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RESEARCH ARTICLE

Elicitation and Isolation of Gallic Acid from Cell Suspension Culture of *Pseudarthria viscida* (L.) WIGHT & ARN.

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Abstract

Pseudarthria viscida (L.) Wight & Arn. is a perennial semi erect diffuse under shrub, belonging to the family Fabaceae. This plant is an essential component of many famous Ayurvedic formulations like Dashamoola, Mahanarayana taila and Dhantara taila. Major chemical compounds present in the roots include gallic acid, ferulic acid, caffeic acid, rutin, quercetin and oleic acid. Of which, gallic acid was the major phenolic compound present in highest quantity than other phenolics in *Pseudarthria viscida.* Gallic acid is a poly hydroxyl phenolic compound widely distributed in various plants, fruits and foods. It acts as an antioxidant, antimutagenic, lipid lowering, anti atherosclerotic, antiliver injury, antitumor, and anticarcinogenic agent. The main aim of this work is the enhanced production of this important secondary metabolite gallic acid using biotic and abiotic elicitors in cell suspension culture of *Pseudarthria viscida*. For secondary metabolite production, the four week old callus were cultured in suspension using different concentration of abiotic (Salicylic acid, Calcium chloride) and biotic elicitors (Chitosan, Pectin) for 24, 48 and 72 hrs of treatment duration. The results were analyzed spectrophotometrically. The identification and quantification of bioactive phenolic compound (gallic acid) was carried out by HPTLC and HPLC methods. Isolation was carried out by Column chromatography**,** Preparative TLC, UV & IR methods. The optimal fold increase of phenolic compound was noticed in 48 hour callus culture by 1.5 mg chitosan treatment. These chitosan treated cell suspension were further used for isolation purpose of phenolic compound. Future works should focus on evaluation of medicinal properties of the isolated compound.

Introduction:-

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. The production of these compounds is often low with less than 1% dry weight and depends greatly on the physiological and developmental stage of the plant (Oksman and Inze, 2004). Currently one-fourth of all prescribed pharmaceuticals in industrialized countries contain compounds that are directly or indirectly, via semi-synthesis, derived from plants (Rates, 2001). Plant derived drugs in western countries also have a huge market value. Many plants containing high value compounds, but are difficult to cultivate or are becoming endangered because of overharvesting (Rates, 2001). Furthermore, the chemical synthesis of plant derived compounds is often not economically feasible because of their highly complex structures and the specific stereochemical requirements of the compounds. The biotechnological production of valuable secondary metabolites in plant cell or organ cultures is an attractive alternative to the extraction and destruction 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 approaches had been experimented for enhanced production of secondary metabolites from medicinal plants. Some of these include screening of high yielding cell line, media modification, precursor feeding, elicitation, large scale cultivation in bioreactor system, hairy root culture, plant cell immobilization and biotransformation (Dornenburg and Knorr, 1995). Cell cultures have been established from many 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. It has opened up a new area of research which could have important economical benefits for pharmaceutical industry (Poulev, 2003). Elicitors can be classified on the basis of their 'nature' like abiotic elicitors or biotic elicitors, or on the basis their 'origin' like exogenous elicitors and endogenous elicitors. 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.

Pseudarthria viscida (L.) Wight and Arn. is a perennial semi erect diffuse under shrub, belonging to the family Fabaceae. It is distributed throughout India especially found in river basins and in hills up to above 900m (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 to 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, 2010). On the basis of phytochemical screening and HPLC analysis of crude extract of *Pseudarthria viscida*, gallic acid was the major phenolic compound present in highest quantity compared to other phenolics present in *Pseudarthria viscida* (Thinagaran and Suriyavathana, 2013). Gallic acid is a poly hydroxyl phenolic compound widely distributed in various plants, fruits and foods, where it is present either in free form or as an ingredient of tannins. It acts as an antioxidant, antimutagenic, lipid lowering, anti atherosclerotic, antiliver injury, antitumor, and anti carcinogenic agent (Jie and Yaguang, 2014). The main aim of this work was the enhanced production of this important secondary metabolite gallic acid by biotic and abiotic elicitation in cell suspension culture of *Pseudarthria viscida*.

