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IN-VITRO APPLICATIONS OF *SWERTIA* XANTHONE EXTRACTS ON CALLUS GROWTH.

VIJAY DAMODHAR MENDHULKAR*[1] AND ARUNA SHAHAJI NANGARE[2]

[1] Head, Department of Botany, The Institute of Science, Mumbai, India.
[2] Department of Botany, The Institute of Science, Mumbai, India.

ABSTRACT

Swertia species are important botanical sources of xanthones. These compounds have remarkable pharmacological properties such as antipyretic, hypoglycaemic, anti-carcinogenic, antihepatoxic, antimicrobial and antiviral. Xanthones-rich extracts have been widely used for the treatment of various health ailments and used in nutritional supplements, herbal cosmetics and pharmaceutical preparations. The purpose of this study is to examine the *In-vitro* impact of *Swertia* species crude xanthone extracts on callus growth. Seven xanthone concentrations (5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L.) were prepared from three *Swertia* species i.e. *Swertia densifolia, Swertia lawii* and *Swertia minor*. Leaf callus was grown in xanthone supplemented MS media. Results obtained were recorded in the form of growth parameters, biochemical analysis, protein profiling and cell viability. Media supplemented with *Swertia* xanthones were able to develop calli from leaf explants. Fresh and dry weight recorded to be high in all *S. minor* xanthone treated calli, Moisture content was more in many studied xanthone concentrations compared to control (94.36%). From the studied phytochemicals, the polyphenols were accumulated in very high amount. Protein profiling study revealed the expression of low and high molecular weight proteins and the numbers of low molecular weight protein bands were recorded more. *S. densifolia* xanthone supplemented medium (80 mg/L and 100 mg/L) enhances the expression of more proteins. The cell viability parameter was analysed and it was observed that *S. minor* xanthone treated cell cultures indicated 97.6% cell viability against control cell viability of 93.21%.

KEY WORDS: Callus, *Swertia* species, Cell suspension culture, Cell viability.

VIJAY DAMODHAR MENDHULKAR *
Head, Department of Botany, The Institute of Science,
Mumbai, India.

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INTRODUCTION

Swertia is an important genus of family Gentianaceae, *Swertia* species are rich sources of biologically active phytochemicals, like xanthones, flavonoids, iridoid, secoiridoids, glycosides and terpenoids.[1] *Swertia chirayita* is a topmost valuable herb in countries like Nepal, India and China as a tonic. Properties reported by this plant are hypoglycaemic, anti-carcinogenic, antihepatoxic, anthelmintic, anti-inflammatory, antipyretic and antiviral properties. The bitterness, anthelmintic, hypoglycaemic and antipyretic properties are attributed to amarogentin swerchirin, swertiamarin and other active principles of the herb. Herbal medicines such as Ayush-64, Diabecon, Menstrual syrup and Melicon V ointment contain chirayita extract in different amounts for its antipyretic, hypoglycaemic, antifungal and antibacterial properties. *Swertia chirata* is widely used in India to treat fever and malaria. It is also used to treat liver diseases**.** Many herbaceous species are being used as adulterants of *Swertia chirayita* such as *Swertia ciliata, S. bimaculata, S. minor, S. nervosa, S. elegans, S. multiflora, S. lawii, S. densiflora* (*densifolia*) and *Andrographis paniculata*.[2] Xanthones are polyphenolic plant constituents that exhibit well-documented pharmacological properties, mainly due to their oxygenated heterocyclic nature and diversity of functional groups. Xanthones have stronger antioxidant potential compared to vitamins C and E. Our work is focused on *In-vitro* biological activities of *Swertia* xanthones. The use of cell culture techniques for qualitative and quantitative enhancement of active phytoconstituents deserves unique importance in order to achieve the product in short time and large quantity. To this context, several media and hormonal combinations are required to be tried, to achieve good callus induction and its growth. Researchers have focused on the organic growth controlling compounds and salts formulations. It is observed that there is a good scope toward utilizing low cost natural extracts as a substitute over the expensive synthetic nutrient media. The response of callus growth with media supplemented with another phytoconstituents has generated the curiosity and hence the present work was undertaken. Protein synthesis in plant cells plays many important physiological roles in response to unfavourable conditions. Proteins play key role in the mechanisms that bring about their capacity in stress tolerance through various functions from control of synthesis of regulatory components to maintenance of their concentrations and functions accordingly. Protein and secondary metabolites of plant have been found to be promising markers such as water stress-induced protein. In order to study the changes at cell level protein profiling have been carried out. The work presented deals with the *In-vitro* applications of xanthone extracts using MS media to evaluate its possible applications on callus generation, phytochemical status and protein profiling. Primary callus growth was noted by callus induction. Impact on callus was observed in terms of fresh weight, dry weight and moisture content. Extent of severity or enhancement was estimated through cell viability.

