Series2
Series3
Series4
Series5
Series6

PLATE-1

Habit of *Solanum seaforthianum*
a. Mass of Plant **b. Single Plant**
c. Flower **d. Fruits**

PLATE – 2



Zones of inhibition with different solvent extract of *Solanum seaforthianum* stem on pathogenic bacteria

a. Ethyl acetate / *Klebsiella pneumoniae* **b. Methanol / *Salmonella typhi***
c. Ethyl acetate / *Proteus mirabilis* **d. Chloroform / *Staphylococcus aureus***
e. Ethyl acetate / *Staphylococcus aureus* **f. Ethyl acetate / *E.coli***

The methanol extracts of *Solanum seaforthianum* stem showed high degree of inhibition than the other solvents used. Though, the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteriostatic abilities [10] mentioned

that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents. Similar results showing that the alcoholic extract having the best antimicrobial activity [11] *in Leucas aspera, Holarrhena antidysenterica.* Seyydnejad also studied the effect of different alcoholic viz. ethanol and methanol for antimicrobial activity and observed that this difference in the activity between different alcoholic extract is due to the difference between extract compounds in this two extract.
The study also revealed that Petroleum ether extract shows moderated and aqueous extract shows minimum antimicrobial activity. However, [12] showed that petroleum ether extract of plant *Memecylon umbellatum* Burm. f. shows significant antimicrobial activity. Furthermore, water extract from leaves of P. *acerifolium* had been reported to have prominent antimicrobial activity against several gram positive and gram negative human pathogenic bacteria.

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