Materials and methods:-

Elicitation of secondary metabolite:-

For elicitation of secondary metabolite, gallic acid which is a phenolic compound in *Pseudarthria viscida,* four weeks old callus was cultured in suspension. Two abiotic elicitors (Salicylic acid, calcium chloride) and two biotic elicitors (Chitosan, Pectin) were used for enhancing the production of phenolic compound. Different concentration of Salicylic acid (0.25mM, 0.5mM, 2.5mM and 5mM), Calcium chloride (0.025µM, 0.05 µM, 0.25 µM, 0.5 µM), Chitosan (0.5mg, 1mg, 1.5mg, 2mg) and Pectin (0.5mg, 1mg, 1.5mg, 2mg) were used for 24, 48 and 72 hrs of treatment duration. Phenolic compound was tested in callus and medium. The results were analyzed spectrophotometrically (Poulev, 2003). All experiments were done thrice in triplicates.

Identification and quantification of Gallic acid:-

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

HPTLC analysis:-

HPTLC studies were carried out following Sasikumar et al. method (2009). 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 WinCATS- 4 software were used. The samples were spotted in the form of bands of width 8 mm with a Camag 25 µL microlitre syringe on pre-coated silica gel glass plate 60F-254 (20 × 10 cm with 50 µm thickness (E. Merck) using a Camag Linomat IV. 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 photodocumentation 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 photodocumentation of the derivatised plate was carried out in 366 nm (Sasikumar et al., 2009).

HPLC analysis:-
For HPLC analysis, about 1mg of the samples (Crude root extract, callus extract, and elicitor treated cell cultures) 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).

Isolation of secondary metabolite (Gallic acid):-
After the identification and quantification of phenolic compound (gallic acid) in crude root extract, callus extract and different elicitors (biotic and abiotic) treated cell cultures of *Pseudarthria viscida*, isolation of gallic acid were carried out. From the result of HPTLC and HPLC analysis, gallic acid quantity was highest in chitosan treated cell cultures of *Pseudarthria viscida* than that of crude root extract, callus extract and other elicitors treated cell cultures. So chitosan treated cell suspension cultures of *Pseudarthria viscida* were further used for isolation purpose of phenolic compound.

Isolation was carried out by Column chromatography**,** Preparative TLC, UV & IR methods.

Column chromatography:-
The column chromatography (Length: 450 mm; Bore: 30 mm) was performed using 60-120 mesh silica gel to elute out individual components from the chitosan treated cell suspension cultures of *Pseudarthria viscida.* The column was rinsed with hexane and completely dried before use. The packing was performed after activating the silica gel at 100 °C for 1 h and gently poured on the top of the column with constant tapping to avoid air bubbles and cracks after mixing with hexane. The column was run with varying solvent polarities, elution was started with hexane followed by gradient mixtures of Hexane: Toluene then pure Toluene followed by gradient mixtures of Toluene: Ethyl acetate then pure Ethyl acetate followed by gradient mixtures of Ethyl acetate: Formic acid then pure Formic acid followed by gradient mixtures of Formic acid: Methanol and at last with pure Methanol, loaded with methanol extract of chitosan treated suspension callus mixed with 10-20 g of activated silica gel. The flow rate was maintained at 1 ml per min and the fractions collected were tested by TLC for single spot. Each fraction was tested for the presence of gallic acid (Vijayalakshmi and Ravindhran, 2012).

Gallic Acid Test:-
To detect Gallic acid, 1 ml extract was mixed with 10 ml of distilled water and filtered. Ferric chloride reagent (3drops) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic acid (Vijayalakshmi and Ravindhran, 2012).

Thin layer chromatography (TLC):-
TLC is one of the most widely used and potent techniques to resolve mixture of plant compounds. The TLC plate supplied by Merck, Germany (TLC Silica gel 60 F254) was used to observe the separation of individual compounds as a single spot from the selected crude extract. Various solvent systems commonly used for gallic acid separation, such as Toluene:Ethyl acetate:Formic acid:Methanol (3:3:0.8:0.2), Chloroform:Ethyl Acetate:Formic acid (7.5:6:0.5), Ethyl acetate:Glacial acetic acid:Formic acid:Water (7:3:3:1).were tried for getting purity of compound. The developed TLC plate after visualizing with different staining procedures (Iodine, 5% Ferric chloride, UV and Ninhydrin), served as a reference to identify and confirm the compounds eluted through column chromatography (Vijayalakshmi and Ravindhran, 2012).