MATERIALS AND METHODS

Plant material

The authenticated samples were collected from three different localities; *Swertia densifolia* from Kas- Satara Dist., *Swertia lawii* from Panhala- Kolhapur Dist. and *Swertia minor* from Sinhagad- Pune Dist. *Swertia densifolia* (*Griseb.*) matches with voucher no. 129 of M. R. Almeida and *Swertia minor* with NI4485 of N. A. Irani. Voucher specimens of these species have been deposited in Blatter Herbarium, Mumbai India. *Swertia lawii* identified and authenticated by standard morphological characters (keys) according to the Flora of Kolhapur District.

Extraction of crude xanthone extract

Fresh and mature leaves of *Swertia* species were collected, cleaned and shade dried. The dried leaves were powdered and macerated in dichloromethane and methanol (1/1, v/v) for 48 hrs. At the end each extract was passed through Whatman filter paper no.1. The filtrate obtained were concentrated in vacuo using rotary evaporator at 30^{o}C to obtain crude extract. The part of this extract was reextracted with ethyl acetate, concentrated and stored in the fridge for further use.[3]

Administration of xanthone treatment

Different concentrations of xanthone rich extracts of three species of *Swertia* were prepared like 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L. 50 ml of media was poured in Dippy's jar and supplemented with these concentrations separately. MS medium without xanthone supplement was kept as control. The leaf explants *(Phaseolus vulgaris)* were inoculated and incubated for callus induction in all the sets. Impact on callus was determined by studying the growth parameters like callus induction, fresh weight and dry weight of callus and moisture content. Phytochemical analysis, protein profiling and cell viability was also studied. Callus induction = Number of explants with callus/ Total number of explants inoculated X 100.

Cell viability count

The cell viability in 25 days old suspension culture was determined by Guava Via count Assay using Guava Easy CD4 system. Cell suspension culture medium was supplemented with different concentrations of xanthone rich extracts of three *Swertia* species viz. 5 mg/L, 10 mg/L, 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L. Cell viability was counted after 48 hrs. Cell suspension culture without treatment was taken as control. For assay, 10 µl of suspension culture was taken in eppendrof tube and added with 190 µl of viability counting solution. The content was incubated for 5 min at room temperature. The analysis was performed using Cytosoft Software for Guava Via count.

STATISTICAL ANALYSIS

All the results were analysed using statistical formulations and mean presented with standard deviation. The data was analyzed with one-way ANOVA at the level $P<0.05$ using MS excel software.

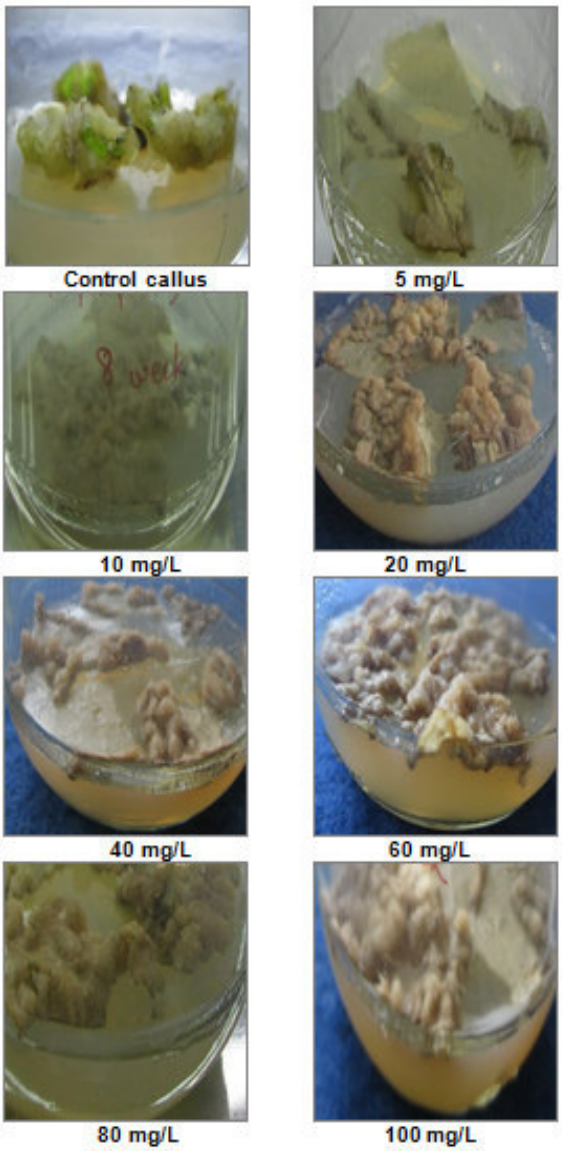
RESULTS AND DISCUSSION

Under *In-vitro* condition, the impact of *Swertia* xanthone extract on callus was analyzed in *Phaseolus vulgaris.* Inferences were made on the basis of growth parameters, phytochemical analysis, protein profiling and cell viability. The crude xanthone extract of studied *Swertia* species was able to developed calli from leaf explants when supplemented with MS medium (Photoplate- 1).

