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Allelopathic effect of Swertia xanthone extracts on protein profiling and protein quantity in Vigna radiata leaves.

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Abstract : *Swertia* species are demanding medicinal plants used in India and China. The herbal drug chiretta is obtained by drying the *Swertia* plants. Medicinal importance of this plant is due to the presence of xanthones, irridoids, secoiridoids, flavonoids and terpenoids. Xanthones comprises the main bioactives. *S. chirayita* is used as antipyretic, anthelmintic and in treatment of asthma and leucorrhoea. In the present study allelopathic effect of crude xanthone compounds was observed in *Vigna radiata* seedlings, with special reference to protein quantity and protein profiling. Our main objective was to trace out the possibility of variations in the protein banding pattern on account of allelopathic effect in leaves obtained from the seedlings grown in two different conditions. Treatment was administered with 0.05%, 0.1% and 0.5% under lab condition and culture condition. Leaves obtained from the seedlings grown in both conditions were analysed for protein estimation and protein profiling. Protein content was observed to be decreased in lab condition. An enhancement in protein content was observed in culture condition. Highest protein content (24.52±0 mg/g) was observed in 0.5% *S. densifolia* xanthone treatment. SDS-PAGE Protein profiling indicated occurrence of new protein bands in leaves in both the experimental condition when compared to control. Lab condition treatment gives rise to synthesis of more proteins (13 bands) when compared to culture condition (11 bands) mostly due to the defragmentation of complex protein structures. In both the conditions proteins were de novo synthesized and more of low molecular weight.
Key words- *Swertia,* allelopathy, protein profiling.

Introduction-

Swertia is a large genus of herbs occurred in the mountains. In India it is known as chirayita. Many components such as xanthone derivatives, irridoids and secoiridoids, flavonoids, triterpenoids and alkaloids have been isolated and biological activities have been investigated. *S. chtrayita* is used as antipyretic, anthelminitic, antiperiodic, cathartic and in asthma and leucorrhoea in Ayurveda and as harsh, analeptic, stomachic, mitigate inflammation, relaxing to pregnant uterus and never ending fevers.[1] It is a remedy for ulcers, gastrointestinal diseases, skin diseases, cough, hiccup, liver and Kidney diseases, Neurological disorders and urinogenital tract disorders. It is also used as Breast milk purifier and a laxative and carminative.[2]

Plants are known to synthesize allelochemicals that affect germination, growth, metabolism, development, distribution, behavior, and reproduction of other organisms.[3] Allelopathic compounds play important role in the determination of plant diversity, dominance, succession, and climax of natural vegetation and in the plant productivity of agroecosystems. Allelopathy also may be one of several attributes which enable a plant to establish in a new ecosystem. This type of interaction was named phytochemical ecology or ecological biochemistry.[4]

SDS- PAGE is a dependable method for determining the presence of soluble proteins. Variations in the protein structure and properties are considered as an indication of protein polymorphism and phenotypic variation such phenotypic variation is the base for the separation of individual species in the population that lead to creation of different taxa. Proteins are considered to be direct products of genes and can be taken as markers of these genes.[5] There is a strong correlation between protein band patterns and species diversity among the species. Gene activation due to environmental stimuli plays an important role in the adaptation of plants to stress conditions and promotes the appearance of stress resistant specific proteins.[6] In addition, tissue culture, inclusive of *in vitro* cells, tissues and organs, is a convenient and effective means in physiology and biochemistry of medicinal plants and it contribute to the production of biomass and metabolites. In the present study the allelopathic effect of *Swertia* xanthone was investigated in leaf protein under lab condition and culture condition.

Materials and methods-

Plant material-

Three *Swertia* species i.e. S. densifolia, S. lawii and S. minor were collected from Kas (Satara), Panhala (Kolhapur) and Sinhagad (Pune) respectively. Specimens were identified and deposited as Voucher specimens in Blatter herbarium Mumbai.

Extraction of crude xanthone-

The leaves of the *Swertia* species were collected. They were washed and cut into small pieces and shade dried. Powdered leaves were macerated in dichloromethane and methanol in 1:1 proportion for 48 hrs. The resulting solution was filtered and concentrated with Rotary evaporator. This solution is again reextracted with ethyl acetate. The resulting solution was labelled as crude xanthone extract and stored in fridge until used.[7]

Treatment under lab condition-

One experimental set was conducted by administrating the treatment of xanthone extract of three *Swertia* species to the seeds of *Vigna radiata* directly. Three xanthone concentrations 0.05%, 0.1% and 0.5% of the respective *Swertia* species were selected for the treatment. The treated seeds were germinated using blotting paper method.[8] Fifty seeds from each treatment were placed on petri dish of 9.0 cm diameter containing water soaked blotting paper. Each treatment was replicated thrice. Leaves of the plants grown under lab conditions were used for the protein estimation and SDS- PAGE protein analysis.

