Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 07, pp.1718-1725, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

STUDY OF SECONDARY METABOLITES FROM CELL SUSPENSION OF *Gymnema Sylvestre* (RETZ.) R. BR. EX. SCHULT. BY USING ELICITORS

***Asha, V. Jaybhaye and Deokule, S. S.**

Department of Botany, University of Pune, Ganeshkhind, Pune-411007, India

ARTICLE INFO

Article History:
Received 16th April, 2013
Received in revised form
14th May, 2013
Accepted 20th June, 2013
Published online 18th July, 2013

Key words:
Suspension Culture,
Elicitor: Salicylic acid (SA),
pH variation; Yeast extract
(YA),gymnemic acid etc.

ABSTRACT

The present study aimed to explain the effect of biotic and abiotic elicitor derived from yeast extract and Salicylic acid on cell suspensions evolved from superior callus lines of *G. sylvestre* (Retz) R. Br. ex. Schult. Cell suspension cultures maintained in MS liquid medium were treated with 265 cells/ml and 530 cells/ml homogenate of the elicitor on the 14th day. Growth of *G. sylvestre* callus in terms of biomass revealed 5 fold enhancements when treated with 530 cells/ml**.** elicitor on fourteenth day in callus line-1. The type of callus line and concentration of the elicitor influence the biomass growth of *G. sylvestre* callus in suspension culture and gymnemic acid production was accomplish with the help of biotic and abiotic elicitor treatment. (as confirmed by HPTLC analysis)

INTRODUCTION

Herbs and medicinal plants which form the backbone of traditional medicine many researchers investigated that medicinal plants and their individual constituents plays similar role to modern drugs and sometimes better without the side effects.(Anonymous, 1995) *Gymnema sylvestre* (Retz.) R.Br. ex. Schult. (Asclepiadaceae) is a perennial woody climber of tropical and subtropical regions of India (Santapau *et al*., 1960 ; Anonymous, 1997). The leaves are used in herbal medicine preparations. In Hindi the word Gymnema is referred to as "gurmar". Gurmar means "sugar killer". When leaves of Gymnema chewed it has ability to taste sweetness. Hence, it has long been used in diabetes. *Gymnema Sylvestre* leaves mainly contain gymnemic acids. Gurmar can interact with receptors on the tongue to decrease the sensation of sweetness.(Saxena, *et al*., 2004) It is also useful in the various treatments of asthma, eye complaints and also in family planning and snake bite. (Grover, *et al*., 2002) In nature the over exploitation of *Gymnema Sylvestre* caused the depletion. The extracts of this plant are widely used by the Australian, Japananese, Vietnamese and Indian folk medicine. (Cooke, 1958; 1997) However, *gymnema Sylvestre* is best known for its benefits in diabetes therefore, the *in-vitro* culture is another method is used for the production of secondary metabolites of *Gymnema sylvestre*. Hence, in the present study method of in vitro callus growth kinetin and cell suspension by using different biotic and abiotic elicitor for production of gymnemic acid attempted and reported in the present paper.

MATERIAL AND METHODS

Collection of Explants

Efforts were made to collect plant material for the tissue culture experiments fromecologically different localities of Western Ghats of Maharashtra such as high rain fall localities like Dapoli and low rain fall localities like Botanical Garden of Department of Botany, Pune University, Pune.

Corresponding author: Asha Vithoba Jaybhaye
Department of Botany, University of Pune, Ganeshkhind, Pune-411007, India

Nutrient media

MS (1962) with different auxine and cytokinins (Komalavalli, *et al*., 1997).

Culture Conditions: Photoperiod: 8 hour light/16 hour dark. Light intensity: 30 µmol.
Temperature: 25±2°C

Surface sterilization

Leaves were surface sterilized with 0.1% (w/v) mercuric chloride (HgCl2) solution for 5 minutes followed by washing with sterilized distilled water for 7 times. (Bhojwani, *et al.*, 1996)Leaf segment were inoculated on MS medium with different auxine and cytokinins for callus induction. After 4-5 subculture well established callus line used for cell suspension as well as for biotic and abiotic elicitor treatment (Kumar, *et al.*, 2002).

Establishment and maintenance of Callus/cell suspension culture

Leaf segment were inoculated on MS medium with different auxine and cytokinins for callus induction. After 5-6 subculture callus were transferred on liquid (MS) medium with different concentrations and combinations of Cytokinins and Auxins in combinations as well as cultures incubated under controlled conditions on a gyratory shaker (120-150 rpm). Cell suspensions cultures maintained by sub-culturing the cultures (0.5gm) on fresh parental media at 14 days interval for elicitation.

Elicitation for production of Gymnemic acids

The secondary metabolite production can be induced or triggered in the plant cell and organ culture by exposing the culture to different biotic and abiotic elicitors

Biotic elicitors

Fungal elicitor: Yeast with 100-400 mg/L concentrations

Abiotic elicitors

1) Salicylic acid with 0.1-0.50 mM concentrations
2) pH variation with (3.7-6.5).

Analysis of cell fresh weight and dry weight

After separation of cell from liquid medium properly washed with distilled water for three times and cells were drying in oven at 60°C temperature for 6 hrs.

Experimental design and statistical analysis

The experiment was carried out in completely randomized design with 3 replicates each. Variability in data has been expressed otherwise as mean ± standard error.

Quantitative estimation of gymnemic acids

200 mg of each powdered material was mixed in 4 ml of methanol. Mixture is sonicated for 2-3 minute.

Centrifuged at 10,000 rpm for 5 minutes Supernatant collected 20 µl samples is used for HPTLC (Gopi, *et al.,* 2006).

HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY (HPTLC)

HPTLC Technique was followed for the confirmation of phytochemical present in the studied plants (Passera *et al*., 1964 and Sethi, 1996).

Instrument: CAMG Automatic TLC sampler (ATS4)

Plate Size and Stationary Phase: 20 X 10 cm precoated Silica gel TLC plate Merk 60F 254. Mobile Phase: N butanol: methanol: Water: 3: 1 : 1

Developing distance: 80 mm. Tank saturation: 10 min. Scanning wavelength: 580nm.

Derivatization Reagent: Anisaldehyde-Sulphuric Acids reagent. Rf.: 0.61

Standard gymnemic acids concentration: 1µg/µl.

Standard gymnemic acids loaded: 20 µl. (Stahl, *et al.,* 1969; Kanetkar, *et al.,* 2006).