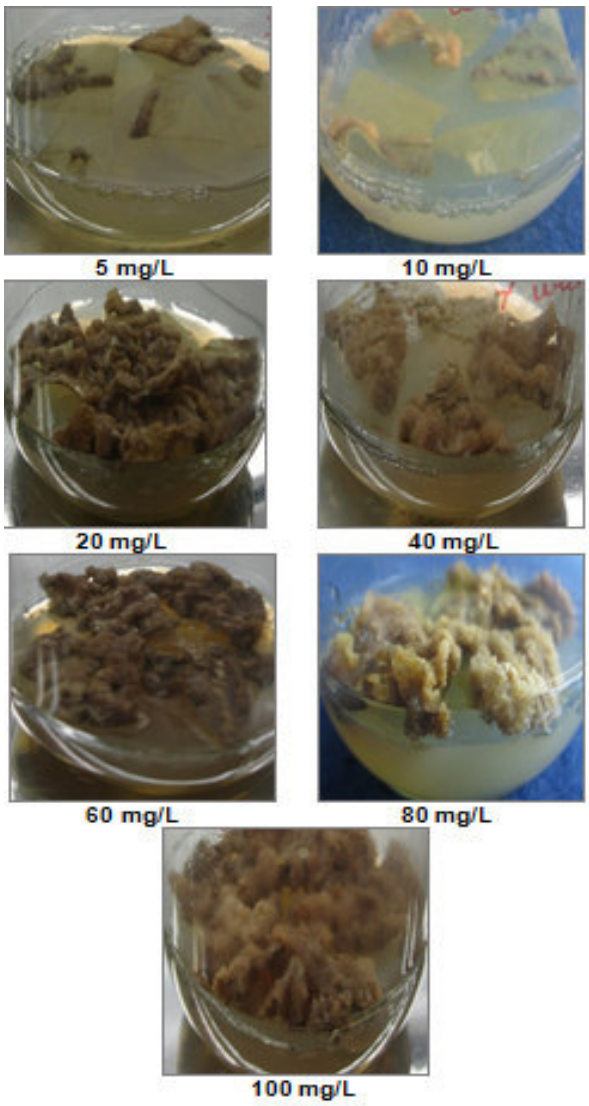
Photoplate-1

Callus culture in Phaseolus vulgaris leaves grown in MS medium supplemented with different concentrations of Swertia xanthone extracts.

A - Swertia densifolia xanthone extracts.



B - Swertia lawii xanthone extracts.



C - Swertia minor xanthone extracts.

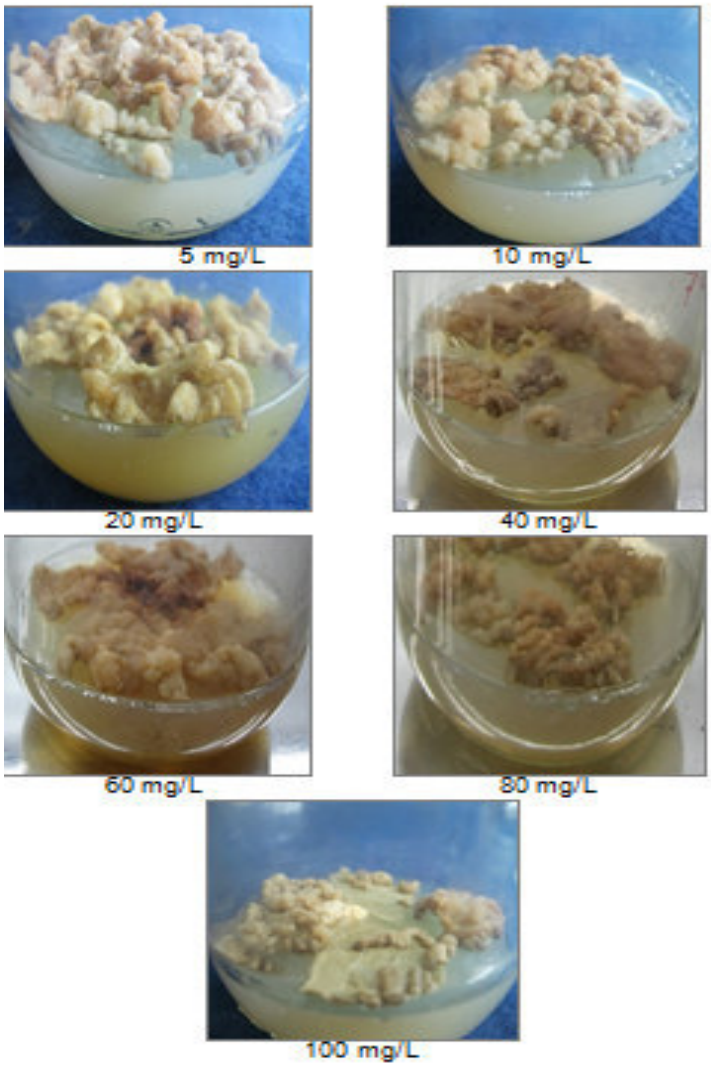


Table 1
Effect of crude xanthone extracts of Swertia species on callus induction in Phaseolus vulgaris