Treatment under culture condition-

In another set, the seeds of *Vigna radiata* were grown in MS media supplemented with 0.05%, 0.1% and 0.5% of *Swertia* xanthone extracted from three different species. For control, seeds were grown on MS medium without xanthone extract. Leaves generated from the seedlings in culture were used for the protein estimation and profiling.

Extraction and estimation of proteins-

The extractions of proteins were carried out in phosphate buffer. 0.1 gm of leaf material was extracted in 10 ml sterile distilled water boiled for 5 min. Cooled and filtered broth was used for protein estimation. Bovine Serum Albumin (0.1 mg/ml) was used as a standard protein. Different volumes with 0.2, 0.4, 0.6, 0.8, 1 ml of standard; 0.2 ml of leaf extract in the test tubes was taken. The volume was adjusted to 1 ml with distilled water. 5 ml of Biuret reagent (50 ml of 2% Na_2CO_3 in 0.1N aqueous NaOH+1 ml of 0.5% CuSO4 in 1% potassium sodium tartarate.) was added to all the test tubes. Addition of 0.5 ml diluted Folin's reagent was followed after incubation at room temperature for 10 min. and further incubation at 25^0C for 30 min. The blue colour developed was measured by recording OD at 660 nm. A standard graph of BSA was plotted and the amount of protein in the sample was calculated.[9]

Electrophoretic profiling of protein-

Protein profiling was done by SDS-PAGE method.[10] Samples for electrophoresis were prepared by adding sample buffer (5X) and protein samples in 1:4 ratio. Standard protein marker was also prepared by same method. Samples were heated in boiling water bath for 2 to 3 min. Samples were cooled and carefully injected using micro syringe into wells through electrode buffer. The gel was run in refrigerator for proper cooling of electrode buffer and plates. The gel was run at 50 mA current until the bromophenol blue reaches to the bottom of the gel. After completion of run, the gel was carefully removed from the plates and immersed in staining solution for overnight. Staining solution contained 0.125% CBB R-250, 50% methanol and 10% glacial acetic acid. The gel was then transferred to destaining solution containing methanol and glacial acetic acid in 5:1 proportion in distilled water. After proper destaining the gel was photographed by geldoc (Alpha Imager HP).

Results and discussion-

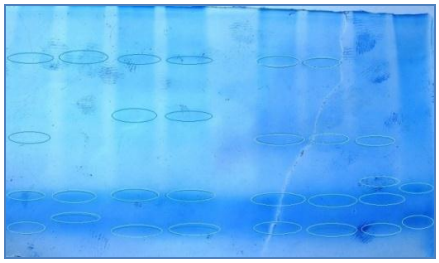
In lab condition, protein content was decreased compared to control. Elevation in protein content was observed in culture condition except for 0.1% *S. densifolia* and 0.5% *S. minor* xanthone concentrations compared to control (Table-1). Highest protein content (24.52±0 mg/g) was observed in 0.5% *S. densifolia* xanthone treatment.

Table-1 Effect of crude xanthone extracts of *Swertia* species on protein content in lab condition and culture condition.

Sr. No.	***Swertia* species**	**Xanthone Conc. (in %)**	**Protein (in mg/g)**	
			Lab condition	Culture condition
1	Control	-	13.08±0.02	15.22±0
2	*S. densifolia*	0.05	10.01±0	15.44±0
		0.1	9.86±0	13.37±0.01
		0.5	13.22±0	24.52±0
3	*S. lawii*	0.05	8.72±0	19.09±0
		0.1	10.01±0	17.16±0
		0.5	9.22±0	16.44±0.01
4	*S. minor*	0.05	10.08±0	17.87±0
		0.1	10.22±0	15.30±0
		0.5	10.01±0	12.29±0

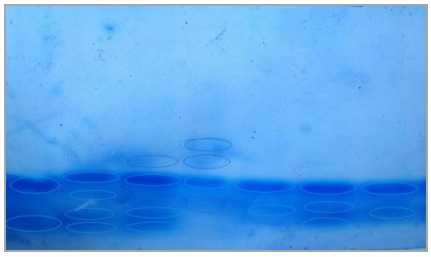
Protein profiling analysis indicated the disappearance of some protein bands, selective increase in some other protein bands and the synthesis of new set of low molecular weight proteins in the xanthone treated experimental set under lab condition and culture condition (Table 2 & 3, Figure 1-3.). In control leaves developed under lab condition, total of four bands were visible. Band no. 1, 2, 3 and 4 appeared at Rf 0.46, 0.54, 0.76 and 0.9 with the molecular weights of 132 kDa, 76 kDa, 24 kDa and 11 kDa, respectively. In the leaves obtained from the seeds treated with *S. densifolia* xanthone extracts in lab condition total of five proteins (135 kDa, 87 kDa, 41 kDa, 33 kDa and 19 kDa) were de novo synthesized, while 76 kDa protein was disintegrated. Results also showed the appearance of six novel protein bands having molecular weights of 56 kDa, 41 kDa, 31 kDa, 28 kDa, 17 kDa and 15 kDa on treating the seeds with *S. lawii* xanthone extract in lab condition. *S. minor* xanthone concentration induces expression of four proteins with 135 kDa, 98 kDa, 56 kDa and19 kDa of molecular weight.