Preparative TLC:-

The glass plate measuring 20 x 20 cm was used for the preparative TLC, Silica gel G for TLC was used as an adsorbent. Layers of 0.5-2mm were prepared and air dried to avoid cracking of thick layers which was later moved to oven for activation at 110 ^{0}C for 1 hour. Then the plate were taken out from the oven and spot the sample in the form of band, and kept the plate for some time for drying, meanwhile the chamber with solvent was saturated, after that the plate was kept in the chamber and allowed to develop. After development the plate was taken out and bands were scrapped off with spatula and washed with an appropriate solvent to remove the components. Methanol was used because the components are soluble in methanol. A couple of washing was made to get the entire component from the silica gel followed by centrifugation and supernatant was collected leaving the silica gel behind (Vijayalakshmi and Ravindhran, 2012). The supernatant was concentrated to get pure compound, which was used for UV& IR analysis.

Ultra Violet Analysis & Infrared (IR) spectrophotometric analysis of isolated compound:-

The UV (MeOH) spectra were recorded on a Varian Cary 3E spectrophotometer. Settings made with spectral bandwidth of 5nm, wavelength accuracy of ± 1.0nm and wavelength ranging from 190- 800nm. Absorbance ranged from 0.00-0.80Aand 0.00-1.00A. The samples were prepared as a 0.1% solution in methanol. For IR analysis, about 1mg of isolated compound was finely ground in a small mortar with about 10 times its bulk of pure potassium bromide and the mixture pressed into a disc using a special mould and a hydraulic press. The functional group was determined using FTIR-820IA single beam laser Shimadzu Infrared Spectrophotometer (Sravan Kumar et al., 2015)

Result and discussion:-

Elicitation of secondary metabolites:-

Elicitors are compounds which stimulating any type of physiological abnormality of plant. Elicitors 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). Two abiotic (Salicylic acid, calcium chloride) and two biotic elicitors (Chitosan, Pectin) were used for enhanced production of phenolic compounds in suspension culture of *Pseudarthria viscida* [Fig 1].

Salicylic acid otherwise known as 2-hydroxy benzene carboxcylic acid is a colorless crystalline organic acid, which has important role to play in growth and development, photosynthesis, transpiration, ion uptake and solute transport. It has been reported to induce gene regulation related to biosynthesis of secondary metabolites in plants which 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 which is 5 fold increase in 48 hrs treatment from callus whereas only 4 fold increase of phenolic compound were noticed from the suspension medium [Table 1].

Elicitation of flavonoids by Salicylic acid in *Andrographis paniculata* cell culture was reported by Vijay and Moinuddin (Vijay and Moinuddin, 2013) where 0.05 mM of salicylic acid showed 1.39 fold increments in total flavonoid content in *Andrographis paniculata.* But in *Pseudarthria viscida*, phenolic compound production were maximum (5.84 mg/g) in 48 hrs treatment of 0.5mM concentration of salicylic acid.

Calcium chloride is an important abiotic elicitor used for the production of important secondary metabolite 'Forskolin' from *Coleus barbatus* (Heena Patel and Krishnamurthy, 2013) *and* phenols in *Marsilea quadrifolia* was reported by Manjula & Mythili (2012). In *Pseudarthria viscida,* calcium chloride was used for elicitation of phenolic compound. Maximum phenolic compound production (3 fold increase) was noticed in 0.05µM concentration in 48 hrs treatment duration from both suspension callus and medium [Table 2].

Natural elicitors include polysaccharides such as pectin and chitosan which may enhance phenyl propanoid pathway, and are being frequently used in a number of plant cell cultures for efficient induction of pharmaceutically important secondary metabolites. Chitosan is an exogenous biotic elicitor that is derived from the fungal cell wall (Montesano, 2003) has been studied for their effects on phenylpropanoid metabolic enzymes (Chakraborty, 2009) and secondary metabolite production. Pectin was also found to enhance induction of oleanolic acid biosynthesis in cell suspension cultures of *Calendula officinalis* (Wiktorowska, 2010). Chitosan and pectin

involved in interactions between plants and microorganisms gave the best results by activating secondary metabolism in 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 [Table 3]. Pectin treatment showed very little phenolic production in both cultured callus and medium [Table 4].

Chitosan mediated elicitation treatment was superior over the other three elicitors in *Pseudarthria viscida*. Increased chitosan concentration caused an increase in metabolite release, which was correlated with cell death. It has been reported that if the changes in gene expression include decreased total protein synthesis, induction of hypoxia associated proteins, and glucose regulated proteins are inadequate to prevent ATP depletion. Membrane ion pumps fail and membrane integrity is lost. Increased intercellular Ca^{2+} occurs and varieties of degradation processes are initiated, leading to cytoplasmic swelling and eventually cell death (Dornenburg and Knorr, 1995).