RESULT AND DISCUSSION

For the present investigation, the plant materials were procured from two ecologically different localities such as Dapoli (High rainfall) and Botanical garden of the Department of Botany, University of Pune, Pune-07 (Maharashtra).

Cell Suspension Cultures and Secondary Metabolites

For determination of the active principles (total gymnemic acids) cell was extracted by using methanol and analyzed through HPTLC. Accumulation of gymnemic acids is much higher levels in cultured cells than in the intact plants. Cell suspension cultures were initiated from friable tissues using callus lines developed on MS basal media supplemented with different concentrations and combinations of plant growth regulators (Table 1). The cells in suspension culture obtained on IAA + BAP combinations were friable and white in color. Thus from the above mention results liquid MS combine with 1.0 mg/L IAA+ 0.5 mg/L BAP gave more biomass as well as more quantity of gymnemic acids. (Graph 1) (Devi *et al*., 2006) For efficient separation of secondary metabolites, good selective and sensitive detection, together with the capability of providing online structural information high performance thin layer chromatography techniques are preferred with 0.84 - 0.87 Rf value range (Hostettmann *et al*. 1997). (Fig. 1) Cell suspension culture of garden leaves explants with MS medium and 0.5 mg/L BAP + 1.0 mg/L IAA shows the more quantity of gymnemic acids having 81.9 maximum height of HPTLC and 2041.6 areas covered as compared to the standard gymnemic acids.). (Graph 2, 3). The Table and Graph shows the maximum FWDW of dapoli leaf explants cultured on MS + 1.0 mg/L IAA + 0.5 mg/L BAP in 14 old cell suspension culture as compared to other concentration and combinations.

Cell Suspension Cultures and secondary metabolites

Cell suspension cultures were initiated from friable tissues using callus lines developed on MS basal media supplemented with different concentrations and combination of plant growth regulators. The average fresh weight and dry weight recorded in 14 day old culture of *G. sylvestre* with 1.0 mg/L IAA + 0.5 mg/L BAP concentrations along with gymnemic acids percentages detected through high performance thin layer chromatography. The fresh weight (11.41±0.387gm) and dry weight (0.62±0.028 gm) (Graph 4) in Dapoli explants also it give maximum gymnemic acids percentages (0.065%) as compare to control and other combination of plant growth regulators. (Fig. 2) (Graph 5) The other combination of plant growth regulators along with different concentrations in both the localities shows the less biomass production as well as less gymnemic acids quantities. The plant growth regulators having six combinations with different conc. such as 1.0 mg/L 2 4- D + 2.0 mg/L NAA, 1.5 mg/L 2 4-D + 2.5 mg/L NAA, 1.0 mg/L IAA + 0.5 mg/L BAP and 1.5 mg/L IAA + 1.0 mg/L BAP, 0.5 mg/L 2 4-D + 2.0 mg/L BAP +2.5 mg/L NAA and 1.0 mg/L 2 4-D + 2.5 mg/L BAP + 1.0 mg/L NAA and control. (Table 1 and 2) The cells in suspension culture obtained on IAA + BAP combinations were friable and white in color. Thus from the above mention results liquid MS combine with 1.0 mg/L IAA+ 0.5 mg/L BAP gave more biomass as well as more quantity of gymnemic acids. (Devi, *et al.,* 2006) For efficient separation of secondary metabolites, good selective and sensitive detection, together with the capability of providing online structural information high performance thin layer chromatography techniques are preferred with 0.84 - 0.87 Rf value range (Hostettmann, *et al.,* 1997) (Graph 5).

Effect of biotic elicitor (Yeast Extract)

Composition and selection of medium and concentrations of elicitor plays an important role in elicitation process. The effect of biotic elicitor i.e. yeast with fresh and dry weight along with gymnemic acids % in cell cultures of *Gymnema Sylvestre* is shown in the Table 5. The 14 days old cultures were used for elicitor treatment. After addition of yeast in cultures, cultures were turned to brown. The treatment with different concentrations of yeast extract resulted in less biomass accumulation as compare to the control. The control shows the maximum fresh weight and dry weight at 48 hr time duration. (FW 3.32±0.31gm and DW 0.23±0.009 gm)(Graph 6) but affected the gymnemic acids percentage, control shows the less 0.01% gymnemic acids. After addition of yeast with different concentrations such as 100mg/L, 200 mg/L and 400 mg/L at different time duration. (Fig. 3-4 and Graph 6-7) The maximum gymnemic acids 0.15% in 100mg/L yeast treatment (36 hr time duration) with minimum biomass accumulation (FW 1.11±0.11 gm and DW 0.10±0.003 gm) (Fig. 4).In this experimental work the elicitor treatment was not effective for the growth of cell biomass in *Gymnema sylvestre* cell suspension culture. None of the elicitor used so far showed the growth of the cell biomass over the control but the quantity of gymnemic acids is more as compared to control because it is known that during elicitation process, plant cell firstly recognizes the elicitor by molecular interaction between plant receptor at the cell membrane surface or cytoplasm and low molecular signal legends from fungal cells (Funk, *et al.,* 1987; Nurnberger*et al.,* 1994). Thus it has been shown that the

Table 1. Comparative account for growth of cell cultures using FW (gm) and DW(gm) with percentage of gymnemic acid on different medium concentrations in *G. sylvestre* (Retz.)R.Br.ex. Schult. collected from Botanical Garden of department of Botany Pune University

S. No.	Medium composition (mg/L)	Conc. mg/L	FW(gm)			DW(gm)			Gymnemic in acid%
			1Week	2Week	3week	1Week	2Week	3week	
M1	2 4-D+NAA	1.0+2.0	1.88±0.08	3.89±0.2	5.38±0.3	0.16±0.007	0.34±0.0	0.38±0.023	0.0096
M2	2 4-D+NAA	1.5+2.5	2.45±0.14	4.14±0.8	4.41±0.4	0.31±0.110	0.34±0.0	0.32±0.030	0.0073
M3	IAA+BAP	1.0+0.5	2.62±0.22	6.58±0.7	7.04±0.5	0.20±0.007	0.39±0.0	0.40±0.079	0.017
M4	IAA+BAP	1.5+1.0	3.1±0.05	5.2±0.5	4.99±0.3	0.27±0.019	0.34±0.0	0.30±0.052	0.011
M5	24D+BAP+NAA	0.5+2.0+2.5	1.92±0.07	2.43±0.2	3.14±0.6	0.13±0.020	0.14±0.0	0.28±0.023	0.0083
M6	24D+BAP+NAA	1.0+2.5+1.0	1.54±0.12	2.57±0.3	2.68±0.3	0.16±0.021	0.16±0.0	0.20±0.007	0.0046
C7	Control	Ms basal	2.77±0.08	3.41±0.4	4.50±0.4	0.2±0.01	0.23±0.0	0.18±0.003	0.0094

Each value is the mean of ±S.E. of 3 replicates.