SDS-PAGE protein profiling of *Vigna radiata* leaves obtained after seed treatment with xanthone extracts of *Swertia* species under lab condition and culture condition.



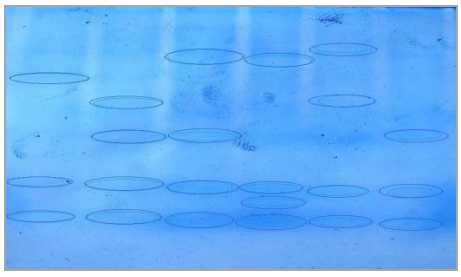
a b c d e f g

Figure 1. a-control (lab condition), *S. densifolia* xanthone extracts under lab condition, b- 0.05%, c- 0.1% and d- 0.5%, *S. densifolia* xanthone extracts under culture condition, e- 0.05%, f- 0.1% and g- 0.5%.



a b c d e f g

Figure 2. *S. lawii* xanthone extracts under lab condition, a- 0.05%, b- 0.1% and c- 0.5%, d-protein marker, *S. lawii* xanthone extracts under culture condition, e- 0.05%, f- 0.1% and g- 0.5%.



a b c d e f

Figure 3. *S. minor* xanthone extracts under lab condition, a- 0.05%, b- 0.1% and c- 0.5%, *S. minor* xanthone extracts under culture condition, d- 0.05%, e- 0.1% and f- 0.5%.

Table- 2: SDS-PAGE protein profiling of *Vigna radiata* leaves after seed treatment with xanthone extract of *Swertia* species and grown under lab conditions-

Sr. No.	Name of sample	Concentration of xanthone extract	Total no. of Bands	Distance travelled by each band (in-mm)	RF value	Molecular weight (in kDa.)
1	Control	-	4	Band No.1-23 mm	0.46	132
				Band No.2-27mm	0.54	76
				Band No.3-38mm	0.76	24
				Band No.4-45mm	0.9	11
2	*S. densifolia*	0.05	3	Band No.1-22 mm	0.44	132
				Band No.2-35 mm	0.7	33
				Band No.3-40 mm	0.8	19
		0.1	4	Band No.1-22 mm	0.44	135
				Band No.2-26 mm	0.52	87
				Band No.3-33 mm	0.66	41
				Band No.4-44 mm	0.88	12
		0.5	4	Band No.1-22 mm	0.44	135
				Band No.2-26 mm	0.52	87
				Band No.3-38 mm	0.76	24
				Band No.4-45 mm	0.9	11

3	*S. lawii*	0.05	2	Band No.1-32 mm	0.71	31
				Band No.2-40 mm	0.88	17
		0.1	4	Band No.1-30 mm	0.66	41
				Band No.2-33 mm	0.73	28
				Band No.3-38 mm	0.84	15
				Band No.4-40 mm	0.88	12
		0.5	4	Band No.1-27 mm	0.6	56
				Band No.2-30 mm	0.66	41
				Band No.3-34 mm	0.75	25
				Band No.4-38 mm	0.84	15
4	*S. minor*	0.05	3	Band No.1-25 mm	0.5	98
				Band No.2-40 mm	0.8	19
				Band No.3-46 mm	0.92	10
		0.1	4	Band No.1-22 mm	0.44	135
				Band No.2-30 mm	0.6	56
				Band No.3-40 mm	0.8	19
				Band No.4-46 mm	0.92	10
		0.5	4	Band No.1-26 mm	0.52	76
				Band No.2-30 mm	0.6	56
				Band No.3-40 mm	0.8	19
				Band No.4-46 mm	0.92	10

Table- 3: SDS-PAGE protein profiling of *Vigna radiata* leaves obtained from the seeds grown in MS media supplemented with xanthone extracts of three different *Swertia* species.

