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Optimization of -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.

-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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