Table 2. Comparative account for growth of cell cultures using Fresh Weight (gm) and Dry Weight(gm) with percentage of gymnemic acid on different medium concentrations in *G. sylvestre* (Retz.) R. Br. ex. Schult. Collected from Dapoli Maharashtra Locality

S. No.	Medium composition (mg/L)	Conc. mg/L	FW (gm)			DW (gm)			Gymnemic acid in%
			1Week	2Week	3week	1Week	2Week	3week	
M1	24-D+NAA	1.0+2.0	5.32±0.320	7.85±0.366	6.59±0.38	0.27±0.032	0.58±0.049	0.43±0.119	0.0094
M2	24-D+NAA	1.5+2.5	6.64±0.854	7.82±1.278	7.23±0.42	0.34±0.053	0.41±0.058	0.40±0.052	0.0092
M3	IAA+BAP	1.0+0.5	6.36±0.767	11.41±0.387	8.89±0.10	0.43±0.042	0.62±0.028	0.56±0.112	0.065
M4	IAA+BAP	1.5+1.0	3.71±0.139	6.32±0.168	5.02±0.03	0.26±0.012	0.36±0.025	0.47±0.065	0.032
M5	24D+BAP+NAA	0.5+2.0+2.5	5.22±0.006	7.94±1.512	6.58±0.08	0.32±0.003	0.61±0.091	0.4±0.121	0.0031
M6	24D+BAP+NAA	1.0+2.5+1.0	3.03±0.036	4.50±0.003	3.77±0.30	0.17±0.015	0.28±0.003	0.42±0.009	0.0048
C7	Control	MSbasal	5.30±0.535	6.40±0.134	5.85±0.41	0.55±0.032	0.44±0.025	0.48±0.021	0.0095

Each value is the mean of ± S.E. of 3 replicates

Table 3. Effect of biotic elicitor yeast with fresh and dry weight along with gymnemic acid % in cell cultures of *G. sylvestre* (Retz.) R. Br. ex. Schult

S. No.	Yeast conc.mg/L	Fresh wt(gm)			Dry wt(gm)			Gymnemic acid in %		
		24hr	36hr	48hr	24hr	36hr	48hr	24hr	36hr	48hr
1	100(Y1)	1.54±0.04	1.80±0.38	1.11±0.11	0.10±0.003	0.12±0.022	0.11±0.017	0.029	0.15	0.02
2	200(Y2)	1.36±0.17	1.85±0.05	1.36±0.32	0.10±0.021	0.13±0.012	0.10±0.019	0.018	0.013	0.019
3	400(Y3)	2.13±0.75	1.85±0.19	1.31±0.03	0.16±0.042	0.12±0.009	0.10±0.003	0.01	0.01	0.01
4	Control	1.37±0.02	2.24±0.03	3.32±0.31	0.14±0.022	0.14±0.012	0.23±0.009	0.006	0.004	0.010

Each value is the mean of ± S.E. of 3 replicates

Table 4. Effect of salicylic acid on cell growth along with FW and DW and gymnemic acid % in Cell cultures of *G. sylvestre* (Retz.) R. Br. ex. Schult

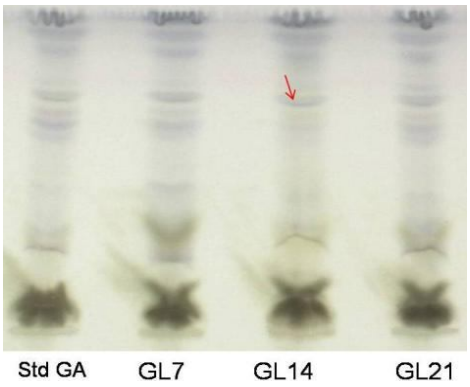
S. No.	SA (µM)	Fresh weight (gm)			Dry weight (gm)			Gymnemic acid in %		
		24hr	36hr	48hr	24hr	36hr	48hr	24hr	36hr	48hr
1	0.1 (S1)	1.17±0.21	1.57±0.15	1.41±0.22	0.10±0.006	0.11±0.006	0.18±0.070	0.012	0.014	0.013
2	0.25 (S2)	1.35±0.30	1.73±0.34	1.31±0.08	0.13±0.012	0.13±0.018	0.11±0.010	0.011	0.015	0.012
3	0.50 (S3)	1.29±0.19	2.08±1.01	1.3±0.15	0.11±0.012	0.15±0.065	0.12±0.012	0.01	0.09	0.03
4	Control	1.37±0.02	2.24±0.03	2.85±0.37	0.14±0.022	0.14±0.012	0.23±0.009	0.006	0.004	0.010

Each value is the mean of ±S.E. of 3 replicates.

Table 5. Effect of pH Variation on cell growth along with Fresh and Dry weight and gymnemic acid % in Cell cultures of *G. sylvestre* (Retz.) R. Br. ex. Schult

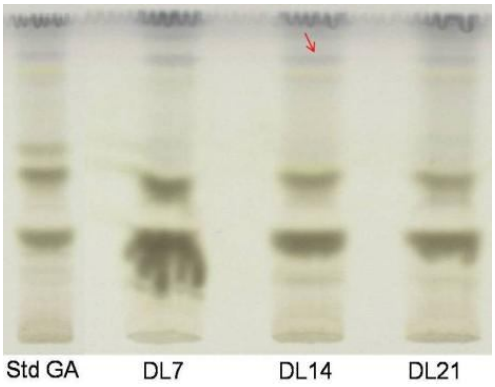
S. No.	pH range	Fresh weight (gm)			Dry weight (gm)			Gymnemic acid in%		
		7days	14days	21days	7days	14days	21days	7days	14days	21days
1	Control	3.03±0.18	5.1±0.1	5.07±0.02	0.16±0.01	0.60±0.10	0.49±0.04	0.005	0.013	0.01
2	3.7	2.88±0.45	4.43±0.58	3.79±0.34	0.23±0.05	0.35±0.05	0.26±0.03	0.0082	0.0072	0.008
3	5.6	2.47±0.28	4.17±1.05	4.99±0.40	0.15±0.07	0.32±0.09	0.34±0.04	0.0066	0.0091	0.5
4	6.5	2.70±0.41	4.59±0.37	4.37±0.64	0.21±0.05	0.37±0.03	0.36±0.06	0.0105	0.0085	0.009

