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Optimization of ς-glucosidase assay and protein estimation from various parts of *Rauvolfia serpentina*

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Rauvolfia serpentina is gifted with unique alkaloids that have remarkable medicinal properties and is being pronounced as "Wonder drug of India". Several Ayurvedic preparations containing Rauvolfia plant parts are available in the market. The activity of crude enzyme in *Rauvolfia serpentina* was highest in mature leaf-1 (100%) followed by very young leaf (94.715%), whereas lowest in root (9.829%). It was noticed that very young leaf and young leaf have highest protein 7.4573 and 3.8344 mg/ml, respectively and lowest protein content was found in stem (0.1689 mg/ml) of *Rauvolfia serpentina*. Young leaf contained highest number of isoforms (3).

Key words : *Rauvolfia serpentina,* Protein, Alkaloid, β-glucosidase

INTRODUCTION

Rauvolfia serpentina is a medicinal plant and popularly known as Sarpagandha or snakeroot. This medicinal plant occurs in hot and humid regions with sufficient rainfall and soil containing high nitrogenous content (Sahu, 1983). According to Ayurveda root is bitter, heating, sharp, pungent and anthelminic. Rauvolfia preparations such as sarpagandha ghanvati, sarpagandha yoga, sarpagandha churna and mashesvari vati are used as antihypertensive and as sedative. It is also used for the treatment of various central nervous system disorders associated with psychosis, schizophrenia, insanity, insomnia and epilepsy.

MATERIALS AND METHODS

Plant material:

Rauvolfia serpentina was used for the β-glucosidase extraction and protein estimation (Esen, 1978). Rauvolfia was grown and maintained in the field, at Allahabad Agricultural Institute, Deemed University, Allahabad. The plants were grown following standard agronomic practices. The plant material was freshly harvested for use and processed immediately after harvest to avoid tissue breakage and loss in enzyme activity.

Chemicals:

All chemical were of high analytical grade and purchased from Hi-media.

ς-*Glucosidase assay:*

The plant parts of *Rauvolfia serpentina* was ground to fine powder in a chilled mortar and pestle using liquid N_2 and extraction buffer (1ml buffer / 1g tissue) was mixed to it. This was centrifuged at 12000 rpm / 4^0C / 30 min. The supernatant was taken and it was used for activity and protein estimation. For activity estimation, para nitophenyl-β-D-glucopyranoside (pNPG) was used as a substrate. Optical density was taken using spectrophotometer at 405 nm for experimental and control reaction. For experimental, 165 µl citrate phosphate buffer (0.1 M, pH 4.8), 10 µl crude enzyme and 25 µl substrate (pNPG) was mixed at room temperature. After 15 min 800 µl, 1 M Na_2CO_3 was mixed to stop the reaction and O.D. was taken. For control, 165µl citrate phosphate buffer (0.1 M, pH 4.8), 10 µl enzyme, 800 µl Na_2CO_3 (1M) and 25ml substrate (pNPG) was mixed at room temperature and after 15 min, O.D. was taken (Mahadaven and Sridhar, 1986).

Protein estimation:

Soluble protein estimation was performed calorimetrically with BSA as standard using Lowry's method (Lowry *et al.,* 1951). The protein / enzyme extract (100 ml) was precipitated with 100 µl T.C.A. (12%). After 30 min centrifugation was done at 10,000 RPM, 4^0C for 5 min. Thus, obtained pellet was dissolved in 200 µl NaOH (0.1 N). Using these samples, protein was estimated. Taking replicates in different volumes such as, 50 µl and 100 µl, and 950µl and 900 µl of 0.1 N NaOH was added. There after, 5 µl alkaline Cu -reagent was mixed to it. After 10 min, 0.5 ml F.C.C. reagent (1N) was added to it. After 30 min., absorbance was taken at 660 nm. Alkaline Cu-

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reagent and Folin Ciocalteus phenol dilution was done at the time of estimation only. All the samples including blank and standard BSA are done in triplicate.

RESULTS AND DISCUSSION

Optimization of extraction and assay of S-glucosidase enzyme:

The enzyme from each part of *Rauvolfia serpentina* was extracted with procedure described in Materials and Methods. The volume of supernatant and weight of respective tissues taken for different part of plants were tabulated in Table 1.

