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In vitro micropropagation and antimicrobial activity of *Solanum trilobatum*

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ABSTRACT

Solanum trilobatum is one of the oldest known therapeutic herbs belonging to the family solanaceae. To establish a suitable protocol for micropropagation in S. trilobatum leaf and node explants were used. The explants were surface sterilized with 1% Bavistin and 0.1% Mercuric chloride for 3min and it was found to be satisfactory in controlling the contamination. MS medium supplemented with BAP 2.0mg/l and Kinetin 2.0mg/l was found to initiate the shoot from node explants with in10-15days. The root induction was obtained in MS media containing 2, 4-D (2.0mg/l) and IBA (2.0mg/l). For callus initiation node and leaf explants are used, NAA + Kinetin (2.0+2.0mg/l) was found good combination for induction of callus from node, NAA + Kinetin(2.0+2.0mg/l) and NAA + BAP (2.0+2.0mg/l) was found good combination for induction of callus for leaf explants. The n-butanol extract of S. trilobatum showed highly effective against K. pneumoniae, B. cereus, S. boydii, S. aureus. There is no comparative study of in vivo and in vitro plants activity. In this study we found that in vitro grown plants showed better activity when compare to in vivo plants.

Keywords: Micro propagation; *solanum trilobatum*; BAP; Kinetin; IBA; Antimicrobial activity.

INTRODUCTION

Solanum trilobatum Linn (Family: Solanaceae) is one of the important medicinal plant, more commonly available in Southern India and has been used in herbal medicine to treat various diseases like respiratory problems, bronchial asthma and tuberculosis. The leaf extract seems to increase male fertility and counteracts snake poison Govindan *et al*., [1999, 2004].

The major alkaloids identified in the alcoholic extract from leaves and stem part of *S. trilobatum* has been shown to possess antimitotic and antimicrobial activity against bacteria and fungi

Subramanian *et al*., [1983], Swapna latha *et al*., [2006]. Biological screening of the alkaloid mixture of this plant revealed anticancer activity against certain type of cancer and its effectiveness as an adjuvant in cancer chemotherapy, Brindha *et al*., [1980].

Previous studies shown that the plant based drugs as good sources of antibiotics, anti-inflammatory and antioxidant agents, A.Mathur *et al*., [2011]; Hindustan Abdul Ahad *et al*., [2011]; Afzal Unnisa and T.Parveen, [2011]. The methanolic extract of *S. trilobatum* has been shown to possess antioxidant activity, hepato protective activity, Shahjahan *et al*., [2005], anti inflammatory activity and anti-ulcerogenic activity, Amir *et al*., [2004] skin repellent activity against *Anopheles stephensi*, Rajkumar *et al*., [2005].

Increased demand due to medicinal properties and depletion of natural sources has initiated the development of plants through micropropagation, Manisha Sharan *et al*., [2010]. Only limited success has been reported for *in vitro* micropropagation and organogenesis of *S. trilobatum*, Arulmozhi *et al*., [1997]. There are only a few reports on *S.trilobatum* for micropropagation to propagate plants from leaf and node explants. In the present study an attempt was made to standardize the protocol for micropropagation of *S. trilobatum* by using different plant growth regulators and to evaluate the antimicrobial activity from *in vivo* and *in vitro* growing plants.

MATERIALS AND METHODS

Micropropagation

Leaf and node explants were collected from healthy plants, washed with running tap water, treated with 1% Bavistin and washed with sterile distilled water. Then surface sterilized with 0.1% Mercuric chloride in different concentration and time duration. The surface sterilized explants were washed thrice with sterile distilled water. Leaf and node explants were inoculated in MS medium containing 30g/l sucrose. The different combination of plant growth regulators were used to find the effect on induction of shoot, root and callus. For shoot regeneration different concentrations of 2, 4-D (1.0, 2.0 mg/l), IAA, BAP, BAP+Kn, IAA+BAP were used. For root induction well matured node explants were choosed for initiating rooting and also the different growth hormones like 2,4-D, IAA in different concentration were used. The rooted plants (20days) were transferred to plastic cup contain soilrite mix ^{TC} : garden soil mix (1:1), hardend in rectangular box in growth room for 15 days and later it was transferred to room temperature for future establishment. The different combination of growth regulators were also used to find out the effect on callus induction from leaf and node. In this experiment the MS media supplemented with and without auxin and cytokinin.