Each value is the mean of ± S.E. of 3 replicates



Cell suspension culture of Garden Leaf Sample with the MS medium.
GL+0.5 mg/LBAP +1.5mg/L IAA Std GA-Standard Gymnemic acids
GL7 -seven day old sample
GL14- Fourteen day old sample.
GL21-Twenty-onedayold sample

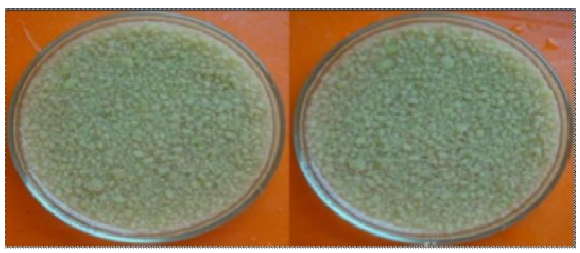
Fig. 1. Qualitative analysis of Gymnemic acids by using HPTLC techniques



Cell suspension culture of Dapoli Leaf Sample with this medium
DL+0.5 mg/LBAP+1.0 mg/LIAA. Std GA-Standard Gymnemic acids
DL7 - seven day old sample.
DL14 -Fourteen day old sample.
DL21 -Twenty-one day old sample.

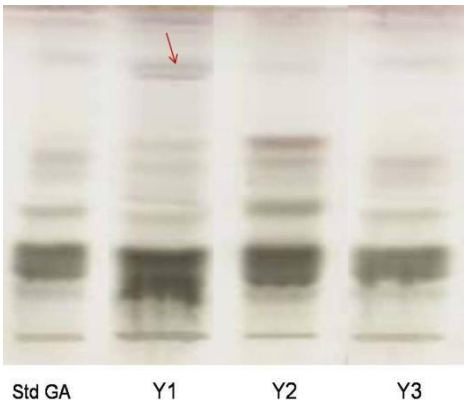
Fig.2. Qualitative analysis of Gymnemic acids by using HPTLC techniques

Control **100 mg/L(Y1)**



200mg/L(Y2) **400mg/L(Y3)**

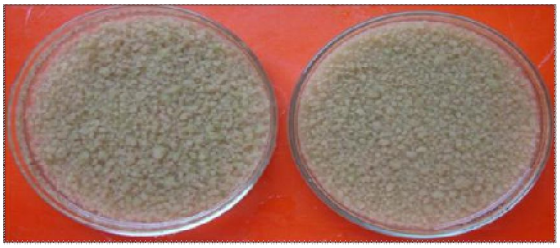
Fig. 3. Cell suspension culture by using Biotic Elicitor (Yeast extract) mg/L



Std GA-Standard Gymnemic acids. Yeast with different conc. with different time durations.
Y1 – 100 mg/L(36hr)
Y2 -200 mg/L (48hr)
Y3 – 400 mg/L(24hr)

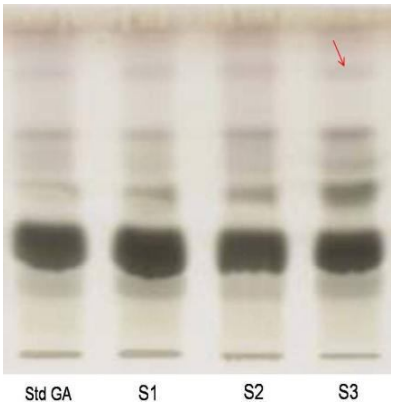
Fig. 4. Qualitative analysis of Gymnemic acids after biotic elicitor (YEAST) treatments

Control **0.1 µM (S1)**



0.25 µM (S2) **0.50 µM(S3)**

Fig. 5. Cell suspension culture by using a biotic Elicitor Salicylic acid



Std GA- Standard g. acids Salicylic acids with different concentrations and different time durations.
S1-0.1µM (48hr)
S2 -0.25 µM (24hr)
S3 – 0.50 µM (36hr)

Fig. 6. Qualitative analysis of Gymnemic acids after abiotic elicitor Salicylic acids treatments

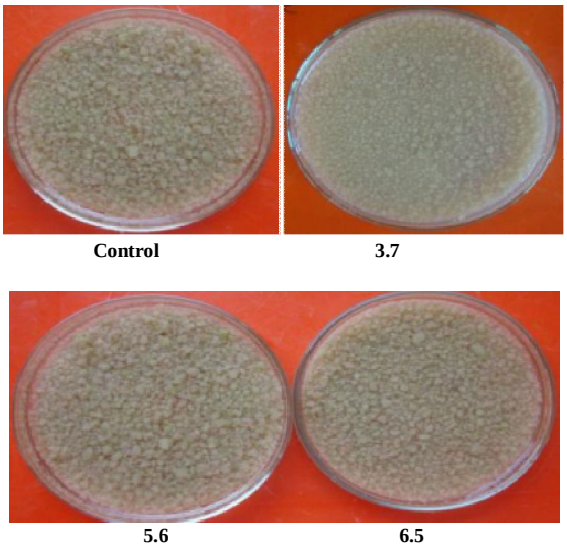
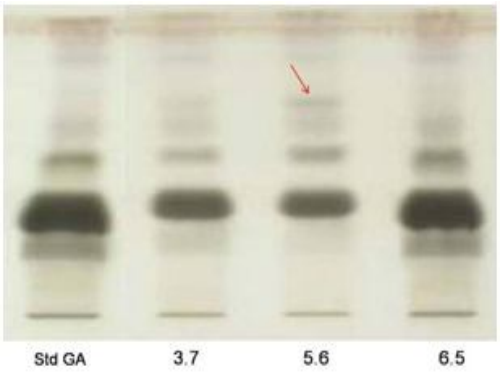
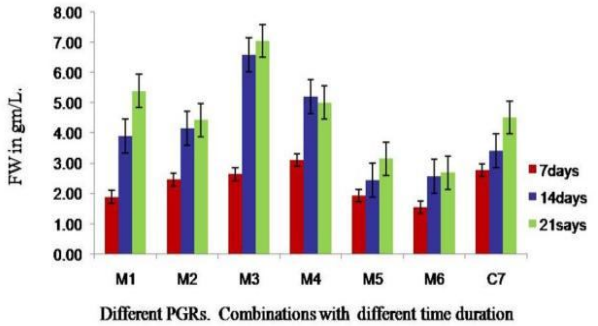


