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RESEARCH ARTICLE

AN EFFICIENT PLANT REGENERATION AND *IN VITRO* SECONDARY METABOLITE PRODUCTION IN *PSEUDARTHRIA VISCIDA* (L.) WIGHT and ARN

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ABSTRACT

Pseudarthria viscida (L.) Wight and Arn. belonging to the family Leguminosae is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhantara taila. Major chemical compounds present in the roots are gallic acid, ferulic acid, caffeic acid, rutin, quercetin and oleic acid. Since *Pseudarthria viscida* has high commercial and medicinal values, the excessive collection has resulted in gradual disappearance of this plant from natural habitat and at present its number is highly reduced in the wild. To conserve the genetic stocks of this plant *in vitro* propagation can be utilized successfully. The main aim of this work is to identify an efficient regeneration and *in vitro* secondary metabolite production in *Pseudarthria viscida* using MS medium. Leaf, node and inter nodal segments were used as explants for callus induction, subsequent shoot regeneration and adventitious roots induction. Ninety eight percentage of callus induction observed from leafy segment on MS medium supplemented with 1.5 mgl^{-1} 2,4-D + 0.5 mgl^{-1} Kinetin and 2.5 mgl^{-1}NAA+1 mgl^{-1}BAP. Maximum number of shoot regeneration from nodal segments noticed on medium with 0.5 mgl^{-1} NAA+2.5 mgl^{-1}BAP after 28 days. Maximum number of rooting noticed on medium with 2.5 and 2 mgl^{-1}IBA and NAA respectively after 29 days. The plantlets were then transferred to field after acclimatization. For secondary metabolite production, the four week old callus were cultured in suspension using abiotic (Salicylic acid) and biotic elicitors(Chitosan) for enhancing the production of phenolic compound. The optimal fold increase of phenolic compound were noticed in 48 hour callus culture by 1.5 mg Chitosan treatment. The bioactive phenolic compound (gallic acid) was then isolated from the different elicitors treated callus extract and its identification and quantification was carried out by HPTLC and HPLC methods.

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INTRODUCTION

Hundreds of medicinal plants are at risk of extinction, threatening the discovery of future cure for disease. Interest and support for the conservation and development of medicinal plants is increasing in all parts of the world. As per World Health Organisation (WHO) estimates, almost 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs. In India, medicinal plants sector has traditionally occupied an important position in the socio cultural, spiritual and medicinal arena of rural and tribal lives. The conservation of plant genetic resources has long been realized as an integral part of biodiversity conservation (Hoareau and Silva, 1999). Plant secondary metabolites can be defined as compounds that have no recognized role in the maintenance of fundamental life processes in the plants that synthesize them, but they do have an important role in the interaction of the plant with its environment. Many plants containing high value compounds are difficult to cultivate or are becoming endangered because of overharvesting (Rates, 2004). The biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an attractive alternative to the extraction of whole plant material. However, the use of plant cell or organ cultures has had only limited commercial success (Vanishree *et al*., 2004). Many biotechnological strategies like high yielding cell line culture, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization and biotransformation have been experimented for enhanced production of secondary metabolites from medicinal plants (Dornenburg and Knorr, 1995). Cell cultures have been established from many

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plants but often they do not produce sufficient amounts of the required secondary metabolites (Rao and Ravishankar, 2002). However, in many cases the production of secondary metabolites can be enhanced by the treatment of the undifferentiated cells with elicitors such as methyl jasmonate, salicylic acid, chitosan and heavy metals (Dicosmo and Misawa, 1985). *Pseudarthria viscida* (L.) Wight and Arn. belonging to the family Leguminosae is a perennial viscid pubescent semi erect diffuse under shrub. It is distributed throughout India especially found in hills up to above 900m and in river basins (Krithikar and Basu, 1918). The roots are with astringent, thermogenic, digestive, anthelmintic, antiinflammatory, antifungal, antidiarrhoel, antioxidant, aphrodisiac, nervine, febrifuge, cardio and rejuvenating properties (Warrier *et al*., 1996). They are useful in vitiated conditions of cough, bronchitis, asthma, tuberculosis, helminthiasis, cardiopathy, fever, hemorrhoids, gout, diabetes, hyperthermia and general debility. Major chemical compounds reported be present in the roots are 1,5 dicaffeoyl quinic acid, oleic acid, tetradecanoic acid, rutin, quercetin, gallic acid, ferulic acid and caffeic acid (Vijayabaskaran *et al*., 2010). Since *Pseudarthria viscida* has high commercial and medicinal values. The excessive collection has resulted in gradual disappearance of this plant from the wild. Beside its high medicinal value, this plant has not been cultivated for commercial purposes due to low seed viability, germination rate and high mortality of seedlings in young stages. To conserve the genetic stocks of this plant *in vitro* propagation can be utilized successfully. The main aim of this work is to identify an efficient regeneration system for *Pseudarthria viscida* and *in vitro* secondary metabolite production in MS medium.