Sr. No.	Experimental material	Xanthone Concentrations (in mg/lit.)	1st week	2nd week	3rd week	4th week	5th week
1	Control	-	*	+	++	**+++**	**+++**
2	*Swertia densifolia*	5	-	*	+	+	++
		10	*	+	++	++	**+++**
		20	-	*	+	++	++
		40	*	+	++	++	++
		60	*	+	++	**+++**	**+++**
		80	*	+	+	++	++
		100	-	*	+	++	++
3	*Swertia lawii*	5	-	-	*	+	+
		10	-	-	*	+	+
		20	-	*	+	++	++
		40	-	*	+	++	++
		60	*	+	+	++	++
		80	*	*	+	++	+++
		100	*	+	++	++	+++
4	*Swertia minor*	5	*	+	++	++	++
		10	*	+	++	++	**+++**
		20	-	*	+	++	++
		40	-	*	+	++	++
		60	*	+	++	**+++**	**+++**
		80	*	+	++	++	**+++**
		100	*	+	++	++	++

*** Swelling of explants,+ Callus initiation, ++Good callus growth, +++Very good callus growth.**

Table 2
Growth parameters of Phaseolus vulgaris callus grown in MS medium supplemented with xanthone extracts of Swertia species.

Sr. No.	*Swertia* species	Xanthone Concentrations (in mg/lit.)	Callus induction (%)	Fresh weight (g)	Dry weight (g)	Moisture content (%)
1	Control	-	90±0.00	0.71±1.32	0.04±0.00	94.36
2	*Swertia densifolia*	5	80±0.67	0.5±2.00	0.02 ±0.05	96
		10	60±0.04	1.46±0.78	0.05±0.05	96.57
		20	100±0.08	0.85±0.06	0.04±0.01	95.29
		40	80±0.06	0.45±0.02	0.02±0.00	95.55
		60	100±1	1.57±0.02	0.04±0.00	97.45
		80	60±0.35	0.43±0.02	0.02±0.06	95.34
		100	80±0.00	0.33±0.05	0.02±0.68	93.93
3	*Swertia lawii*	5	40±0.01	0.5±0.05	0.06±0.56	88
		10	60±0.03	0.64±0.02	0.04±1.20	93.75
		20	80±0.01	0.73±0.02	0.04±0.98	94.52
		40	60±0.78	0.82±0.01	0.04±0.04	95.12
		60	100±0.07	1±0.02	0.04±0.98	96
		80	60±0.89	1.32±0.01	0.04±0.34	96.96
		100	80±0.00	2.05±0.01	0.05±0.00	97.56
4	*Swertia minor*	5	100±0.00	0.55±0.01	0.048±0.35	91.27
		10	60±0.01	1.2±0.01	0.09±0.01	92.5
		20	100±0.02	1.4±0.01	0.06±0.78	95.71
		40	80±0.06	1.5±0.01	0.07±0.34	95.33
		60	100±0.03	1.67±0.01	0.04±0.34	97.60
		80	60±0.01	2.23±0.03	0.05±0.07	97.75
		100	100±0.05	1±0.01	0.08±0.05	92

Results are statistically significant at level P<0.05.

Table 3
Phytochemical analysis of cultured cells of Phaseolus vulgaris grown in MS medium supplemented with Swertia crude xanthone extract.

Sr. No.	Species	Xanthone Concentration (in mg/lit.)	Proteins (in mg/gm.)	Flavonoids (in mg/gm.)	Polyphenols (in mg/gm.)	Saponins (in mg/gm.)
1	Control	-	6.27±0.46	6.27±0.09	12.16±0.00	1.05±0.00
2	*Swertia densifolia*	5	9.86±0.00	15.93±0.01	58.56±0.01	4.97±0.00
		10	10.11±0.00	13.86±0.13	74.48±0.01	1.59±0.02
		20	10.04±0.00	14.78±0.04	80.08±0.01	1.29±0.00
		40	4.62±0.00	10.69±0.21	65.6±0.01	2.92±0.00
		60	7.85±0.00	26.26±0.01	98.64±0.01	1.75±0.01
		80	8.16±0.00	16.47±0.13	98.24±0.01	1.23±0.00
		100	4.32±0.06	30.81±0.01	78.16±0.01	3.53±0.00
3	*Swertia lawii*	5	11.05±0.00	7.98±0.02	83.68±0.01	2.00±0.01
		10	5.81±0.08	13.15±0.11	104.24±0.02	1.34±0.01
		20	16.51±0.04	11.13±0.02	125.12±0.02	2.49±0.00
		40	12.57±0.12	5.79±0.04	59.92±0.01	1.56±0.01
		60	7.91±0.09	31.58±0.09	102.08±0.02	4.52±0.00
		80	11.61±0.05	13.07±0.06	110.96±0.02	1.38±0.02
		100	8.10±0.08	12.46±0.01	91.52±0.01	1.68±0.00
4	*Swertia minor*	5	5.12±0.06	9.42±0.00	61.36±0.01	1.81±0.00
		10	9.15±0.03	10.42±0.03	50.16±0.01	1.62±0.00
		20	8.94±0.06	6.18±0.01	62.08±0.01	2.23±0.00
		40	9.13±0.22	7.92±0.01	91.28±0.01	2.22±0.01
		60	11.19±0.06	16.53±0.01	127.76±0.02	4.14±0.01
		80	12.51±0.08	9.96±0.02	88.56±0.01	2.44±0.02
		100	12.96±0.00	1.55±0.02	119.36±0.02	0.63±0.01