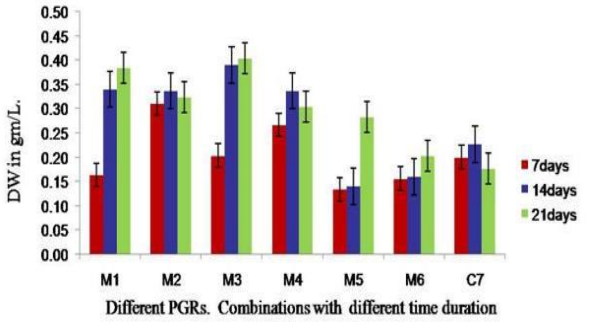
Fig. 7. Cell suspension culture at different pH variations



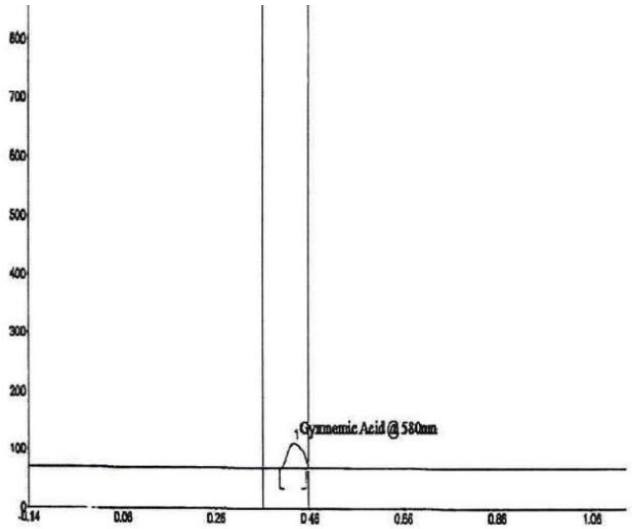
Std GA-Standard Gymnemic acids pH variation with time duration
pH -3.7 (21 days) pH – 5.6 (14 days) pH -6.5 (7days)

Fig. 8. Qualitative analysis of Gymnemic acids at different (pH) variation



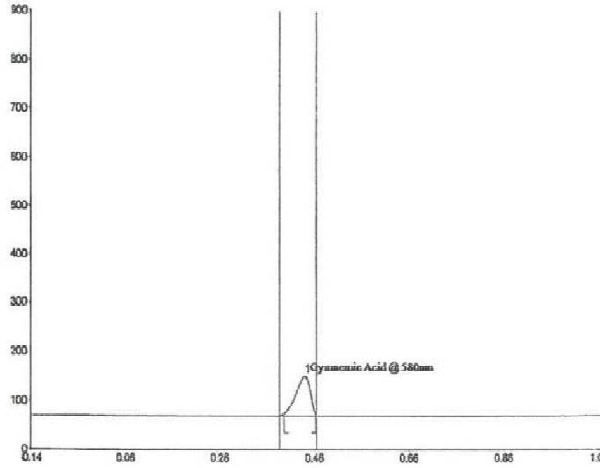


Graph 1. The growth rate of cells in suspension culture of Garden explants (FW& DW)



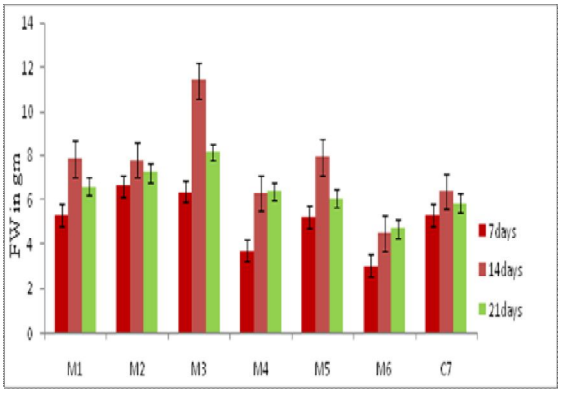
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	0.0	0.42	44.3	100.00	0.45	16.6	1098.2	100.00	Gymnemic Acid

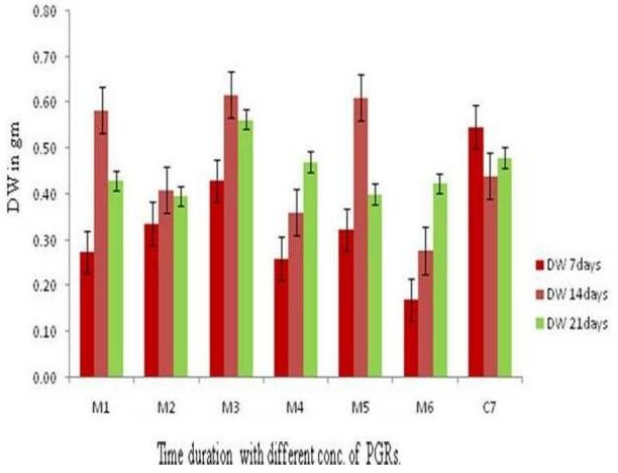
Graph 2.Quantitativeestimationof Standard Gymnemic acids by usingHPTLC Techniques



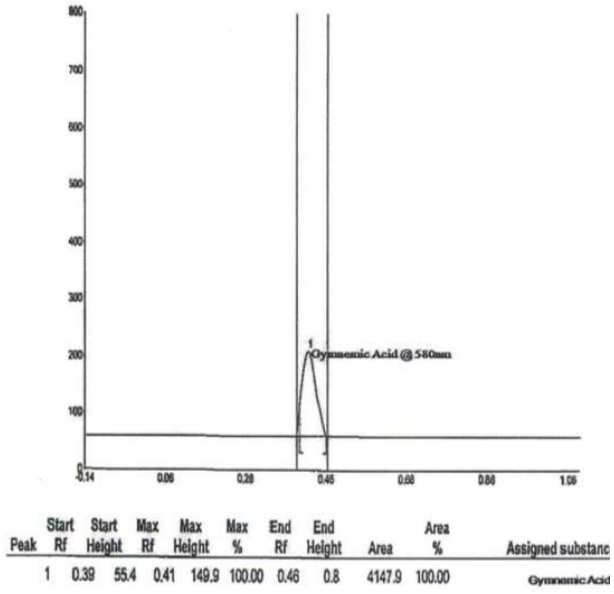
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	5.6	0.44	81.9	100.00	0.46	2.9	2041.6	100.00	Gymnemic Acid