MATERIALS AND METHODS

In vitro culturing of *Pseudarthria viscida*

Explants and media

Fresh young leaves, nodes and internodal segments were used as explants for *in vitro* propagation of this plant in MS medium (Murashige and Skoog, 1962). Fresh explants were washed thoroughly with tap water; the explants were surface sterilized in 70% (v/v) ethanol for one minute, subsequently in 1% (w/v) $HgCl_2$ or 3% sodium hypochlorite for 2, 5, or eight minute duration. Finally, the explants were washed with sterile double distilled water thrice. For *in vitro* culturing, the sterilized explant segments were inoculated in medium supplemented with auxins like IAA, IBA, NAA, 2,4-D and cytokines like BAP and Kinetin, tried in range between 0.5- 2.5 mgL^{-1}. Combinations of different concentrations of auxin and cytokinin like 2, 4-D and BA, IBA and BA, NAA and BA, 2, 4-D and kinetin, IBA and kinetin as well as NAA and kinetin were tested. Media was adjusted to *p*H 5.8 and autoclaved (121 °C, 15 min). All media contained 0.8% agar, 3% sucrose. All hormonal combinations were examined to determine the optimal conditions for initiation and indefinite growth of callus and for regeneration (Murashige and Skoog, 1962). All experiments were repeated thrice. Callus formed during the first culture was used for inoculation in sub culture. The callus was cut into pieces and inoculated into the corresponding fresh media. After sub culture the tubes were placed in the incubation room. Temperature of the room was maintained at $25^oC \pm 2^oC$. Light was given for 12 hours per day.

In vitro Organogenesis

For shoot regeneration, the sterilized explant segments were inoculated in medium supplemented with cytokinins- BAP and Kinetin, tried in range between 0.5- 2.5 mgL^{-1}. Combinations of auxins and cytokinins in different concentrations of 2, 4-D and BA, IBA and BA, IAA and BA, NAA and BA, 2, 4-D and kinetin, IAA and kinetin, IBA and kinetin as well as NAA and kinetin were tested. The explants were routinely subcultured in fresh medium. The multiplied microshoots after elongation were transferred for root formation. For *in vitro* root formation, 2, 4- D, IAA, IBA and NAA at a range of 0.5- 2.5mgL^{-1} were used in MS medium. The elongated shootlets were individually cultured in culture tubes containing the rooting media. After eight weeks, percentages of root formation and root numbers of each treatment were recorded.

Acclimatization of Plantlets

Well developed plantlets were transferred to *in vivo* conditions. The plantlets were carefully taken out and the washed with tap water. The plants were transferred to paper cups containing sand, garden soil and farmyard manure in the ratio of 1:1:1 and kept for one month in culture room. After one month, plants were transferred to plastic bags, and maintained at room temperature for 10 days and later moved to the green house.

Elicitation of secondary metabolites

The enhanced production of the secondary metabolites from plant cell cultures through elicitation has opened up a new area of research which could have important economical benefits for pharmaceutical industry (Poulev, 2003). Abiotic elicitors are the substances of non-biological origin, predominantly inorganic salts, and physical factors acting as elicitors like Cu and Cd ions, $Ca^{2+}$and high pH whereas biotic elicitors are substances with biological origin, they include polysaccharides derived from plant cell walls (pectin or cellulose) and micro-organisms (chitin or glucans) and glycoproteins or G-protein or intracellular proteins whose functions are coupled to receptors and act by activating or inactivating a number of enzymes or ion channels (Veersham, 2004).

