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Optimization of β-Glucosidase assay and protein estimation from various parts of *Rauvolfia tetraphylla*

Abha Singh and Om Prakash Verma

Abstract
Rauvolfia tetraphylla is one of the medicinally most important plant. β-glucosidase enzyme was extracted from different plant parts of *Rauvolfia tetraphylla,* its crude enzyme activity was found highest in very young leaf (100%) followed by mature leaf-1 (87.16%) and mature leaf -2 (92.16%) whereas lowest in old leaf (15.0%). It was also noticed that flowers of *Rauvolfia tetraphylla* have highest soluble protein content in mature leaf–1 (9.241 mg/ml) which was followed by very young leaf (9.216 mg/ml). *Rauvolfia tetraphylla* have maximum 3 isoforms were found in flower steam, very young and mature -1 leaves.

Keywords: *Rauvolfia tetraphylla,* β-glucosidase enzyme, total protein, specific activity,

Introduction
Rauvolfia tetraphylla is a plant in the Apocynaceae family, growing as a bush or small tree. It is commonly known as the devil-pepper. The plant is native to the tropical Americas. It has been cultivated widely as both an ornamental and as a source of pharmaceuticals and is now naturalized throughout the tropics including Australasia, Indochina and India. This medicinal plant occurs in hot and humid regions with sufficient rainfall and soil containing high nitrogenous content (Sahu, 1983) [8]. β- glucosidase (β-D-glucoside glucohydrolases; EC 3.2.1.21) occur ubiquitously in plant, fungi, mammals and microorganisms (Woodward and Wiseman, 1982) [9]. It have been reported that β-glucosidase uses in improvement of fruit juices (Roitner *et al.,* 1984) [7]**.** They are also used in the production of synthetic substrate (Gunata *et al.,* 1997) [2] and flavour of wine (Riou *et al.,* 1998) [6].The present study was carried with optimization of β-glucosidase assay and protein estimation from various parts of *Rauvolfia tetraphylla.*

Materials and Methods
Plant Material
Rauvolfia tetraphylla was used for the β-glucosidase extraction and protein estimation (Esen, 1978).

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

β-Glucosidase assay
β-Glucosidase assay was performed by using standard method (Mahadaven and Sridhar, 1986) [5].

Protein estimation
Soluble protein estimation was performed calorimetrically with BSA as standard using Lowry's method (Lowry *et al*., 1951) [4].

Results and Discussion
Optimization of extraction and assay of β-glucosidase enzyme
The enzyme from each part of *Rauvolfia tetraphylla* 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 tetraphylla*

S. No.	Sample	Wt. of tissue taken (g)	Volume of supernatant (ml.)
1	Fruit	2.38	**2.96**
2	Flower	0.94	0.61
3	Flower stem	0.80	0.54
4	Very young leaf	1.20	0.82
5	Mature leaf-1	3.10	2.65
6	Mature leaf-2	3.11	2.5
7	Old leaf	1.16	0.74
8	Stem	0.641	0.46
9	Root	1.06	0.40

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 in Table 2. The activity of crude enzyme in *Rauvolfia tetraphylla* was found highest in very young leaf (100%) followed by mature leaf-1 (87.16%) and mature leaf -2 (92.16%) whereas lowest in old leaf (15.0%) as shown as Table 2.

Table 2: β-glucosidase activity profile from various parts of the plant in *Rauvolfia tetraphylla*

S. No.	Sample	Change in O.D./10µl	Activity Units/ml/min.	Activity (%)
1	Fruit	0.141	940	78.33
2	Flower	0.065	433	36.08
3	Flower stem	0.093	620	51.67
4	Very young leaf	0.180	1200	100
5	Mature leaf-1	0.157	1046	87.16
6	Mature leaf-2	0.166	1106	92.16
7	Old leaf	0.027	180	15.0
8	Stem	0.035	233	19.42
9	Root	0.031	206	17.17

Total soluble protein estimation from extracts of *Rauvolfia tetraphylla*

Total soluble proteins estimated as per details in Materials and Methods section and are represented in Table 3. From protein estimation data, it was noticed that flowers of *Rauvolfia tetraphylla* have highest protein content in mature leaf–1 (9.241 mg/ml) which was followed by very young leaf (9.216 mg/ml) as shown in Table 3.