Graph 3. Quantitative estimation of Gymnemic acids by using HPTLC Techniques in 14 days old cell culture 1.0 mg/L IAA +0.5mg/LBAP of Garden explants



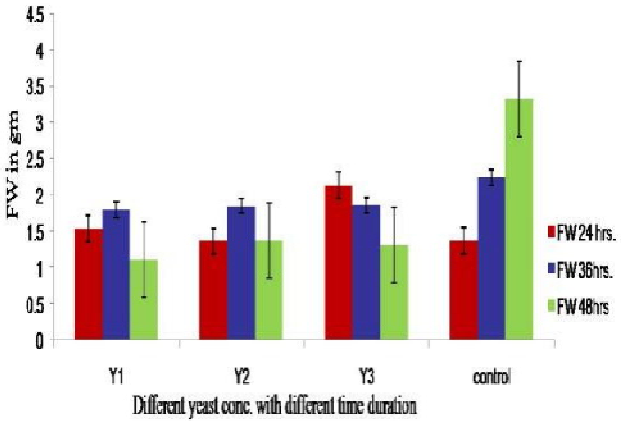


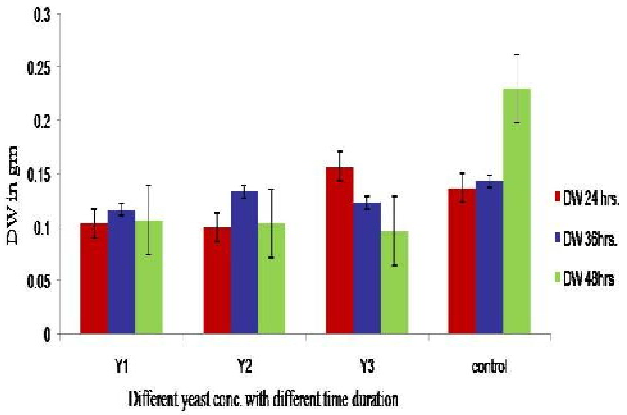
Graph 4. The growth rate of cells in suspension culture of Dapoli explants along with Fresh and Dry weight



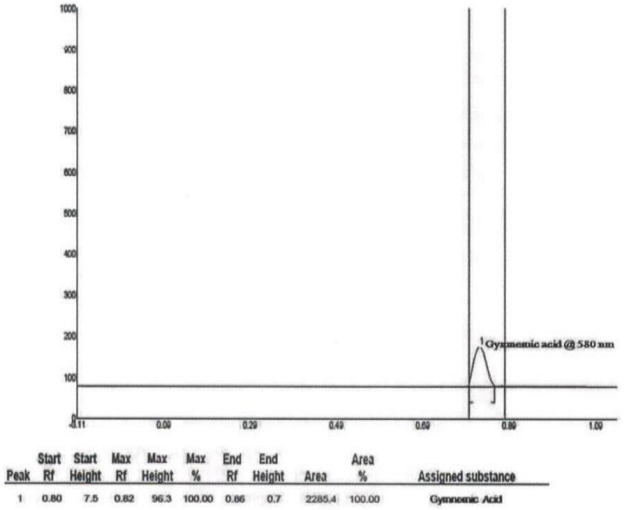
Graph 5. Quantitative estimation of Gymnemic acid by using HPTLC techniques in 14 days old cell culture at concentrations1.0 mg/L IAA +0.5 mg/L BAP of Dapoli explants

use of various biotic elicitors' affects the growth of the cell suspension culture but it does not affects the secondary metabolites accumulation.





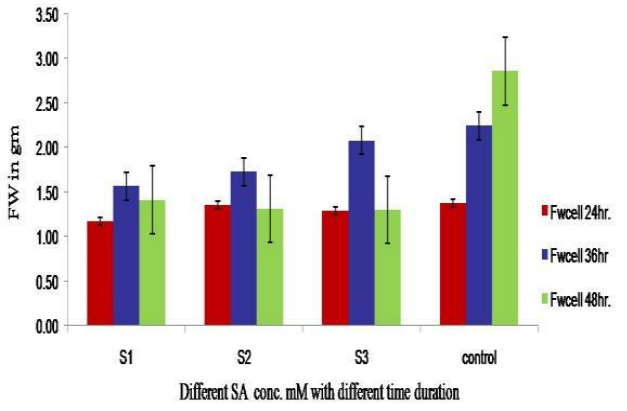
Graph 6. Effect of biotic elicitor Yeast on cell growth along with FW and DW

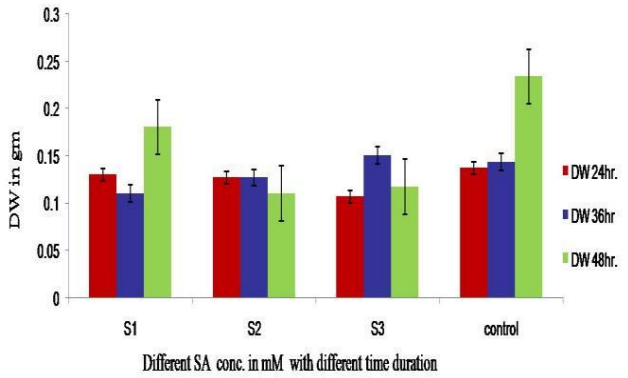


Graph 7. Quantitative estimation of Gymnemic acids with 100 mg/L by using biotic elicitor Yeast after 36 hr time duration

Effect of salicylic acid on cell cultures. (SA)

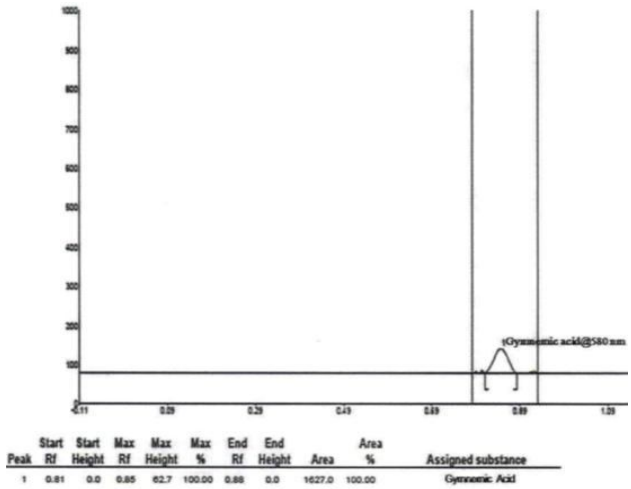
Salicylic acids is considered as one of the key endogenous signals involved in the activation of numerous signals involved in the activation of various plant defense responses (Shah *et al.* 1999). The effect of salicylic acids (SA) on cell growth along with Fresh and Dry weight and gymnemic acids % in Cell cultures of *Gymnema sylvestre* (Table 4). For SA highest biomass was obtained in control (FW 2.85±0.37gm and DW 0.23±0.009 gm) (Graph 8) at 48hr time duration.