Results are statistically significant at level P<0.05.

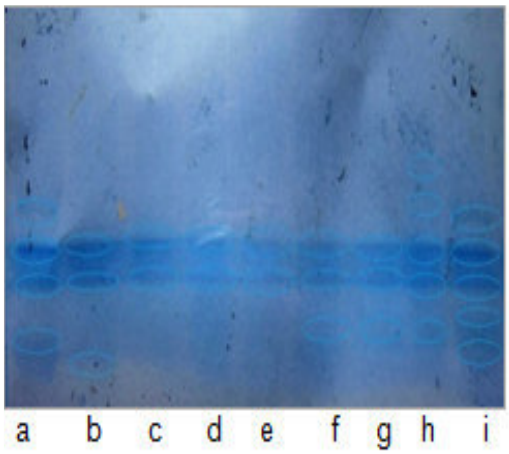
According to our findings, 1: 0.5 mg/L 2-4-D: BAP combination was most favourable to obtain callus biomass from leaf explants. First calli were formed from the cut edges of the explants. In first week there was a swelling of explants, in second week initiation of callus followed by progressive growth of callus in control. In first week, the swelling of explants was observed in 10 mg/L, 40 mg/L, 60 mg/mL and 80 mg/L *S. densifolia* xanthone supplemented medium (Table-1). 5 mg/L, 20 mg/L and 100 mg/L *S. densifolia* xanthone concentrations showed swellings in second week. Hence, these concentrations showed poor callus growth. Although swelling was occurred earlier in 40 mg/L and 80 mg/L concentrations, the growth of callus was slow (Photoplate-1A). *S. lawii* xanthone extract showed less enhancing effects on callus growth. Poor callus growth was observed in all studied concentrations except for 80 mg/L and 100 mg/L concentrations were proved to be callus growth enhancers (Photoplate-1B). Although swelling was noted earlier in 5 mg/L and 100 mg/L *S. minor* xanthone concentrations, the growth of callus was slow. Progressive callus growth was observed in 10 mg/L, 60 mg/L and 80 mg/L *S. minor* xanthone supplemented medium (Photoplate-1C). Well grown calli were found in 10 mg/L and 60 mg/L *S. densifolia* xanthone concentration, 80 mg/L and 100 mg/L *S. lawii* xanthone concentration and 10 mg/L, 60 mg/L and 80 mg/L *S. minor* xanthone supplemented

media. Although swelling was occurred earlier in some concentrations but the growth of callus was slow. *S. densifolia* 20 mg/L, 60 mg/L and *S. lawii* 60 mg/L xanthone concentrations were able to produce 100% callus induction. In studied *Swertia* xanthone concentrations, more of *S. minor* xanthone concentrations gave rise to 100% callus induction from leaf explants. It is more compared to control (90 ±0%). *S. minor* xanthone concentration of 5 mg/L, 20 mg/L, 60 mg/L and 100 mg/L revealed 100% callus induction with maximum fresh weight of 2.23±0.3 g. in 80 mg/l and maximum dry weight of 0.09±0.01 g. in 10 mg/l concentration compared to other studied species (Table-2). Our findings in the present work indicated that there was differential response in various concentrations supplemented to growth media for callus but all *Swertia* species able to produce increased fresh weights like *S. densifolia* 10 mg/L, 20 mg/L and 60 mg/L, *S. lawii* 40 mg/L, 60 mg/L, 80 mg/L and 100 mg/L compared to control (0.71±1.32 g). All *S. minor* xanthone concentrations have enhanced effect on fresh weight except 5 mg/L. Increased dry weights were recorded in calli like *S. densifolia* 10 mg/L, *S. lawii* 5 mg/L, 100 mg/L, *S. minor* 10 mg/L, 20 mg/L, 40 mg/L, 80 mg/L and 100mg/L. Moisture content was more in all studied xanthone concentrations compared to control (except for 100 mg/L *S. densifolia*, 5 mg/L, 10 mg/L *S. lawii* and 5 mg/L, 10 mg/L and 100 mg/L *S. minor* xanthone concentrations). The high protein and flavonoids content was observed in 20 mg/L and 60 mg/L *S. lawii* xanthone supplemented calli. The level of polyphenols content was enhanced in 60 mg/L *Swertia minor* xanthones concentrations compared to other studied species. The highest saponins content was recorded in *S. densifolia* xanthone treated calli at 5 mg/L concentration (Table-3).