Exogenous elicitors are substances originated outside the cell like polysaccharides, polyamines and fatty acids whereas endogenous elicitors are substances originated inside the cell like galacturonide or hepta-β-glucosides etc. For elicitation of secondary metabolites (phenolic compound) in *Pseudarthria viscida,* four weeks old callus were cultured in suspension, using an abiotic elicitors (Salicylic acid) and a biotic elicitors (Chitosan) for enhancing the production of phenolic compound. Different concentration of Salicylic acid (0.25mM, 0.5mM, 2.5mM and 5mM) and Chitosan (0.5mg, 1mg, 1.5mg, 2mg) were used for 24, 48 and 72 hrs of treatment duration. The results were analyzed spectrophotometrically.

Identification and quantification of Gallic acid in *Pseudarthria viscida*

Crude root extract, callus extract and different elicitors (biotic and abiotic) treated cell cultures of *Pseudarthria viscida* were subjected to HPTLC analysis for the identification and HPLC analysis for the quantification of bioactive phenolic compound (gallic acid).

HPTLC analysis

HPTLC studies were carried out following Sasikumar *et al*., (2009) method. For the present study, CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by Win CATS- 4 software were used. The samples were spotted in the form of bands of width 8 on pre-coated silica gel glass. The standard Gallic acid was also dissolved in methanol and applied as 8 mm band (5 micro litre). It is then developed upto 85 mm in a twin trough glass chamber using the Mobile Phase: Chloroform-Ethyl Acetate-Formic acid (7.5:6:0.5). After development the plate is scanned at 292 nm using TLC Scanner 3 equipped with WinCats software. The photo documentation of the plate were carried out at 254 nm, 366 nm. The plate was then derivatised using Natural product Reagent and Poly Ethylene Glycol. The photo documentation of the derivatised plate was carried out in 366 nm (Sasikumar *et al*., 2009).

HPLC analysis

For HPLC analysis, about 1mg of and standards were dissolved in 50 ml of methanol and 20µl of the resulting solution was injected into the analytical HPLC system. The corresponding peaks of the individual components within the sample were produced by the chromatograph.

The gallic acid was detected at 254 and 366 nm with a flow rate of 1 ml/min. The column was operated at a temperature of 30°C. Separations were carried out in mobile phase of Methanol/ 0.2%Phosphoric Acid (65:35) at flow rate 1.0 ml /min. The phenolic compounds were analysed by matching the retention time and their spectral characteristics against those of standards (Sawant *et al*., 2010).

RESULTS AND DISCUSSION

In vitro* propagation of *Pseudarthria viscida

Ninety six percentage of callus induction was observed from leafy segment on MS medium supplemented with 1.5 mgl^{-1} 2,4-D + 0.5 mgl^{-1} Kinetin and 2.5 mgl-1 NAA+1 mgl^{-1} BAP. Maximum number of shoot regeneration from nodal segments noticed on MS medium with 0.5 mgl^{-1} NAA + 2.5 mgl^{-1} BAP after 28 days. Maximum number of rooting noticed on medium with 2.5 and 2 mgl^{-1} IBA and NAA respectively after 29 days (Table1-3 and Fig 1-3).Well developed plantlets were transferred to *in vivo* conditions. The plantlets were carefully taken out and the washed with tap water. The plants were transferred to paper cups containing sand, garden soil and farmyard manure in the ratio of 1:1:1 and kept for one month in culture room. After one month, plants were transferred to plastic bags, and maintained at room temperature and later moved to the green house (Fig 2). An efficient plant regeneration system through callus for *Pseudarthria viscida* was reported by Meena and Dennis (2011) from cotyledonary node explants on MS medium supplemented with 1.5 mgl^{-1} 2,4-D. But in the present study ninety six percentage of callus induction was observed in leafy explants with1.5 mgl^{-1} 2,4-D + 0.5 mgl^{-1} Kinetin. In an earlier investigation by Vinoth kumar *et al*., (2009) maximum number of shoots obtained on MS medium supplemented with 0.6mgl^{-1} IBA.

Table 1. Callus culturing of *Pseudarthria viscida* (L.) Wight & Arn.