Identification and quantification of Gallic acid:-

Gallic acid is an interesting natural compound because of its antioxidant, antiinflammatory, antifungal and antitumor properties. Gallic acid is a trihydroxybenzoic acid, a type of phenolic acid. The physical properties of gallic acid are: chemical formula $C_6H_2(OH)_3COOH$, molecular weight 170 and boiling point 250^oC. Gallic acid and its catechin derivatives are also present as one of the main phenolic components in many plants including both black and green tea. Gallic acid also has therapeutic applications for inflammatory allergic diseases, such as asthma, allergic rhinitis, sinusitis due to its ability to inhibit histamine release and the expression of proinflammatory cytokine. In addition, gallic acid is employed as a source material for inks, paints and colour developers (Jie and Yaguang, 2014). Gallic acid accumulation was reported in cell suspension culture of some plants like *Acer ginnala* (Jie and Yaguang, 2014). HPTLC and HPLC results from suspension culture of *Pseudarthria viscida* were showed in figures 2-5.

Isolation of secondary metabolite (Gallic acid):-

Column chromatography:-

Methanol extract of chitosan treated suspension callus (8g) subjected to silica gel column chromatography eluting with different mixtures of hexane, Toluene, Ethyl acetate, formic acid and methanol. Elution of 60 fractions of 5 ml each was collected in dry glass tubes and then subjected for phytochemical tests for gallic acid. Fractions 29 to 34 gave positive result for gallic acid phytochemical test. These fractions along with standard gallic aid were subjected for HPTLC analysis, for knowing its presence in the extract [Fig 6]. Fractions 31 to 33 gave the bands similar to standard gallic acid at Rf value - 0.47. These fractions were pooled together and used for further analysis.

Thin Layer Chromatography (TLC):-

Different solvent systems were used to isolate the active constituent. Of which, Toluene: Ethyl acetate: Formic acid: Methanol (3: 3: 0.8: 0.2) gave better separation of gallic acid. This solvent system was used in the preparation of preparative TLC for the isolation of gallic acid.

Ultra Violet Analysis & Infrared (IR) spectrophotometric analysis of isolated gallic acid:-

Result of UV and IR analysis of isolated gallic acid were shown in figure 7 & 8. The isolation and characterization of gallic acid were reported in the fruit extract of *Terminalia chebula* by Sravan Kumar *et al.* (2015) using similar procedures as in *Pseudarthria viscida*. In *Terminalia chebula* isolation and characterization were done by Column chromatography**,** Preparative TLC, UV & IR methods. The UV (MeOH) spectra show that λ max at 271.2 nm and 218nm. The IR spectrum showed intense absorption bands at 3281cm^{-1} and 3495 cm^{-1} corresponding to hydroxyl group of carboxylic acid and phenolic-OH group respectively with an absorption band corresponding to aromatic – C-H at 3062 cm^{-1}. Absorption bands at 1667 cm^{-1} and 1611 cm^{-1} correspond to carnonyl and aromatic C=C functional groups similar to that of *Pseudarthria viscida*.

Conclusion:-

In the present study thus concluded that the chitosan gave best result for elicitation of phenolic compound (gallic acid) in the cultured callus of *Pseudarthria viscida* whereas salicylic acid gave the best result in suspension medium. The optimal fold increase of phenolic compound was noticed in 48 hours of 1.5 mg of chitosan treatment. Maximum gallic acid content (45.4mg/g of callus) was quantified by HPLC method and its isolation was done by Column

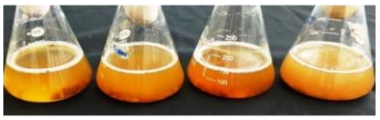
chromatography**,** Preparative TLC, UV & IR methods. This will be helpful for the maximum *in vitro* production and isolation of useful bioactive molecule- gallic acid from *Pseudarthria viscida.*

Fig 1:- Elicitation of phenolic compound from suspension culture of *Pseudarthria viscida* (L.) Wight & Arn.