Table 1 : Volume of crude enzyme of *Rauvolfia serpentina*

Sr. No.	Sample	Wt. of tissue taken (g)	Volume of supernatant (ml)
1.	Fruit	2.748	2.95
2.	Flower	3.472	2.7
3.	Flower stem	0.653	0.24
4.	Very young leaf	0.4052	0.24
5.	Young leaf	1.511	1.50
6.	Mature leaf-1	1.673	1.0
7.	Mature leaf-2	2.958	2.1
8.	Old leaf	3.418	3.3
9.	Stem	1.235	0.11
10.	Root	4.080	3.0

Activity estimation:

The activity was estimated using spectrophotometer at wave length 405 nm. For the reaction, pNPG was used as a substrate. The reaction mixture was incubated for 15 min at room temperature. Changes in optical density were calculated and the activity was estimated (Table 2). The activity of crude enzyme in *Rauvolfia serpentina* on per gram fresh weight basis was highest in mature leaf -1(100%) followed by very young leaf (94.715%), where as lowest in root (9.829%).

Table 2 : S-glucosidase activity profile from various plant parts in *Rauvolfia serpentina*

Sr. No.	Sample	Change in O.D./10µl	Activity Units/ml/min.	Activity (%)
1.	Fruit	0.031	206	11.17
2.	Flower	0.164	1093	62.1
3.	Flower stem	0.061	400	23.5
4.	Very young leaf	0.25	1667	94.715
5.	Young leaf	0.075	500	28.40
6.	Mature leaf-1	0.264	1760	100
7.	Mature leaf-2	0.094	627	35.625
8.	Old leaf	0.132	880	50.0
9.	Stem	0.034	227	12.897
10.	Root	0.026	173	9.829

Total soluble protein estimation from extracts of Rauvolfia serpentina:

Total soluble proteins were estimated and are presented in Table 3. From protein estimation data, it was noticed that young leaf and mature leaf-2 have highest protein 7.4573 mg and 3.8344 mg, respectively and lowest protein content was found in stem (0.1689 mg) of *Rauvolfia serpentina*.

Table 3: Protein estimation in *Rauvolfia serpentina*

Sr. No.	Sample	Protein mg/ml	Protein mg/gfw
1.	Fruit	3.1412	3.3721
2.	Flower	2.148	1.6703
3.	Flower stem	1.7816	0.6547
4.	Very young leaf	6.234	3.6923
5.	Young leaf	7.512	7.4573
6.	Mature leaf-1	5.567	3.6563
7.	Mature leaf-2	5.401	3.8344
8.	Old leaf	3.678	3.5510
9.	Stem	1.897	0.1689
10.	Root	1.891	1.3904

Specific activity:

Specific Activity was calculated by using the value of activity (International unit) and total protein. The data are presented in Table 4 and also represented through Fig. 1.

In *Rauvolfia serpentina* glucosidase activity was highest in very young leaf (53.0 IU) followed by mature

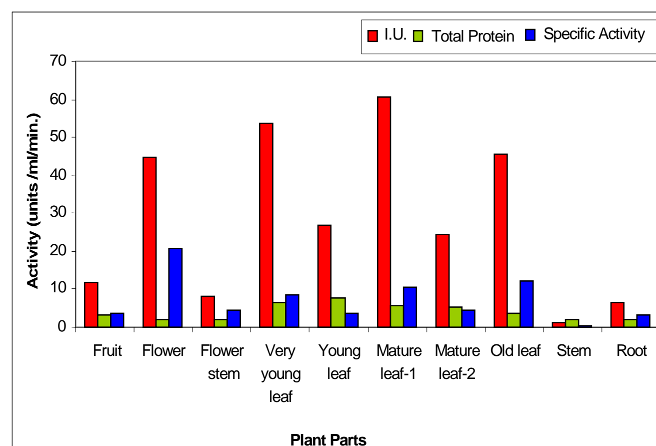

Fig. 1 : IU/g, Total protein and specific activity -glucosidase from various parts of the plant in *Rauvolfia serpentina*

Table 4: Estimation of specific activity of S- glucosidase in *Rauvolfia serpentina*