Hormonal Concentration (mgl^{-1})						Explant	Response (%)	Callusing	No of days
2,4-D	NAA	IBA	IAA	BAP	KIN				
0.5	-	-	-	-	2.5	L	30.00±0.04	++	20
1.0	-	-	-	-	2.0	L	87.33±0.02	+++	23
1.5	-	-	-	-	1.5	IN	55.66±0.03	++	25
2.0	-	-	-	-	1.0	L	66.00±0.04	++	18
2.5	-	-	-	-	0.5	IN	56.66±0.03	+	19
0.5	-	-	-	2.5	-	L,IN	49.16±0.02	+	18
1.0	-	-	-	2.0	-	L	43.33±0.01	+	20
1.5	-	-	-	1.5	-	L	79.16±0.03	+++	24
2.0	-	-	-	1.0	-	IN	62.50±0.29	++	25
2.5	-	-	-	0.5	-	L	58.33±0.03	+	21
	0.5	-	-	2.5	-	L	39.26±0.02	+	19
-	1.0	-	-	2.0	-	L	58.33±0.02	+	21
-	1.5	-	-	1.5	-	IN	65.00±0.39	++	19
-	2.0	-	-	1.0	-	IN	51.56±0.04	++	20
-	2.5	-	-	0.5	-	L	29.16±0.03	+	26
-	-	0.5	-	2.5	-	L	72.50±0.02	+	19
-	-	1.0	-	2.0	-	L,IN	68.33±0.02	++	29
-	-	1.5	-	1.5	-	IN	76.66±0.03	+	18
-	-	2.0	-	1.0	-	IN	39.16±0.03	+	20
-	-	2.5	-	0.5	-	L	50.00±0.29	+	29
-	-	-	0.5	2.5	-	L	41.66±0.03	++	29
-	-	-	1.0	2.0	-	L	22.32±0.02	+	23
-	-	-	1.5	1.5	-	L,IN	33.56±0.04	+	25
-	-	-	2.0	1.0	-	L	28.30±0.01	++	19
-	-	-	2.5	0.5	-	L	36.40±0.03	+	26

L-Leaf segment, IN- Inter Nodes, ± Values represent the standard deviation

Table 2. *In vitro* root induction of *Pseudarthria viscida* (L.) Wight & Arn.

Hormonal Concentration (mgl^{-1})					Explant	Response (%)	Shooting	No of days
NAA	IBA	IAA	KIN	BAP				
-	-	-	3.0	-	N	80.00±0.04	++	20
-	-	-	2.5	-	N	87.00±0.02	+++	23
-	-	-	2.0	-	N	75.60±0.03	++	25
-	-	-	1.5	-	N	66.00±0.04	++	18
-	-	-	1.0	-	N	56.46±0.03	+	19
-	-	-	-	3.0	N	85.36±0.02	++	18
-	-	-	-	2.5	N	83.33±0.01	++	20
-	-	-	-	2.0	N	79.60±0.03	++	24
-	-	-	-	1.5	N	62.50±0.29	+	25
-	-	-	-	1.0	N	58.23±0.03	+	21
0.5	-	-	-	3.0	L	79.23±0.02	+	19
0.5	-	-	-	2.5	L	58.33±0.02	+++	21
0.5	-	-	-	2.0	L	85.00±0.39	++	19
1.0	-	-	-	3.0	L	51.46±0.04	++	20
1.0	-	-	-	2.5	L	49.16±0.03	+	26
-	0.5	-	-	3.0	L	52.00±0.02	+	19
-	0.5	-	-	2.5	L	48.30±0.02	++	29
-	0.5	-	-	2.0	L	26.36±0.03	+	18
-	1.0	-	-	3.0	L	39.26±0.03	+	20
-	1.0	-	-	2..5	L	20.00±0.29	+	29
-	-	0.5	-	3.0	L	21.76±0.03	++	29
-	-	0.5	-	2.5	L	35.00±0.02	+	27
-	-	0.5		2.0	L	27.43±0.01	+	26
-	-	1.0	-	3.0	L	30.73±0.04	+	28
-	-	1.0	-	2..5	L	20.34±0.04	+	29

L-Leaf segment, N-Nodes, ± Values represent the standard deviation.