Solvent extraction for dried sample

The 1gm of leaf and stem dried powder was weighed, then it was transferred into a sterile screw cap tube containing (10ml) of different organic solvents like methanol, n-butanol, chloroform and hexane which were allowed to soak for 48 hours at room temperature, The mixture was then centrifuged at 2000 rpm for 10min at 4˚C . The supernatants were filtered through a sterile funnel containing sterile whatman filter paper No.1 and then filter sterilized using syringe filter containing 0.2µ cellulose acetate membrane.

Solvent extraction for fresh sample

The fresh leaves and stems were collected from the well grown plants, and then the sample was washed in distilled water for many times, again the sample was washed in sterile distilled water, the sample is allowed for air dried for few minutes. Then 1gm of weighed sample was taken and grinded well by using pestle and mortar. The similar procedures of dried sample solvent extraction procedure were followed.

Solvent extraction for callus and fresh sample

The callus which was obtained from leaf and node explants were collected from cultured tube, the leaf and stem were collected from the *in vitro* grown plants. The fresh calluses were washed with sterile distilled water in an aseptic condition to remove the agar residues, and then it was allowed to air dried for minutes aseptically. The 1gm of air dried fresh callus, leaf and stem was taken for solvent extraction. The weighed sample was grinded well by using pestle and mortar. The similar procedures of dried sample solvent extraction procedure were followed.

Test microorganism

The bacterial species, *Staphylococcus aureus* (MTCC 87), *Escherchia coli* (MTCC 452), *Bacillus cereus* (MTCC 430), *Klebsiella pneumonia* (MTCC 432), *Vibrio parahaemolyticus* (MTCC 451) and clinical isolate of *Shigella boydii* were used as test organisms and they are maintained on Muller Hinton Agar solid media.

Antimicrobial activity of *S. trilobatum* extracts

To detect the antimicrobial activity of *S .trilobatum* four types of plant sample were used. Fresh plant, dried plant, in vitro fresh plant and callus sample and also different types of solvents are used for extractions such as Methanol, n-butanol, chloroform and Hexane. All test organisms were inoculated in nutrient broth (NB) and incubated overnight at 37°C to make a homogenized suspension. The antimicrobial activity was performed by following agar disc diffusion assay, Gaurav *et al*., [2010]; Abishek Mathur *et al.,* [2011]. The plates were incubated at 37^{o}C for 24hrs and anti microbial activity were evaluated by measuring the diameter of the zone of inhibition.

RESULTS

The effects of sterilants on surface sterilization of the explants were assessed based on the contamination percentage and the percentage of explants response (callus and shoot). In this study it was found that, 1% Bavistin (3min) and $HgCl_2$ (3min) was considered as the suitable combination of sterilants for explants response, since its resulted in 17% contamination with higher frequency of callus and shoot response. The details of contamination levels and regeneration of explants were shown in the Table.1

To establish micropropagation of *S. trilobatum*, node and leaf explants were cultured on different combination of PGR's in MS media. The different combination of growth regulators were assessed based on the explants response. The most of growth regulators showed callus and shoot induction. The NAA, NAA +Kinetin and 2, 4-D+Kinetin have showed good callus induction in node and leaf explants. The effect of different plant growth regulators on explants was mentioned in the Table. 2. From our study it was clear that BAP 2.0mg/l and Kinetin 2.0mg/l combination in MS media were significantly more effective for inducing shoot organogenesis.

Table.1 Contamination levels and Regeneration of explants

TREATMENT	BAVISTIN (1%)	$HgCl_2$ (0.1%)	CONTAMINATION (%)	REGENERATION (%)
To	-	-	95%	10%
T1	1min	3min	80%	25%
T2	2min	3min	47%	27%
T3	3min	3min	17%	80%
T4	4min	3min	12%	35%
T5	5min	3min	10%	27%

Table.2 Plant growth regulators on explants

TREATMENT	PGR'S(mg/l) in MS MEDIA	EXPLANT RESPONSE	
		LEAF	NODE
2,4-D+NAA	2.0+2.0	Initiation of callus within 4 days	Initiation of callus within 4 days
NAA+IAA	2.0+2.0	Browning and death of explants after 1-2 weeks	Callus initiation after 25 days
IAA+2,4-D	2.0+2.0	Curling of leaves in 1-2 weeks	Sprouting of shoot was initiated with in 10days, callus also initiated after 15 days
BAP+KINETIN	2.0+2.0	Browning and death of explants	Shoot was initiated with in 7 days
2,4-D+BAP	2.0+2.0	Explant remain dull in media	Shoot response was initiated after 15 days
2,4-D+KINETIN	2.0+2.0	Curling of leaves after 10 days	Shoot response was initiated after 15 days
NAA+BAP	2.0+2.0	Callus was initiated after 7 days	Callus was initiated after 10 days
NAA+KINETIN	2.0+2.0	Curling of leaves after 20 days	Callus was initiated after 15 days
IAA+KINETIN	2.0+2.0	Callus response was very slow	Shoot was initiated after 13 days
IAA+BAP	2.0+2.0	Explant remain dull	Shoot response was initiated after 15 days