Photoplate-2

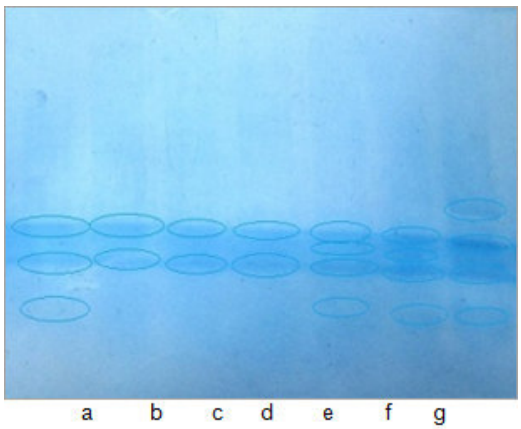
SDS-PAGE protein profiling of Phaseolus vulgaris callus grown in MS media supplemented with Swertia xanthone extracts.

A-Swertia densifolia xanthone extracts.



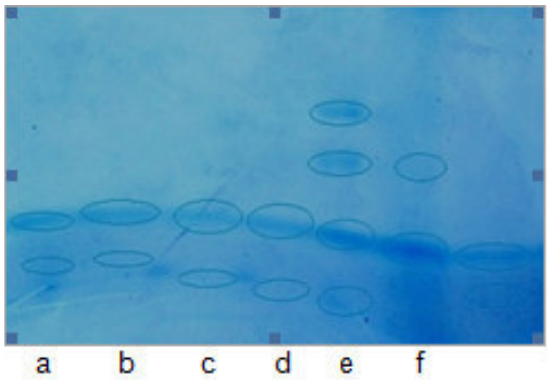
a-Protein marker, b- control callus,
c- 5 mg/L, d- 10 mg/L, e- 20 mg/L, f- 40 mg/L,
g- 60 mg/L, h- 80 mg/L and i- 100 mg/L.

B-Swertia lawii xanthone extracts



a- 5 mg/L, b- 10 mg/L, c- 20 mg/L, d- 40 mg/L,
e- 60 mg/L, f- 80 mg/L and g- 100 mg/L

C-Swertia minor xanthone extracts



a-5 mg/L, b- 10 mg/L, c- 20 mg/L, d- 40 mg/L, e- 60 mg/L, f- 80 mg/L and g- 100 mg/L

Table 4
Electrophoretic protein profiling of Phaseolus vulgaris* callus *grown in MS media supplemented with Swertia xanthone extracts

Sr. No.	Name of sample	Conc. of xanthone extract	Total no. of bands	Distance travelled by each band (in-mm)	RF value	Molecular weight in kDa
1	**Protein marker**	-	4	Band no. 1 - 23 mm	0.575	66
				Band no. 2- 26 mm	0.65	43
				Band no. 3- 30 mm	0.75	29
				Band no. 4-35 mm	0.875	14
2	**Control**	-	3	Band no. 1- 26 mm	0.65	43
				Band no. 2- 30mm	0.75	29
				Band no. 3-38 mm	0.95	8
3	***S. densifolia* xanthone concentration**	5 mg/L	2	Band no. 1 - 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		10 mg/L	2	Band no. 1 - 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		20 mg/L	2	Band no. 1 - 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		40 mg/L	3	Band no. 1 - 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
				Band no. 3 - 33 mm	0.825	17
		60 mg/L	3	Band no. 1 - 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
				Band no. 3- 32 mm	0.8	19
		80 mg/L	5	Band no. 1- 22 mm	0.55	74
				Band no. 2 - 25 mm	0.625	49
				Band no. 3 - 26 mm	0.65	43
				Band no. 4- 30 mm	0.75	29
				Band no. 5- 32 mm	0.8	19
		100 mg/L	5	Band no. 1 - 23 mm	0.575	66
				Band no. 2- 26 mm	0.65	43
				Band no. 3- 30 mm	0.75	29
				Band no. 4-33 mm	0.825	17
				Band no. 5 - 36 mm	0.9	11
4	***S. lawii* xanthone concentration**	5 mg/L	3	Band no. 1 -26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
				Band no. 3- 32 mm	0.8	19
		10 mg/L	3	Band no. 1 -26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
				Band no. 3- 32 mm	0.8	19
		20 mg/L	3	Band no. 1 -26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
				Band no. 3- 31 mm	0.775	22
		40 mg/L	2	Band no. 1- 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		60 mg/L	2	Band no. 1- 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		80 mg/L	2	Band no. 1- 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	29
		100 mg/L	4	Band no. 1- 26 mm	0.65	43
				Band no. 2- 28 mm	0.7	33
				Band no. 3- 30 mm	0.75	29
				Band no. 4-32 mm	0.8	19
5	***S. minor* xanthone concentration**	5 mg/L	2	Band no. 1- 26 mm	0.65	43
				Band no. 2- 30 mm	0.75	25