Table 3. *In vitro* root induction of *Pseudarthria viscida* (L.) Wight & Arn.

Hormonal Concentration (mgl^{-1})				Explant	Response (%)	Rooting/ Adventitious Rooting	No of days
NAA	IBA	IAA	2,4-D				
0.5	-	-	-	N.L	40.00±0.03	+	25
1.0	-	-	-	N,L,IN	58.30±0.01	+	23
1.5	-	-	-	L,N	72.66±0.02	++	25
2.0	-	-	-	N,L,IN	89.00±0.04	+++	29
2.5	-	-	-	N,L	74.66±0.03	++	27
3.0	-	-	-	N,L	39.16±0.02	+	18
-	0.5	-	-	N,L	53.33±0.01	+	20
-	1.0	-	-	N,L	69.16±0.03	++	25
-	1.5	-	-	N,L,IN	63.50±0.29	++	27
-	2.0	-	-	N,L,IN	68.33±0.03	++	26
-	2.5	-	-	N,L,IN	81.26±0.02	+++	29
-	3.0	-	-	L	38.33±0.02	+	21
-	-	0.5	-	N,L	24.00±0.39	+	23
-	-	1.0	-	N,L	31.56±0.04	+	20
-	-	1.5	-	N,L	29.16±0.03	+	26
-	-	2.0	-	N,L	32.50±0.02	+	27
-	--	2.5	-	N,L	48.33±0.02	++	23
-	-	3.0	-	L	26.06±0.03	-	26
-	-	-	0.5	L	29.16±0.03	-	25
-	-	-	1.0	L	20.00±0.29	+	24
-	-	-	2.0	L	41.66±0.03	+	20
-	-	-	3.0	L	22.32±0.02	-	21

L-Leaf segment, IN- Inter Nodes, N- Node ± Values represent the standard deviation.

Table 4. Effect of salicylic acid on phenolic compound production in *Pseudarthria visida*

Name of Elicitor	Treatment Duration	Concentrations of elicitor	Phenolic compound in callus (mg/g)	Phenolic compound in medium (mg/g)	Fold Increase (callus)	Fold Increase (medium)
		Control	1.33±0.34	0.62±0.07	-	-
		0.25mM	1.52±0.21	1.02 ±0.25	1.14	1.64
	24 hrs	0.5mM	1.88±0.56	1.15±0.53	1.41	1.85
		2.5mM	1.83 ±0.12	1.03±0.24	1.37	1.66
		5mM	1.65 ±0.37	0.96±0.31	1.24	1.54
Salicylic		Control	1.08±0.65	0.93 ±0.61	-	-
acid		0.25mM	3.16±0.40	2.51±0.26	2.92	2.69
	48 hrs	0.5mM	5.84±0.61	3.74 ±0.52	5.40	4.02
		2.5mM	3.20±0.26	2.16 ±0.33	2.96	2.32
		5mM	2.11±0.29	1.08 ±0.03	1.95	1.16
		Control	1.80±0.28	1.81±0.20	-	-
		0.25mM	1.98±0.31	1.75±0.30	1.1	-
	72 hrs	0.5mM	2.23±0.50	2.27±0.28	1.23	1.25
		2.5mM	1.51±0.22	1.64±0.12	-	-
		5mM	1.17±0.41	0.95±0.08	-	-

Table 5. Effect of Chitosan on phenolic compound production in *Pseudarthria visida*

Name of Elicitor	Treatment Duration	Concentrations of Elicitor	Phenolic compound in callus (mg/g)	Phenolic compound in medium (mg/g)	Fold Increase (callus)	Fold Increase (medium)
		Control	1.32 ±0.05	1.46±0.3	-	-
		0.5mg	3.47±0.21	1.10±0.56	2.62	-
	24 hrs	1.0mg	4.33±0.03	1.87±0.34	3.28	1.28
		1.5mg	5.24±0.04	2.43±0.06	3.96	1.66
		2.0mg	3.78 ±0.15	1.69±0.58	2.86	1.15
Chitosan		Control	1.01±0.26	1.62±0.03	-	-
		0.5mg	5.02±0.53	1.73±0.12	4.97	1.06
	48 hrs	1.0mg	5.34±0.46	1.80±0.43	5.28	1.11
		1.5mg	9.17±0.61	2.94 ±0.32	9.07	1.81
		2.0mg	6.19±0.23	1.03±0.14	6.12	-
		Control	2.01 ±0.03	1.76 ±0.57	-	-
		0.5mg	1.86 ±0.01	1.36±0.53	-	-
	72 hrs	1.0mg	2.08±0.45	1.56±0.32	1.03	-
		1.5mg	4.45±0.65	2.12±0.65	2.21	1.20
		2.0mg	2.12±0.73	1.29±0.45	1.05	