Hormonal combination for shoot induction:

To examine the effect of different plant growth regulators on shoot induction, node parts dissected from the mother plant were inoculated in MS medium supplemented with different PGR's like 2,4-D, IAA, BAP, Kinetin, IAA + Kinetin, BAP + Kinetin. In this IAA, BAP and BAP + Kinetin have showed good response for shoot induction. The MS media supplemented with BAP and also in the combination of BAP and Kinetin showed good response. The other PGR's like kinetin and IAA + Kinetin the growth rate of explant was very slow, when compare to other growth regulators (Table-3). In case of internode length, the BAP (2mg/l), BAP + Kinetin IAA + Kinetin (2.0+2.0mg/l) were showed less distance. (Table-3.1).

Root induction:

The well matured node explants were inoculated into MS medium containing different PGR's for *in vitro* rooting, The different PGR's like 2,4-D and IBA in different concentration were used,

root induction occurred in 2,4-D and IBA at the concentration of 2.0mg/l, but the root of biomass showed more in 2,4-D when compare to IBA.Fig-3

Table.3 Shoot Response

PGR'S	PGR'S(mg/l)	LENGTH(cm)	DAYS
BAP	2.0	5.6±0.14	30
IAA	2.0	5.5±0	30
2,4-D	2.0	2.1±0.12	30
KINETIN	1.0	4.1±0.26	30
IAA+KINETIN	2.0+2.0	2.5±0.08	30
BAP+KINETIN	2.0+2.0	6.5±0.08	30

Table. 3.1 Internode response

PGR'S	PGR'S(mg/l)	LENGTH(cm)	DAYS
2,4-D	2.0	1.96±0.12	30
IAA	2.0	1.66±0.23	30
KINETIN	2.0	1.5±0	30
BAP	2.0	0.66±0.23	30
IAA+ KINETIN	2.0+2.0	0.5±0.08	30
BAP+KINETIN	2.0+2.0	0.6±0.08	30

Hardening:

The well developed rooted plants were carefully taken out from the tube, and then it was dipped in fungicide (Bavistin) for few minutes as a prophylactic treatment and transferred to soilrite mix^{TC}: garden soil in plastic cups. Then it was maintained in rectangular box in the laboratory for a week with 90 to 100% humidity. Good establishment of plants was seen after 5-8 days.

Effect of plant growth regulators on callus initiation:

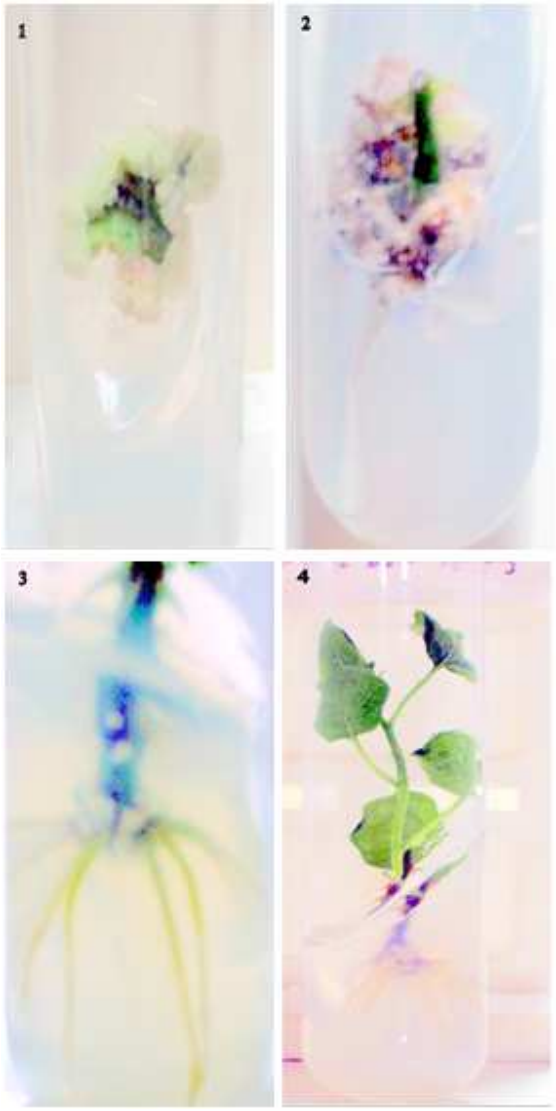
The effect of auxins with cytokinins on callus initiation was studied by culturing explants on MS medium supplemented with 2,4-D and NAA at four different levels (0.5,1.0,1.5.2.0mg/l) and also MS media supplemented PGR's in the combination of NAA and Kinetin and also NAA+BAP at four different concentrations respectively. In this study also the lower concentration of PGR's doesn't induce any callus. The 2, 4-D and NAA alone does not induce any callus in MS media. At higher concentrations of NAA+ KN (2.0+2.0 mg/ l) and NAA+BAP (2.0 +2.0mg/l) the matured leaves induce green callus Fig-1. The node explants inoculated in MS media does not induce any callus. The NAA+ Kinetin was found effective combination for callus induction in leaf.