10 mgL	2	Band no. 1- 26 mm	0.65	43
		Band no. 2- 30 mm	0.75	29
20 mg/L	2	Band no. 1- 26 mm	0.65	43
		Band no. 2- 34 mm	0.85	15
40 mg/L	2	Band no. 1- 27 mm	0.675	38
		Band no. 2- 35 mm	0.875	14
60 mg/L	4	Band no. 1- 17 mm	0.425	145
		Band no. 2- 20 mm	0.5	98
		Band no. 3-31 mm	0.775	22
		Band no. 4-34 mm	0.85	15
80 mg/L	3	Band no. 1- 20 mm	0.5	98
		Band no. 2- 30 mm	0.75	29
		Band no. 3-33 mm	0.825	17
100 mg/L	3	Band no. 1- 32 mm	0.8	19
		Band no. 2-35 mm	0.875	14

Phaseolus vulgaris callus gown in xanthone supplemented MS medium revealed the expression of low and high molecular weight proteins with more numbers of low molecular weight protein bands. Similarly *S. densifolia* xanthone supplemented media enhances the expression of more proteins (in 80 mg/L and 100 mg/L) in addition to the number of bands already present in the control sample (Photoplate-2A). 100 mg/L *S. lawii* xanthone treated calli and 60 mg/L *S. minor* xanthone treated calli indicated presence of more bands as compared to other concentrations of these two species (Photoplate-2B,C). The highest molecular weight protein of 145 kDa was noted in 60 mg/L *S. minor* xanthone treated calli. Highest and lowest Rf value was 0.875 (14 kDa) in 40 mg/L *S. minor* xanthone concentration and 0.425 (145 kDa) in 60 mg/L *S. minor* xanthone concentration, respectively (Photoplate-2C).

Table 5
Comparative effect of crude xanthone extracts of Swertia species on percent cell viability of cultured cells in Phaseolus vulgaris.

Sr. No.	Concentrations of crude xanthone (in mg/l.)	Percent (%) cell viability		
		Swertia densifolia	*Swertia lawii*	*Swertia minor*
1	5	86.15±7.83	80.47±1.99	85.38±4.44
2	10	83.25±2.34	96.00±4.51	91.24±2.46
3	20	80.44±2.75	87.43±2.35	95.65±2.34
4	40	88.01±1.41	92.36±1.03	96.8±0.70
5	60	78.56±2.03	92.31±1.18	97.1±1.55
6	80	85.84±13.16	94.63±0.14	97.6±0.28
7	100	96.29±3.96	90.97±4.55	87.15±17.74

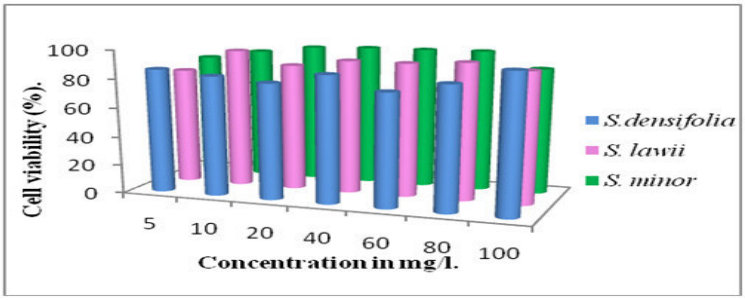


Figure 1
Comparative effects of Swertia species crude xanthones on cell viability in Phaseolus vulgaris.

S. densifolia crude xanthone 100 mg/L concentration showed 96.29% cell viability with slight enhancement over the control count (93.21%). *S. lawii* concentration showed remarkable difference over control specially 80 mg/L and 10 mg/L *S. lawii* crude xanthone concentrations (Table-5). The cell viability was 94.63 % and 96% for these two doses. In *S. minor* xanthone treated cell cultures cell viability was 91.24% to 97.6% for 10 mg/L to 80 mg/L concentrations. All the three studied *Swertia* species enhances the cell viability percentage but *S. minor* proved to be highly significant compared to other studied *Swertia* species (Figure - 1).Various kinds of organic additives have been used in plant tissue culture to promote the growth of the plants including coconut water, banana pulp, potato homogenate and juice, honey, date palm syrup, corn extract, papaya extract and also beef extract.[4] Organic additives help in producing more PLBs (protocorm-like bodies), shoots and leaves,[5] increases the size of somatic embryos,[6] also promotes growth and development of asymbiotic seeds and regeneration of plantlets.[7] Many workers studied the effect of amino acids on the growth of *Datura stramonium* cultured on MS-medium supplemented with 1 mg/L of both 2,4-D and kinetin.[8] They noted that the fresh and dry weights of calli were reduced. Effects of various concentrations