Table 3: Protein estimation in *Rauvolfia tetraphylla*

S. No.	Sample	Protein mg/ml	Protein mg/g
1	Fruit	2.798	3.4798
2	Flower	5.305	3.4426
3	Flower stem	4.681	3.1596
4	Very young leaf	9.216	6.2976
5	Mature leaf-1	9.241	7.8995
6	Mature leaf-2	6.898	5.5450
7	Old leaf	6.590	0.0973
8	Stem	3.584	2.5719
9	Root	2.158	0.8143

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 Figure 1. In *Rauvolfia tetraphylla* glucosidase activity was highest in fruit (63.0 IU) followed by mature leaf –1 and mature leaf –2 (48.0 IU), while specific activity was highest in fruit (18.104). The lowest glucosidase activity was found in root (4.0 IU) and also the specific activity of glucosidase was found lowest in old leaf (1.444) as shown in Table 4. and the maximum numbers of bands of proteins were found in flower stem, very young leaf and mature leaf-1, while lowest in fruit of *Rauvolfia tetraphylla* (Laemmli, 1970) [3].

Table 4: Estimation of specific activity of β- glucosidase in *Rauvolfia tetraphylla*

S. No.	Sample	Activity Units/ml/min.	Activity Units/g	I.U. /g	Protein (mg/ml)	Specific activity
1	Fruit	940	1169	63.0	3.4798	18.104
2	Flower	433	280	15.0	3.4426	4.357
3	Flower stem	620	418	23.0	3.1596	7.279
4	Very young leaf	1200	820	44.0	6.2976	6.986
5	Mature leaf-1	1046	894	48.0	7.8995	6.076
6	Mature leaf-2	1106	889	48.0	5.5450	8.656
7	Old leaf	180	108	6.0	4.1542	1.444
8	Stem	233	167	9.0	2.5719	3.499
9	Root	206	77	4.0	0.8143	4.912

Results of native gels versus *Rauvolfia tetraphylla* plant parts

The patterns of band over native gels of *Rauvolfia tetraphylla* are given in Table 5. From the Table 5, it is apparent that glucosidase exhibited two zones of activities in flowers. Root had no detectable bands under the conditions of gel running and its development (Figure 1). During the native PAGE study, it was found that in *Rauvolfia tetraphylla*, maximum 3 isoforms were found in flower steam, very young and mature -1 leaves, while there were two isoforms in flower, mature -2 and old leaves as shown in Table 5. These results are of physiological significance as they reveal requirement of more than one isoform (root) for the tissue to perform the deconjugation. 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*, isoforms were obtained. These isoforms play an important role in vomiline and ajmaline biosynthesis or may have some new role.

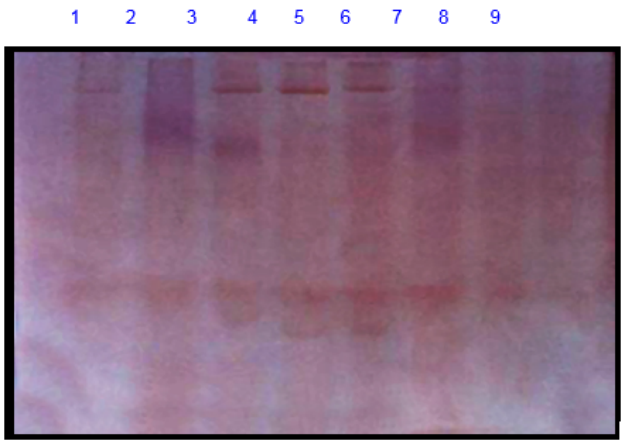

Fig 1: Native PAGE of *Rauvolfia tetraphylla.* Lane 1 – fruit, Lane 2 – flower, Lane 3 - flower stem, Lane 4 - very young leaf, Lane 5 - mature leaf -1, Lane 6 - mature leaf -2, Lane 7 - old leaf, Lane 8 - stem and Lane 9 – root

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

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

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