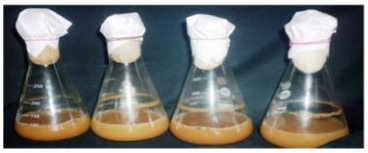
Four weeks old callus

Elicitation process

24 hrs culture



48 hrs culture



72 hrs culture

Fig 2:- HPTLC analysis of *Pseudarthria viscida* (L.) Wight & Arn.

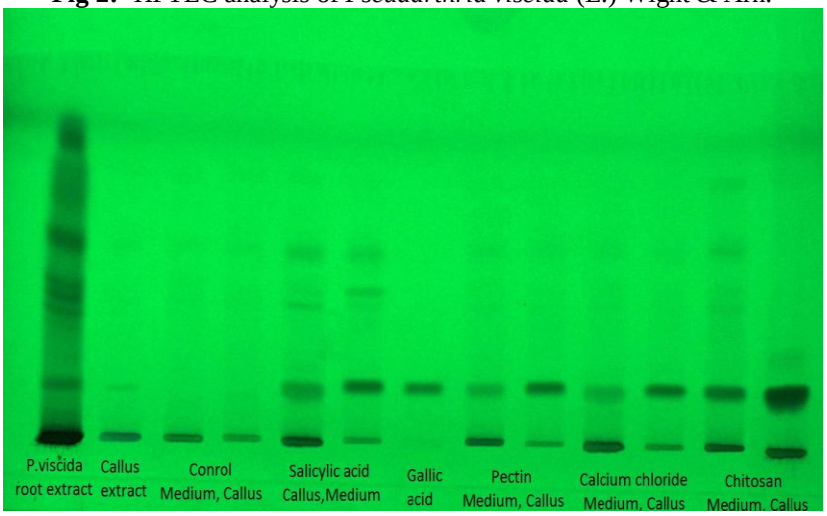
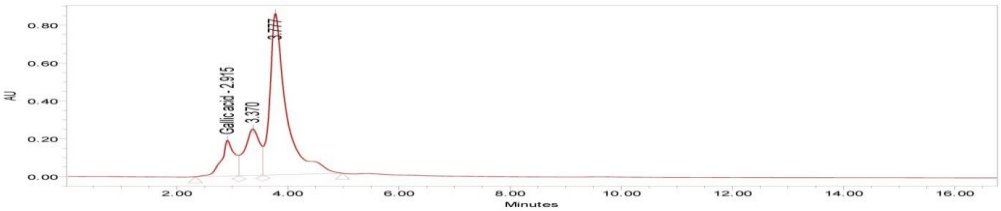


Fig 3:- Quantification of bioactive phenolic compound (gallic acid) in crude extract of *Pseudarthria viscida* by HPLC method

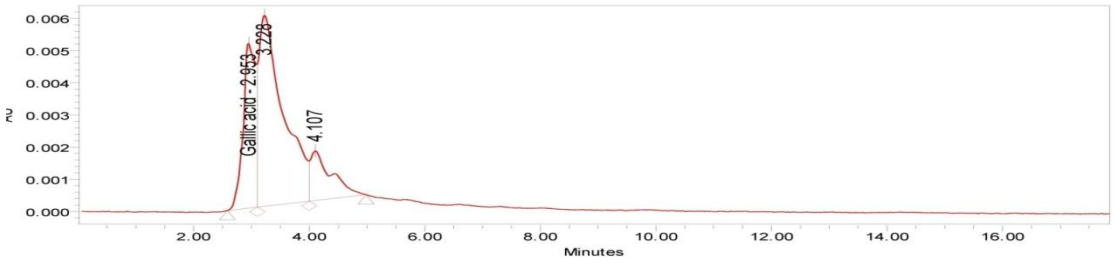


Peak Results

	Name	RT	Area	Height	Concentration	Units
1	Gallic acid	2.915	3144061	189012	0.117	mg/ml
2		3.370	4632079	245452		
3		3.777	17585072	853812		

Amount og Gallic acid- 11.7 mg

Fig 4:- Quantification of bioactive phenolic compound (gallic acid) in callus extract of *Pseudarthria viscida* by HPLC method