Sr. No.	Name of sample	Concentration of xanthone extract	Total no. of Bands	Distance travelled by each band (in-mm)	RF value	Molecular weight (in kDa.)
1	Control	-	4	Band No.1-23 mm	0.46	132
				Band No.2-28 mm	0.56	71
				Band No.3-40 mm	0.8	19
				Band No.4-46 mm	0.92	10
2	*S. densifolia*	0.05	4	Band No.1-23 mm	0.46	132
				Band No.2-28 mm	0.56	71
				Band No.3-40 mm	0.8	20
				Band No.4-45 mm	0.9	11
		0.1	3	Band No.1-28 mm	0.56	71
				Band No.2-39 mm	0.78	21
				Band No.3-45 mm	0.9	11
		0.5	2	Band No.1-38 mm	0.76	24
				Band No.2-44 mm	0.88	17
3	*S. lawii*	0.05	2	Band No.1-35 mm	0.77	22
				Band No.2-40 mm	0.88	17
		0.1	3	Band No.1-35 mm	0.77	22
				Band No.2-38 mm	0.84	15
				Band No.3-40 mm	0.88	17
		0.5	2	Band No.1-35 mm	0.77	22
				Band No.2-38 mm	0.84	15

4	*S. minor*	0.05	3	Band No.1-29 mm	0.58	63
				Band No.2-40 mm	0.8	19
				Band No.3-46 mm	0.92	10
		0.1	4	Band No.1-22 mm	0.44	135
				Band No.2-28 mm	0.56	71
				Band No.3-40 mm	0.8	19
				Band No.4-46 mm	0.92	10
		0.5	4	Band No.1-25 mm	0.5	98
				Band No.2-40 mm	0.8	19
				Band No.3-43 mm	0.86	14
				Band No.4-46 mm	0.92	10

In the control leaves obtained from the seeds grown in MS media, four bands were traced at Rf 0.46, 0.56, 0.8 and 0.92 with the molecular weights of 132 kDa, 71 kDa, 19 kDa and 10 kDa, respectively. In *Swertia densifolia* xanthone concentrations, four novel protein bands with molecular weights of 24, 21, 17 and 11 kDa were traced in addition to the bands recorded in control. In addition, protein bands of molecular weights of 22 kDa, 17 kDa and 15 kDa were induced in the leaves treated with *S. lawii* xanthone extract. Four protein bands of molecular weight 135 kDa, 98 kDa, 63 kDa and 14 kDa were de novo synthesized in the leaves of plants grown in MS media supplemented with *S. minor* xanthone treatment. Comparative analysis between lab condition and culture condition, overall more proteins i.e. 13 bands (10 kDa, 12 kDa, 15 kDa, 17 kDa, 19 kDa, 28 kDa, 31 kDa, 33 kDa, 41 kDa, 56 kDa, 87 kDa, 98 kDa and 135kDa) were traced in Lab condition while 11 bands (11 kDa, 14 kDa, 15 kDa, 17 kDa, 20 kDa, 21 kDa, 22 kDa, 24 kDa, 63 kDa, 98 kDa and 135 kDa) in culture condition in addition to control.

The application of carrot roots extract to the seeds of cowpea grown under salinity conditions has induced a considerable variation in the protein patterns.[11] Treating the seeds with carrot roots extract and salt stress lead to differential expression of the genetic information in cowpea seedlings, resulting in changes in gene products, including protein and isozymes profiles. These changes induced the synthesis of certain proteins and simultaneously decreased the expression of other protein sets. The results also demonstrated that low concentration (25 mg/ L) of carrot root extract was more effective in reducing the adverse effects of salinity through the enhancement of multiple processes. In all the *Swertia* xanthone concentrations studied, only *S. lawii* showed disappearance of proteins while other two species induces the synthesis of new proteins. The effect of kinetin on leaf protein content and its profile in mung bean was observed under salt stress.[12] Gel electrophoretic profile of protein content in leaf of mung bean showed an extra band between 29 kDa and 45 kDa in stress protein profile as compared with control. We understand the possibilities of biomolecular interaction of xanthone phytoconstituents which might be resulted in disintegration of some complex proteins. The fragmented subunits probably appeared as new bands, supportive reports are evident on this aspect. Soluble proteins in rice leaf with molecular weight of more than 100 kDa were reduced as a result of drought stress, but low molecular weight proteins were increased.[13] Under drought stress, some low molecular weight proteins were intensified, while high molecular weight proteins were faint. Our results also showed that low molecular weight proteins increased. The alternation of protein synthesis or degradation is one of the fundamental metabolic processes that may influence drought stress tolerance.[14] Many studies indicate that allelochemicals affect several molecular targets such as membrane permeability, electron transport chains, transport processes, photosynthesis and respiration, DNA and protein biosynthesis as well as hormone metabolism.[15]

Conclusion-

All the studied samples showed variations in leaf protein banding compared to control. In lab condition more proteins were intensified in terms of band numbers and molecular weight compared to culture condition. Overall results indicate that low molecular weight proteins expressed in high amount. Therefore the present work provides an insight to understand the allelopathic impact of *Swertia* xanthone extracts under lab condition and culture conditions on protein constituents in *Vigna* plant. This will help to know about the interaction between species.

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