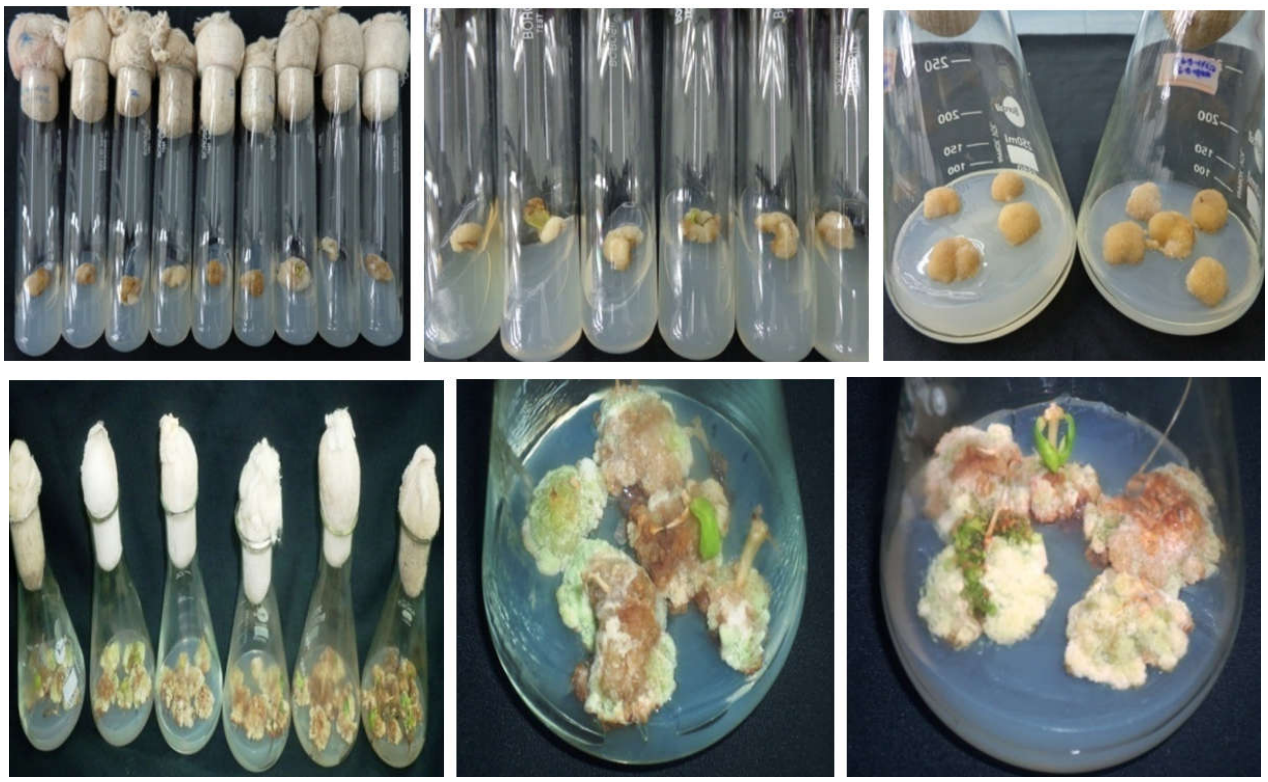


Fig. 1. Callus culturing of *Pseudarthria viscida* (L.) Wight & Arn.

However, in the present study highest response was found on MS medium supplemented with 0.5 mgl^{-1} NAA + 2.5 mgl^{-1} BAP after 28 days. These results indicated that a combination of auxin and cytokinin is essential for getting best response for regeneration in *Pseudarthria viscida* and IBA was superior over NAA in rooting. IBA was generally found to be most effective for induction of roots in legumes as was observed in plants like *Pterocarpus marsupium*. (Chand and Singh, 2004).