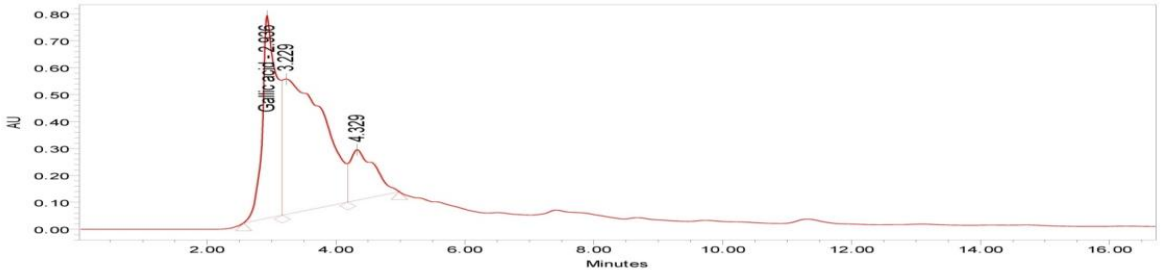


Peak Results

	Name	RT	Area	Height	Concentration	Units
1	Gallic acid	2.953	76369	5126	0.003	mg/ml
2		3.228	176079	5929		
3		4.107	38684	1541		

Amount of Gallic Acid- 0.03 mg

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



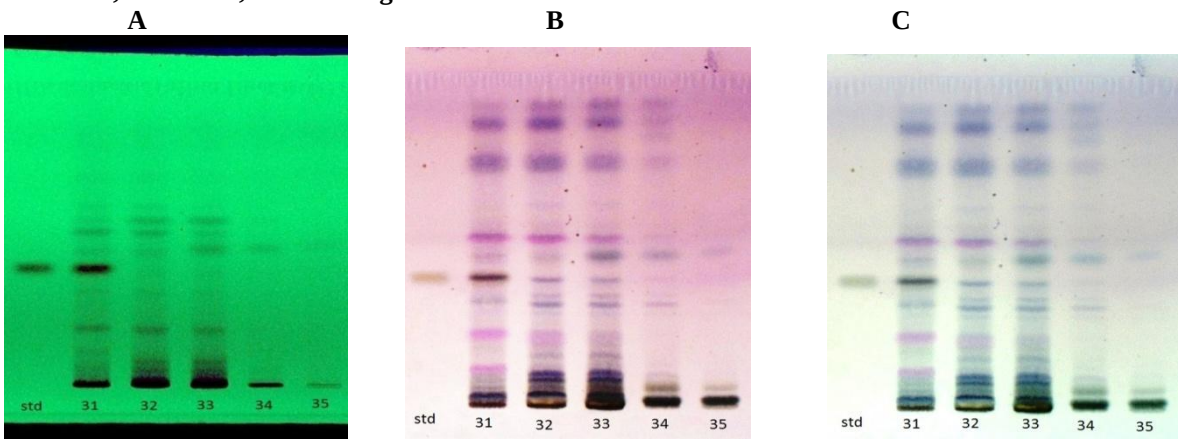
Peak Results

	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		

Amount of Gallic Acid- 45.4 mg

Fig 6:- HPTLC finger print of isolated fractions of Methanol extract of chitosan treated suspension callus of *Pseudarthria viscida* visualised under

A: 254nm; B: 366nm; C: White light



F**ig 7:-** Ultra Violet Analysis of isolated compound-gallic acid

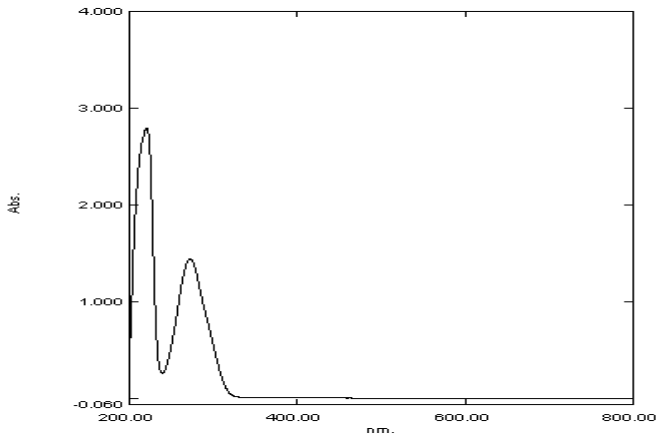


Fig 8:- Infrared (IR) spectrophotometric analysis of isolated compound-gallic acid