Graph 8. Effect of abiotic elicitor Salicylic acid on cell growth along with FW and DW

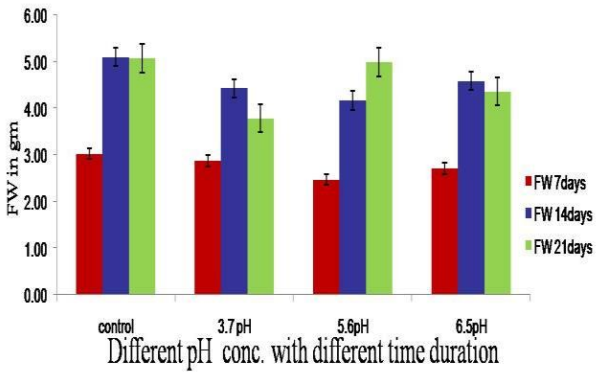
The addition of SA in nutrient media it decreased cell biomass accumulation as compared to control (Fig. 6) but it also shows the maximum quantity 0.09% of gymnemic acids accumulation in 0.50 mM concentrations at 36hr time duration per sample respectively. (Graph 9) Therefore the addition of elicitor in culture affects the cell biomass but enhances the gymnemic acids percentage respectively. (Fig.6 and Graph 8-9). On the basis of above observations it has been shown that the increase in the concentrations of SA conc. induced an increase in gymnemic acids content in cell suspension cultures but decreases biomass accumulation as compared to control.

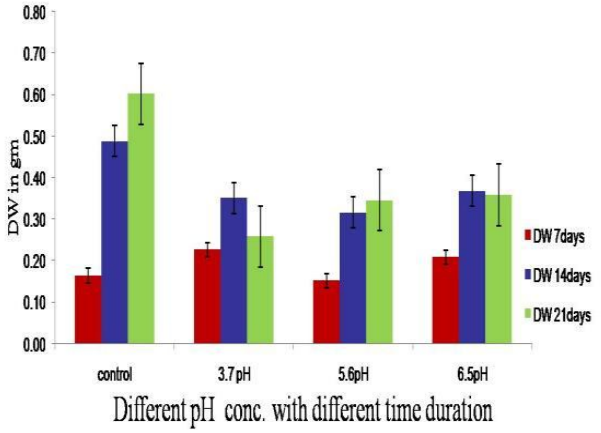


Graph No. 9). Quantitative estimation of Gymnemic acids with 0.50 mM abiotic elicitor Salicylic acid after 36 hr time duration

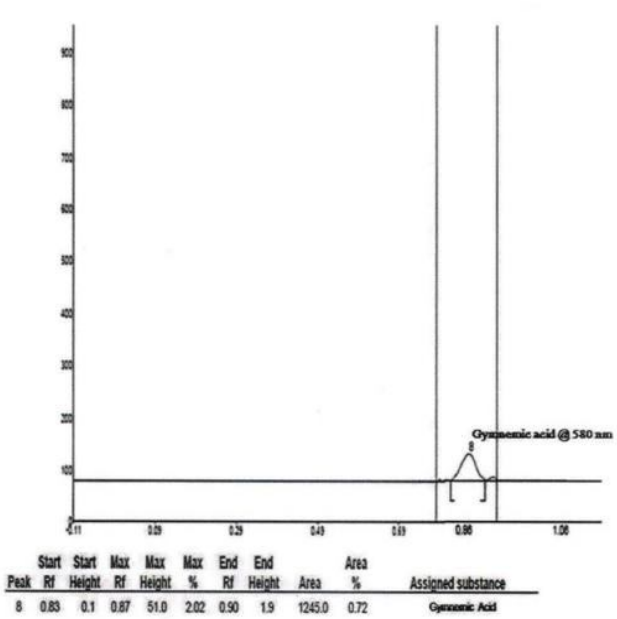
Effect of pH variation on cell cultures

The effect of pH variation ranging from 3.7 to 6.5 along 120 rpm speed with different time duration (Table 5). In our observation the effect of pH on cell biomass production along with fresh and dry weight obtained maximum in control (FW 5.1±0.1 gm and DW 0.60±0.10 gm) in 14 days old cultures (Fig.7 and Graph 10), other pH like 5.6 shows the less (FW 2.47±0.28 gm DW 0.15±0.07 gm) along with less quantity of gymnemic acids 0.0085% estimated through HPTLC (Graph 11). The maximum quantity of gymnemic acids 0.5% is found in 5.6 pH after 21 days but biomass (FW 4.99±0.40 gm DW 0.34±0.04gm) of cell culture is less as compared to control (Graph 10). The pH ranges affects biomass accumulations as compared to control. 3.7 pH ranges shows the less biomass accumulation as well as less gymnemic acids quantities as compared to other pH variations. (Fig. 7) This herb becoming rare and endangered so for the conservation as well as for production of gymnemic acids commercial purpose our investigation will be helpful in further studies.





Graph 10. Effect of pH variation on cell growth along with Fresh and Dry weight



Graph No. 11). Estimation of gymnemic acids after 14 days' time duration with 5.6 pH

Conclusion

Gymnema sylvestre is an age old medicinally important plant useful in diabetics. Our research findings indicated that-

- The well-developed cell line was analyzed for secondary metabolite production.