Elicitation of secondary metabolites

The compounds which stimulating any type of physiological abnormality of plant are elicitors. It could be used as enhance of plant secondary metabolite synthesis and could play an important role in biosynthetic pathways to enhanced production of commercially important compounds (Veersham, 2004). Salicylic acid has been reported to induce gene regulation related to biosynthesis of secondary metabolites in plants. It accumulates at the site of pathogenic attack and plant hypersensitive reaction from where it spreads to different parts of the plant to induce a wide range of defense response (Vijay and Moinuddin, 2013). For the elicitation of phenolic compound in *Pseudarthria viscida*, different concentration of salicylic acid (0.25mM, 0.5mM, 2.5mM and 5mM) were used for 24, 48 and 72 hrs of treatment duration in both suspension callus and suspension medium. Maximum phenolic compound production were noticed in 0.5mM concentration of salicylic acid (5 fold increase) in 48 hrs treatment from callus whereas only 4 fold increase of phenolic compound were noticed from the suspension medium (Table 4). Vijay and Moinuddin (2013) already reported the elicitation of flavanoids by salicylic acid in *Andrographis paniculata* cell culture.

Fig. 2. Mass propagation and Hardening of *Pseudarthria viscida* (L.) Wight & Arn.

Fig. 3. Adventitious roots induction of *Pseudarthria viscida* (L.) Wight & Arn.

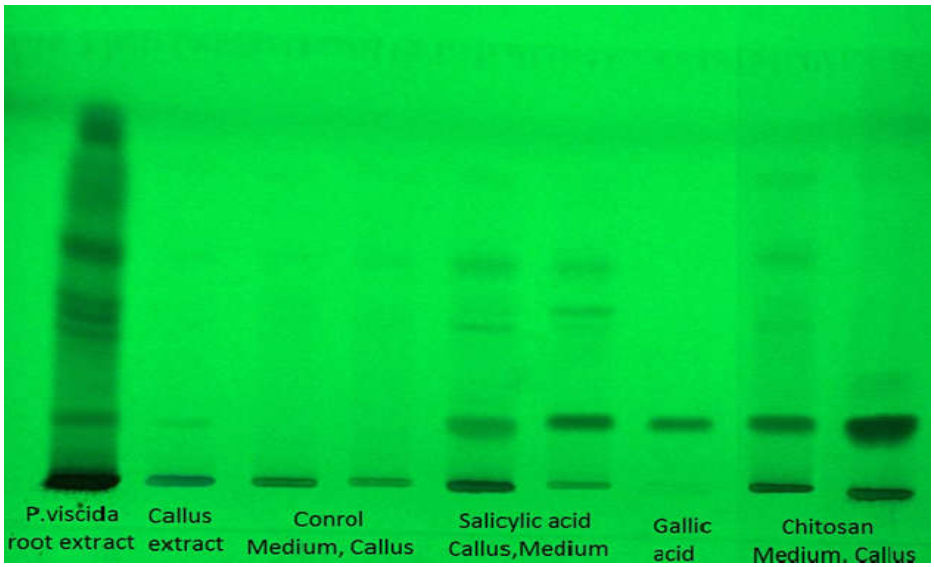
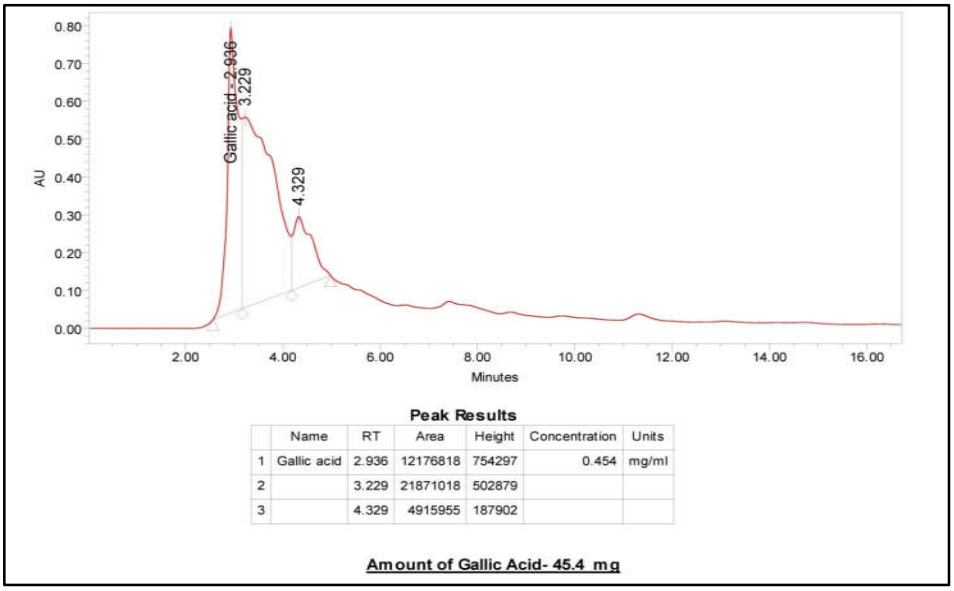