Table.4 Callus from Node

PGR'S	PGR'S (mg/l)	CALLUS WEIGHT	DAYS
NAA	2.0	1.96 ± 0.12	30
2,4-D	2.0	1.5 ± 0.08	30
2,4-D + NAA	2.0+2.0	1.6 ± 0.21	30
NAA + KINETIN	2.0+2.0	4.73 ± 0.12	30

Table 4.1 Callus from leaf

PGR'S	PGR'S (mg/l)	CALLUS WEIGHT	DAYS
NAA + KINETIN	2.0+2.0	2.03 ± 0.12	30
NAA + BAP	2.0+2.0	2.6 ± 0.08	30
2,4-D + KINETIN	2.0+2.0	2.1 ± 0.08	30



Figs 1-4: 1 and 2. Callus from leaf and node explants, 3. Root induction, 4. Shoot induction

Hardening

The rooted plants were dipped in fungicide Bavistin (1%) for few minutes as prophylactic treatment and transferred to a medium with soilrite mix and garden soil in plastic cups. Then it was observed in the laboratory room condition with 90 to 100% humidity, the well grown plants were seen after 5-8 days.

Antimicrobial activity of *Solanum trilobatum* extracts

Antimicrobial activity of leaf extracts and stem extracts from *in vivo*, micropropagated *S. trilobatum* plants and callus sample were tested. The four different types of solvents were used for solvent extraction from *S.trilobatum*. In this study, it was found that n- butanol is highly effective solvent for *S. trilobatum*. The *S.trilobatum* was found highly effective against *Klebsiella pneumonia*, *Bacillus cereus*, *Shigella boydii*, and *Staphlyococcus aureus*. The *Escherchia coli* were showed less zone inhibition to this plant extracts. The *in vitro* fresh and

callus sample showed more zone inhibition when compare to *in vivo* dried and fresh sample. The zone of inhibition was interpreted in millimeter and was tabulated in Table.5

Table. 5: Antimicrobial activity of *Solanum trilobatum* extracts

Organism	Solvents	*In vivo* Dried sample		*In vivo* Fresh sample		*In vitro* Fresh sample		*In vitro* Callus sample	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
S.aureus	n-butanol	13mm	11mm	-	5mm	13mm	20mm	17mm	15mm
	Methanol	-	11mm	-	-	-	-	-	14mm
	Chloroform	-	-	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-	-	-
E.coli	n-butanol	12mm	14mm	-	-	-	-	19mm	-
	Methanol	-	-	-	-	-	-	-	-
	Chloroform	11mm	20mm	-	-	-	-	15mm	17mm
	Hexane	-	-	-	-	-	-	-	-
B.cereus	n-butanol	17mm	15mm	15mm	25mm	16mm	19mm	13mm	20mm
	Methanol	-	-	-	-	-	-	12mm	-
	Chloroform	-	-	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-	3mm	-
K.pneumonia	n-butanol	35mm	30mm	17mm	22mm	20mm	25mm	40mm	30mm
	Methanol	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	22mm	26mm
	Hexane	-	-	-	-	-	-	11mm	19mm
S.boydii	n-butanol	18mm	13mm	16mm	11mm	19mm	22.2mm	19mm	17mm
	Methanol	-	-	-	-	-	-	11mm	-
	Chloroform	-	-	-	-	-	-	-	-
	Hexane	-	-	-	-	-	-	-	-
V. parahaemolyticus	n-butanol	30mm	40mm	-	-	15mm	-	35mm	30mm
	Methanol	-	-	-	-	-	-	-	-
	Chloroform	-	-	-	-	-	-	-	-
	Hexane	-	-	5mm	-	-	-	-	-