of *Azadirachta indica* L. leaf extract was studied in relation to the callus induction and regeneration of green plantlets in *Carica papaya* L. (var. Pusa dwarf).[9] *Azadirachta indica* leaf extract with 100 mg/L concentration was inoculated with stem explants of *Carica papaya* L. and optimized callus regeneration capacity was noted. Our results for *S. lawii* xanthone extract are growth productive. In the media supplemented with 100 mg/ L xanthone extract callus showed optimum growth. For *S. densifolia* and *S. minor* 10 mg/L and 60 mg/L xanthone supplemented media provides optimum callus growth. The effect of *Nicotiana tabacum* L. extracts was studied on tobacco callus cultures and significant positive impact of all kinds of added extracts was noted on increase of callus fresh weight and the organogenetic capability of tobacco cultures.[10] All *Swertia* species able to produce increased fresh weights except few concentrations. Addition of commercial banana powder into the culture medium increased alkaloid production and chlorophyll content in *Solanum laciniatum* but significantly reduced the growth index of the shoots and optimised that it is due to toxic effects caused by certain components found in banana powder.[11] All the studied phytochemicals accumulated in high amount but polyphenols were accumulated in comparatively high amount. Medium supplemented with proline and glutamine was shown to be optimum for callus growth compared to medium without proline and glutamine.[12] The best callusing was observed on Murashige and Skoog (MS) medium supplemented with 2.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 500 mg/L proline and 500 mg/L glutamine. In another study medium was supplemented with homogenates of banana, tomato and coconut water at various concentrations. Coconut water was found to be the best organic additive for the proliferation of PLBs, which showed a four-fold increase of fresh weight.[13] Natural medium prepared from various parts of banana like leaves and stem, proved to be comparable with artificial MS medium.[14] The growth of plantlets was supported by the natural medium. Although the natural media become older early, plantlets were fleshier than grown in MS medium. Protein pattern analysis was carried out in callus cultures of *Citrullus colocynthis* in relation to plant growth regulators.[15] Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis (PAGE) analysis of total soluble protein revealed expression of new protein band (65 kDa) only in stem derived callus. Leaf derived callus showed new protein bands with molecular weight 112 kDa. New proteins were also synthesized in xanthone treated calli. The protein bands with molecular weights between 94 and 20 kDa appeared in *Glycine wightii* callus cultures grown on MS media supplemented with (4.52 mM) 2,4-D and (0.46 mM) kinetin as a response to mechanisms of growth regulators.[16] Proteins were separated by one-dimensional SDS-polyacrylamide gel electrophoresis and revealed the induction of two new protein bands (66 kD and 35 kD) in Slash Pine callus cultures.[17] The water stress, heat-shock and wounding or ABA application has enhanced the synthesis of a 70 kD protein in maize seedlings that was similar to the heat-shock protein of *Drosophila*, and that water stress also led to the appearance of new proteins.[18] On the basis of study conducted revealed that it is possible to use the *Swertia* xanthone extract as organic additives.

CONCLUSION

The experimental findings in the present investigation revealed the fact that the MS media supplemented with moderate or higher concentration of *Swertia* xanthone contributes to generate good amount of callus. The phytoconstituents analysis indicated slight boost in the proteins and flavonoids content whereas polyphenol content was elevated compared to control in the callus grown in *S. minor* xanthone supplemented MS media. Protein profiling also exhibited variations in terms of the de novo occurrence of new protein bands. *S. minor* xanthone extract mediated cell suspension culture has remarkable applications on elevation of cell viability count. The application of such constituents of biological origin may be standardized precisely to minimise the use of expensive chemical components to achieve good results in future in the area of *In-vitro* plant cell culture. There is scope for developing growth potential rich cultures which may enhance the useful metabolite content in the cultured cell lines. Molecular markers have been used to study the extent of genetic variations. The protein profiling of germplasm and genetic markers were successfully applied in achieving taxonomic and evolutionary aspects in several crops. Scientists from all over the world tend to use the natural alternative to the chemical plant hormones, it may enhance the plant growth, but their effects on the human health are still unknown. The excessive use of plant hormones may also harm the developing plants. Natural growth hormone substitutes may be developed which not only enhance the growth but also have less harmful impact on human health and environment.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr. A. B. Ade Ph.D.

Professor, Department of Botany,
Savitribai Phule Pune University,
Ganeshkhind,
Pune 411007 (Maharashtra State)

Mr. Anubrata Paul M.Sc. Biotech (Research)

Department of Biotechnology, Natural Products Research Laboratory, Centre for Drug Design Discovery & Development (C-4D) , SRM University,
Delhi-NCR,Sonepat, India

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