Fig 4. Identification of bioactive phenolic compound (gallic acid) in *Pseudarthria viscida* by HPTLC method



	Name	RT	Area	Height	Concentration	Units
1	Gallic acid	2.936	12176818	754297	0.454	mg/ml
2		3.229	21871018	502879		
3		4.329	4915955	187902		

Fig. 5. Quantification of bioactive phenolic compound (gallic acid) in chitosan treated cell culture by HPLC method

In the cell suspension of *Andrographis paniculata,* salicylic acid was added in different concentrations like 0.05mM, 0.5mM and 1.5mM for 24, 48 and 72 hrs of treatment duration. Of which 24 hrs with 0.05mM concentration of treatment duration showed 1.39 fold increments in total flavonoid content (1.72mg/g). But in *Pseudarthria viscida,* phenolic compound production were maximum in 48hrs treatment (5.48mg/g) of 0.5mM concentration of salicylic acid. An exogenous biotic elicitor that is derived from the fungal cell wall is chitosan (Montesano *et al.,* 2003), has been studied for their effects on phenyl propanoid metabolic enzymes (Chakraborty *et al*., 2009) and secondary metabolite production (Wiktorowska *et al*., 2010). Chitosan involved in interactions between plants and microorganisms gave the best results by activating secondary metabolism in *Morinda citrifolia* cell suspension cultures (Dornenburg and Knorr, 1995).

In *Pseudarthria viscida*, 9 fold increase of phenolic compound were noticed in 1.5 mg concentration of chitosan treatment from cultured callus whereas only 1.8 fold increase from suspension medium (Tables 5) Chitosan mediated elicitation treatment was superior over the treatment of salicylic acid in *Pseudarthria viscida.*

Identification and quantification of Gallic acid in *Pseudarthria viscida*

A poly hydroxyl phenolic compound- gallic acid is widely distributed in various plants, fruits and foods, where it is present either in free form or, more commonly, as an ingredient of tannins. It has been found to be pharmacologically active as an antioxidant, antimutagenic, lipid lowering, anti atherosclerotic, antiliver injury, anti-tumor, and anti carcinogenic agent (Jie and Yaguang, 2011).

HPTLC and HPLC results were showed in Figs. 4 and 5. Gallic acid accumulation in cell suspension culture of *Acer ginnala* was reported by Jie and Yaguang, (2011) using HPLC.

Conclusion

In vitro propagation and secondary metabolite production are important for conservation of *Pseudarthria viscida*. From the results, 2,4-D and KIN and NAA and BAP combinations are most suitable for callus induction, high concentraion of BAP and low concentration of NAA are best for shoot regeneration and high concentration of NAA is good for root induction in *Pseudarthria viscida*. Chitosan gave the best result for elicitation of phenolic compound (gallic acid) in the callus whereas salicylic acid gave the best result for production of gallic acid in medium. The optimal fold increase (9 fold increase) of phenolic compound were noticed in 48 hours of 1.5 mg Chitosan treatment. Maximum gallic acid content (45.4mg/g of callus) was noticed in chitosan treated cell culture which was quantified by HPLC method. This will be helpful for the conservation and maximum utilization of the plant along with the *in vitro* production of useful bioactive molecules.

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