Comparative antimicrobial activity of callus and natural plant extracts of *Solanum trilobatum L.*

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Abstract: *Comparison of natural plant and callus extracts of Solanum trilobatum L. was studied against two bacteria and fungi, for their antimicrobial activity using cup diffusion method. Various solvents such as chloroform, petroleum ether and ethanol were used. The leaf and stem segments of the plant were cultured on Murashige and Skoog basal medium supplemented with various growth regulators. Maximum callus was recorded on medium containing 0.5 mg/l NAA and 0.5 mg/l Kinetin. The results reveals that the stem and leaf callus extracts has shown significant activity against the tested microorganisms than the natural sample.*

Key Words : *Solanum trilobatum*, callus, kinetin, extracts, antimicrobial activity.

INTRODUCTION

Solanum trilobatum L. is an important medicinal plant belonging to the family Solanaceae, widely used in cough, chronic bronchitis and the leaves are cooked as vegetable. The roots and leaves are bitter and astringent. The leaves are roasted along with onion in gingelly oil and used to cure breathing problem. Dried and powdered fruit taken internally cures phlegmatic disorders, rheumatism and gastric problems[1]. The leaves contain rich amount of calcium, iron, phosphorous, protein, fat, crude fibres, minerals and other carbohydrates[2,] solamarine and solasodine have been found in *S.trilobatum* leaves. The requirement of *S.trilobatum* is met from the natural populations, leading to their gradual depletion. Tissue culture techniques can play an important role in the rapid multiplication of the elite clones and germplasm conservation of this medicinal plant.

Plant tissue culture offers an effective method for enhancement of secondary metabolites, which are in high demand for their therapeutic values. It has been reported that callus and regenerated plants have shown enhancement of secondary metabolites, when compared to natural products[3]. These secondary metabolites may show antifungal and antibacterial activity. Hence, an attempt was made to study the antimicrobial activity of callus and the natural plant extracts of *Solanum trilobnatum*.

MATERIALS AND METHODS

Plants were collected from the herbal garden of A.V.C.College. Small/tender stem and leaf were used as explants for the induction of callus. Explants were washed thoroughly in running tap water for 15 minutes and treated with Teepol, for 5 minutes. The explants were then treated with 0.1 % mercuric chloride solution for 3 minutes and finally rinsed with sterilized distilled water to remove the traces of sterilant. The explants were inoculated aseptically on MS[4] medium containing different concentrations of various growth regulators. The cultures were maintained at 25 $\pm$ 2°C in culture room.

The explants viz., stem and leaf were dried under shade and callus were dried at 60°C for 12 hours in an oven and were powdered and subjected to soxhlet extraction separately and successively with ethanol, chloroform and petroleum ether. Each extract was tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and for antifungal activity against *Aspergillus flavus* and *Aspergillus niger.*

For antimicrobial assay, the *in vitro* cup diffusion method[5] was used. Nutrient agar and Potato dextrose agar media were used for growing the test bacteria and fungi respectively. The extracts were dissolved in Dimethyl formamide (DMF) to give a 1mg/1ml solution. The media were poured into plates for solidification and cups (8mm) were made. To each of these cups, 0.1 ml aliquots of test solution, standard solution of Streptomycin for bacteria and Nystatin for fungi were added. For assaying antibacterial activity, plates were incubated at 37°C for 24 hours, whereas for antifungal activity they were incubated at 26°C for 48 hours. The diameter of zone of inhibition was measured as an average of maximum dimensions of zones around the discs. All the experiments were repeated three times.

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RESULTS AND DISCUSSION

Callus was successfully induced from both the explants on MS medium fortified with 0.5 mg /l NAA + 0.5 mg/l Kn. White friable 30 days old callus was used for antimicrobial assay. The antibacterial activity of different extracts was carried out with two bacteria namely, *E.coli* and *S.aureus* (Table 1). From the results, it is evident that the petroleum ether and chloroform extracts of leaf callus showed maximum inhibitory activity against the test bacteria, when compared to natural leaf extracts. Similarly, the stem callus extracts of petroleum ether and chloroform extracts showed more inhibitory activity, when compared to natural stem extracts on the test bacteria. Similar reports of medicinal plants showing antibacterial activity from callus extracts [6,7] and natural plant extracts have been reported[8].

The antifungal activity of different extracts of *S.trilobatum* is shown in Table 2. The petroleum ether extracts of leaf callus showed significant effect on *A.flavus* and *A.niger*. The chloroform and petroleum ether extracts of stem derived callus showed maximum inhibition on *A.flavus* and *A.niger* respectively. Similar reports of callus extracts showing antifungal activity has been made[9] previously. Thus it is obvious that the callus extracts of leaf and stem have maximum antimicrobial activity than the natural leaf and stem extracts.

CONCLUSION

It may be concluded that this enhanced antimicrobial activity shown by leaf and stem derived callus extracts may be due to the presence of more amount of secondary metabolites in callus when compared to natural products. Further work on the isolation and purification of the active principle from the callus would throw new light in to this area of research.

Table 1 : Antibacterial activity of callus and natural plant extracts of *Solanum trilobatum*

S.No	Extract	Zone of inhibition (in mm)	
		E. Coli	S. aureus
1.	**Natural Leaf**		
	Petroleum Ether	15.00 ± 0.33	16.66 ± 0.33
	Chloroform	14.33 ± 0.32	15.00 ± 1.52
	Ethanol	17.00 ± 0.81	17.33 ± 0.32
2.	**Leaf Callus**		
	Petroleum Ether	18.00 ± 0.66	22.00 ± 0.33
	Chloroform	17.00 ± 0.32	19.33 ± 1.52
	Ethanol	13.00 ± 0.33	15.00 ± 0.33
3.	**Natural Stem**		
	Petroleum Ether	17.00 ± 1.52	12.66 ± 1.52
	Chloroform	14.52 ± 0.66	13.66 ± 1.52
	Ethanol	13.33 ± 0.33	15.33 ± 0.66
4.	**Stem Callus**		
	Petroleum Ether	18.00 ± 0.81	17.00 ± 0.81
	Chloroform	17.66 ± 0.31	17.00 ± 0.81
	Ethanol	16.00 ± 0.33	16.66 ± 0.81
5.	**Streptomycin (Standard)**	16.00 ± 0.81	13.00 ± 0.33

Table – 2: Antifungal activity of callus and natural plant extracts of *Solanum trilobatum*

S.No	Extract	Zone of inhibition (in mm)	
		A.flavus	A. niger
1.	**Natural Leaf**		
	Petroleum Ether	23.66 ± 0.88	24.66 ± 0.88
	Chloroform	24.00 ± 0.51	25.00 ± 0.33
	Ethanol	21.00 ± 0.56	24.33 ± 0.5
2.	**Leaf Callus**		
	Petroleum Ether	26.00 ± 0.88	26.00 ± 0.57
	Chloroform	21.33 ± 0.12	23.00 ± 0.52
	Ethanol	21.00 ± 0.12	23.33 ± 0.51
3.	**Natural Stem**		
	Petroleum Ether	24.33 ± 0.66	24.00 ± 0.51
	Chloroform	24.00 ± 0.81	25.00 ± 0.51
	Ethanol	25.33 ± 0.81	23.33 ± 0.56
4.	**Stem Callus**		
	Petroleum Ether	25.00 ± 0.33	27.00 ± 0.52
	Chloroform	27.00 ± 0.88	26.33 ± 0.57
	Ethanol	24.00 ± 0.32	21.00 ± 0.057
5.	**Nystatin (Standard)**	23.00 ± 0.05	24.00 ± 0.66

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