DISCUSSION

In the present study, for micropropagation and callus induction in *S.trilobatum* washing of explants in running tap water for few minutes. Then the explants are surface sterilized with 1% Bavistin and 0.1% $HgCl_2$ for 3minutes was found to good combination for preventing contamination with better response. Arockiasamy *et al*., [2002] reported, *S.trilobatum* seeds are treated with 5% Bavistin sundried and surface sterilized with 0.1% $HgCl_2$ for 3 min was found effective combination for shoot regeneration.

To establish micropropagation of *S.trilobatum* node and leaf explants were cultured on different combination of PGR's on MS media. Some explants are inoculated into hormone free MS media, but there is no response for shoot induction and callus induction in hormone free MS media. The same observation was made in several medicinal plants. The use of BAP and Kinetin alone as growth regulators showed better shoot initiation when compare to other PGR's and also some PGR's did not showed any response to this explants. This was comparable to the reports by Meyer and Staden [1991], that the use of cytokinin, Kn, BAP (or) TDZ as sole growth regulators in the basal media did not support the growth of *Aloe vera.* The shoot responses from node explants were tried in different concentrations, in this study it was found that BAP 2.0mg/l and Kinetin 2.0mg/l showed good response to shoot induction from node explants. Jawahar *et al*.,

[2004] reported the BAP (8.88µm/l) followed by Kinetin (9.28µm/l) in MS medium, in this concentration the node explants of *S.trilobatum* showed better multiple shoot with in 15days after inoculation. BAP and Kinetin individually and in combination induced a higher frequency of adventitious shoots from single explants of *S.xanthocarpum,* Pawar *et al.,* [2002].

From our study it was clear that BAP 2.0mg/l and Kinetin 2.0mg/l combination in MS media were significantly more effective for inducing shoot organogenesis. Kulkarni and Rao [1999] observed that Kinetin did not support the proliferation of shoots in *Acorous calamur*. This result was in contrast the present study were BAP + Kinetin having the ability to increase the shoot length. Alagumanian *et al*., [2004] studied that the BAP was able to induce the shoot proliferation in the culture of trees. IAA also found for shoot elongation, in this concentration the growth rate is very less but it contains more leaves.

In the present study, better response for rooting was observed in full strength MS media. Strong healthy roots 5-6 in number were produced in a very short time 10-15 days in MS media. The root induction was obtained in the media containing 2,4-D and IBA, but the root of biomass showed more in 2,4-D when compare to IBA. Arockiasamy *et al*., [2002] observed that *S.trilobatum,* nodal explants inoculated into LS medium containing different concentration of IBA (0.5-2.5mg/l) for rooting. From our experimental data, it is evident that 2, 4-D also having the ability to induce the root in *S.trilobatum.* In most of the plants the 2, 4-D is a potent hormone which is commonly used for callus induction. *In vitro* developed plants were growned well during hardening, the same results were observed in Manisha sharon *et al.,* [2010].

Traditional healers found more medicinal plants which are highly effective to treat various diseases and also it is necessary to prove scientifically in order to develop new drug molecules. Abhishek Mathur *et al.,* [2011]. Antimicrobial activity of *S.trilobatum* extract against a number of bacteria has been reported. Swapna latha *et al*.,[2006] , they reported the plant extracts from leaves, flowers, stems and fruits of *S.trilobaum* revealed antimicrobial activity against Gram(+) ve bacteria and Gram(-) ve bacteria. Maximal antibacterial activity was seen against *Klebsiella sp.* with aqueous extract and methanol extract of stem showed maximal activity against *S.aureus.* In this study it was proved that *S.trilobatum* was highly effective against to *S.aureus*, *B.cereus*, *K.pneumonia* and clinical isolate of *S. boydii*. In generally it was noticed that *in vitro* grown plants and callus have the high ability when compare to the normal plants. From the studies it may concluded that antimicrobial activity of *S.trilobatum* extract against test organism indicates the medicinal value and supports the claim of the traditional healers that it has been used to relive throat congestion, cough and cold. However, further studies are needed to isolate bio active principle in an aqueous and organic solvent extracts.

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