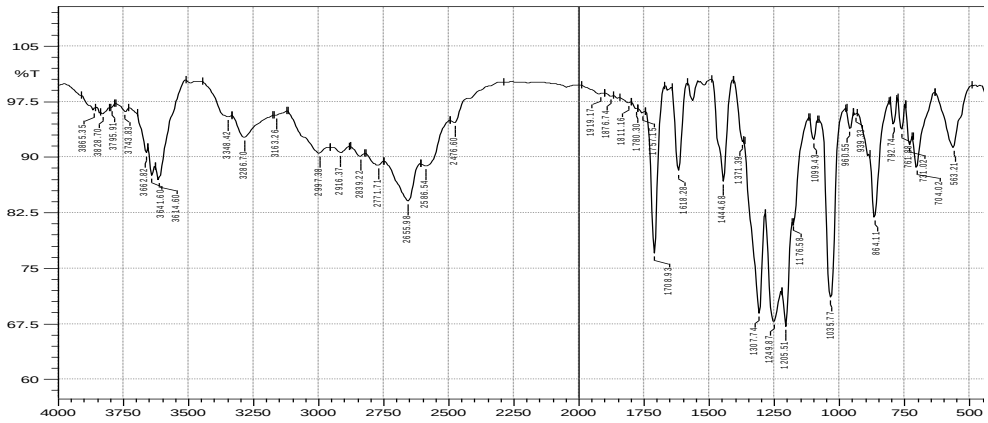


Table 1:- Effect of salicylic acid on phenolic compound production of *Pseudarthria visida*

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

Table 2:- Effect of calcium chloride on on phenolic compound production of *Pseudarthria visida*

Name of Elicitor	Treatment Duration	Concentrations of Elicitor	Phenolic compound in callus (mg/g)	Fold Increase (callus)	Phenolic compound in medium (mg/g)	Fold Increase (medium)
Calcium chloride	24 hrs	Control	1.09±0..21	-	0.64±0.01	-
		0.025µM	1.67±0.06	1.53	0.95±0.23	1.48
		0.05µM	3.04 ±0.26	2.78	1.41±0.22	2.20
		0.25µM	2.15±0.28	1.97	1.21±0.43	1.89
		0.5µM	2.07±0.07	1.89	0.35 ±0.02	-
	48 hrs	Control	1.02±0.28	-	1.10 ±0.01	-
		0.025µM	1.18±0.11	1.15	2.68±0.34	2.43
		0.05µM	**3.85** ±0.04	**3.77**	**4.13**±0.54	**3.23**
		0.25µM	2.47±0.56	2.42	2.92±0.19	2.65
		0.5µM	1.62±0.72	1.58	2.21±0.21	2.00
	72 hrs	Control	1.09 ±0.15	-	1.60 ±0.11	-
		0.025µM	2.10 ±0.23	1.92	1.14±0.09	-
		0.05µM	2.58 ±0.08	2.36	3.91±0.29	2.44
		0.25µM	2.12±0.34	1.94	1.89±0.18	1.65
		0.5µM	1.07 ±0.53	-	1.77±0.03	1.10

Table 3:- Effect of Chitosan on phenolic compound production of *Pseudarthria visida*

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

Table 4:- Effect of pectin on phenolic compound production of *Pseudarthria visida*

Name of Elicitor	Treatment Duration	Concentrations of Elicitor	Phenolic compound in callus (mg/g)	Fold Increase (callus)	Phenolic compound in medium (mg/g)	Fold Increase (medium)
Pectin	24 hrs	Control	1.02 ±0.33	-	0.65±0.01	-
		0.5mg	1.33 ±0.31	1.53	0.97±0.16	1.49
		1.0mg	3.14 ±0.12	2.78	1.45±0.27	2.23
		1.5mg	1.96±0.47	1.97	1.09±0.43	1.67
		2.0mg	1.81±0.76	1.89	0.74 ±0.12	1.13
	48 hrs	Control	1.19±0.52	-	1.87±0.36	-
		0.5mg	2.82±0.43	1.15	1.20±0.55	-
		1.0mg	**3.34**±0.45	**2.79**	**2.37**±0.61	**1.26**
		1.5mg	2.83±0.31	2.42	1.46±0.34	-
		2.0mg	2.08±0.05	1.58	1.22±0.03	-
	72 hrs	Control	4.14±0.23	-	3.39±0.54	-
		0.5mg	2.12 ±0.54	-	2.36 ±0.73	-
		1.0mg	4.62±0.61	1.11	3.49±0.46	1.02
		1.5mg	4.10±0.34		2.13±0.33	
		2.0mg	3.99±0.22	-	1.99±0.12	-

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