Sr. No.	Sample	Activity Units/ml/min.	Activity Units/gfw	I.U. /gfw	Protein (mg/ml)	Specific activity
1.	Fruit	206	221	12.0	3.1412	3.820
2.	Flower	1093	850	46.0	2.148	21.415
3.	Flower stem	400	153	32.0	1.8495	4.450
4.	Very young leaf	1667	987	53.0	6.234	8.507
5.	Young leaf	500	496	27.0	7.512	3.594
6.	Mature leaf-1	1760	1504	62.0	5.561	14.745
7.	Mature leaf-2	627	445	24.0	5.401	4.443
8.	Old leaf	880	850	46.0	3.678	12.506
9.	Stem	227	22	1.0	0.1689	0.5271
10.	Root	173	42	2.0	1.899	1.057

leaf 1(62.0 IU) while specific activity of β-glucosidase was found lowest in stem (0.5271 IU). The highest β-glucosidase activity on per gram fresh weight basis was found in mature leaf-1 of *Rauvolfia serpentina* and the maximum numbers of bands of proteins were found in fruit of *Rauvolfia serpentina* (Laemmli, 1970).

Results of native gels versus Rauvolfia serpentina plant parts:

The patterns of band over native gels of *Rauvolfia serpentina* are given in Table 5. From the table, it is apparent that in all the three species glucosidase exhibited two zones of activities in flowers. Root had no detectable bands under the conditions of gel running and its development.

During the native PAGE study, it was found that in *Rauvolfia serpentina*, maximum number of isoforms of enzyme was detected in young leaves whereas flowers and flower stem were found to have two isoforms each (Kumar *et al.*, 2003). These results are of physiological significance as they reveal requirement of more than one

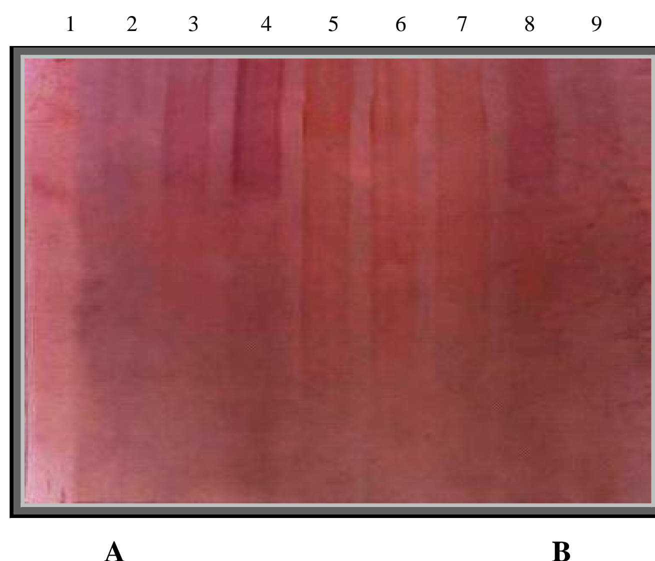

Fig. 2 : Native PAGE of *Rauvolfia serpentina.* Lane 1 - Fruit, Lane 2 - Flower, Lane 3 - Flower stem, Lane 4 - Young leaf,, Lane 5 - Mature leaf - 1, Lane 6 - Mature leaf - 2, Lane 7 - Old leaf, Lane 8 - Stem, Lane 9 - Root.

Table 5 : Number of isoforms of S-glucosidase found in native PAGE

Plant pars	*Rauvolfia serpentina*
Fruit	1
Flower	2
Flower stem	2
Very young leaf	-
Young leaf	3
Mature leaf -1	1
Mature leaf -2	1
Mature leaf -3	-
Mature leaf -4	-
Old leaf	1
Stem	1
Root	0

isoform (root) for the tissue to perform the deconjugation. Interestingly enough, their enhanced amount and presence in flowers and pedicel has not been reported earlier in the literature. This is for the first time we have observed tissue specific activity measurement of β-glucosidase. From native gel developed of crude enzyme, obtained from flowers of *Rauvolfia serpentina*, two isoforms were obtained. These two isoforms were also separated by column chromatography. These isoforms may be RG and SG or some other more isoforms, which may be concluded by further study of β-glucosidase. These isoforms play an important role in vomiline and ajmaline biosynthesis or may have some new role. Looking at the alkaloid biosynthetic pathway (Kutchan, 1998), these two isoforms of enzyme may be strictosidine glucosidase (SG) and raucaffricine glucosidase (RG) or it may be some other

isoform of enzyme. If these are RG and SG, then these play a major role in the pathway of ajmaline and vomiline biosynthesis. If this enzyme has some other isoform, then it may have a new role to play. In Rauvolfia, particular flower it could have even some participation in anthocyanin development. It would be thus interesting to see the enzyme versus flower colour and development.

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