- The maximum average of cell biomass was obtained on MS+1.0 mg/L IAA+0.5 mg/L BAP concentrations along with FW 11.41±0.387 gm and DW 0.62±0.028 gm in 14 days old cell cultures along with percentage of gymnemic acids is 0.065% /sample maximum as compared to other combinations and concentrations of plant growth regulators.
- Salicylic acids had negative effects on growth of cell in suspension culture.
- The biotic (yeast) and abiotic (SA and pH variations) elicitors treatment affects the total biomass production but at certain extent it will helpful for enhancement of secondary metabolites production for commercialization purpose. (Dicosmo, *et al.*, 1996)
- For production of secondary metabolites cell culture is advanced techniques in our plant science. These results shows that plant cell culture systems have potential for commercial exploitation of secondary metabolites by using different biotic and abiotic elicitors so likely to be a significant step towards making cell cultures more generally applicable to the commercial production of secondary metabolites.

Acknowledgment

Both the authors would like to express a sincere thanks to Head, Department of Botany, University of Pune, Pune- 411007 for encouragement and necessary laboratory facilities and The first author is grateful to authorities of Pune university for providing Financial support in the form of research stipend.

REFERENCES

Anonymous, 1995. Pharmacopoeia of India Government of India Ministry of Health manager Publications Delhi edn.1st. 370 and 864.

Anonymous,1997. Medicinal Plants Categorized W/new ICUN Red List Criteria Under the Biodiversity Conservation Priorisation Project, In: CBSG, India News.10

Bhojwani, S. S. and M. K. Razdan, 1996. Plant Tissue Culture: Theory and Practice (Revised Edition), Jones and Bartlett Publishers, Inc., Boston.

Cooke, T., 1958. The flora of Presidency of Bombay, Botanical Survey of India, Calcutta. 2nd edn. II: 224-231.

Cooke, T., 1997. The flora of Presidency of Bombay, published under the authority of the Secretory of the State for India in Council, Bishen Singh; Maindra pal Singh 23-A, Connaught Place, Dehra Dun(India), pp159-160.

Devi, S. C., Murugesh, S., and V. M. Shrinivasan. 2006. Gymnemic Acid Production in Suspension Cell Cultures of *Gymnema sylvestre*. Jour. of Applied Sciences 6(10):2263-2268. ISSN 1812-5654.

Dicosmo F, Misawa M. 1996. Plant cell culture secondary metabolism towards industrial application. CRC, Boca Raton, New York, pp 139–166.

Funk, C., Gugler, R. and P. Brodelius, 1987. Increased Secondary metabolite formation in plant cell suspension cultures after treatment with yeast carbohydrate preparation (elicitor). Phytochemicals 26:401-405.

Gopi, C. and T. M. Vatsala. 2006. In-vitro studies on effect on plants growth regulators on callus and suspension culture biomass yield from *Gymnemasylvestre*. *African Jour. of Biotech.* 5(12):1215-1219.

Grover, J. K., Yadav, S. and V. Vats, 2002. Medicinal plants of India with anti-diabetic potential. *Jour. Of Ethnopharmacology.* 81(1): 81-100.

Haralampidis, K., Trojanowska M. and A. E. Osbourn, 2002. Biosynthesis of triterpenoidsaponins in plants.75:31-49.

Hostettmann, K., Wolfender, J. L. and S. Radrigues, 1997. Rapid detection and subsequent isolation of bioactive constituents of crude plant extracts. Planta med.63:2-10.

Kanetkar, P. V., Singhal, R. S., Laddha, K. S. and M. Y. Kamat, 2006. Extraction and quantification of gymnemic acid through gymnemagenin from callus cultures *Gymnema sylvestre*. Phytochem Anal. 17: 409-413.

Komalavalli, N. and M. V. Rao, 1997. *In-vitro* micropropagation of Gymnemaelegans W. A., A rare medicinal plant. *Indian jour. Exp. Biol*. 35:1088-1092.

Kumar, H. G., Murthy, H. N. and K. Y. Peak., 2002. Somatic embryogenesis and plant regeneration in *Gymnema sylvestre* – Plant Cell, Tissue and organ culture 71 (1): 85-88

Menke, F. L., Parachmann, S., Mueller, M. J., Kijne, J. W. and J. Memelink, 1999. Involvement of octadecanoid pathway and protein phosphorylation in fungal elicitor-induced expression of terpenoidindole alkaloid biosyntheclic genes in catharanthusroseus Plant Physiol. 119:1289- 1296.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15: 473–97.

Namdeo, A. G. 2007. Plant cell elicitation for production of secondary metabolites Pharmacognosy Review 1: 69-79.

Nurnberger, T., Colling, C., Hahlbrock, K., Jabs, T., Rehelt A., Sacks, W. R. and D. Scheel, 1994. Perception and transduction of an elicitor signal in cultured parsley cells. Biochem. Soc. Symp. 60:173-182.

Passera, C.A., Pedrotti and G. Ferrari, 1964. Chromatography, 14:289.

Sahu, N. P. Mahato, S. B., Sarkar, S. K. and G. Poddar, 1996. Triterpenoid saponins from *Gymnema sylvestre*, Photochemistry, 41(4):1181-1185.

Santapau, S.J., and N. A. Irani, 1960. The Asclepiadeceae and Periplocaceae of Bombay, st. Xavier College, Bombay, pp. 12-14, 43-50, 85-88.

Savitha, B. C., Thimmaraja, R., Bhagyalakshmi, N. and G. A. Ravishankar, 2006. Different biotic and abiotic elicitors influence betalain production in hairy root cultures of Beta vulgaris in shake- flask and bioreactor. Process Biochem. 41:50-60.

Saxena, A. and N. V. Kishor, 2004. Role of selected Indian Plants in Management of type 2 Diabetes. *The Jour. Altere. Comp. Medi.*10:369-378.

Sethi, P.D., 1996. High Performance Thin Layer Chromatography, CBS Publisher and Distributors, New Delhi: 3-68.

Shah, J. and D. F. Klessig, 1999. Salicylic acid: Signal perception and transduction. In: Biochemistry and Molecular Biology of Plant Hormones, (eds) K. Libbebga, M. Hall and P. *J. J. Hooykaas (*Elsevier UK) pp 513-541.

Stahl, E., 1969. Thin Layer Chromatography a laboratory Hand Book, Spring Verlog Berlin, Heidenabarg.

Whitaker, R.J., George, C.H., and, Leslie A. Steward 1986. Production of Secondary Metabolites in Plant Cell Cultures, DNA Plant Technology Corporation, 2611 ISBN: 9780841211544 317: 347–362.

Zhao, J., Zhu, W. H. and Q. Hu, 2001. Enhanced Catharanthine production in catharanthusroseus cell cultures by combined elicitor treatment in shake flasks and bioreactors. Enzyme Microbial Technol. 28: 673-681.

****