International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.6, Special Issue.1, pp.86-88, May (2019) **E-ISSN:** 2347-7520
DOI: https://doi.org/10.26438/ijsrbs/v6si1.8688

A Study on Cabbage (*Brassica oleracea*) Peroxidase Activityusing three medicinal plant leaf extracts

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***Abstract*-**Peroxidases are a group of antioxidant enzymes which are widely distributed in nature and have been important both in plants and animals for variousphysiological processes. These enzymes utilize a wide variety of substrates to bring about the redox reactions. In the present study, peroxidase was partially purified from cabbage. In view of the diverse roles catalyzed by this enzyme, it was of interest to identify novel regulators of enzyme activity. Thus the activity of the enzyme was assayed in the presence and absence of three medicinal plant leaf extracts of *Azadirachta indica*(Neem), *Ocimum tenuiflorum* (Tulsi).and *Murraya koenigii* (Curry Leaves). These three leaf extracts are known for their medicinal properties and have been used in treating chronic diseases. Studies conducted revealed that all the plant leaf extracts inhibited the activity of peroxidase to a significant extent. Further studies need to be conducted to ascertain the significance of peroxidase inhibition

Keywords: peroxidase, enzyme inhibition, medicinal plant leaf extract

I.INTRODUCTION

Peroxidases are a large family of enzymes which are classified based on the occurrence of heme in their structure. Peroxidases chiefly function as decomposing enzymes which act on different phenolic and non phenolic substrates. These enzymes have been divided into three super families: Class I,II and III based on their structural and catalytic properties[1].These enzymes play an important role in various physiological processes in plants and animals. In plants, the peroxidases are involved in cell wallmetabolism[2],lignification[3],wound healing [4], metabolism of reactive oxygen species[5], reactive nitrogen species[6,7].

Medicinal plant extracts from various parts of the plant like leaf, stem and bark have long been used for their therapeutic value. Many natural products are enzyme inhibitors; the finding and development are dynamic areas of pharmacology and biochemistry. Medicinal enzyme inhibitors are frequently mediated by its specificity and its effectiveness that designated the absorption desirable to inhibit the enzyme.[8].In view of the role of medicinal plant extracts as enzyme inhibitors, it was of interest to study the effect of three medicinal plant leaf extracts i.e.*Azadirachta Indica* (Neem), *OcimumTenuiflorum*(Tulsi)and *MurrayaKoenigii* (Curry Leaves) on the activity of peroxidase which was partially purified from cabbage (*Brassica oleracea*).

This paper is organized into the following sections. Section I contains the introduction Section II contains Materials and Methods. Section III contains results and discussions. Section IV contains the references.

II.METHODOLOGY

Collection of samples: Fresh Cabbage (*Brassica oleracea*)bud was procured from the local market and was stored at 4^oC until used [9].

Preparation of Crude Extract:10 g of the sample was weighed, washed properly and was cut, homogenized using a homogenizer using 50mL of potassium phosphate buffer (0.2MpH:7.0). The extract was then filtered using layers of cheese cloth, centrifuged at 8,000 rpm for 10mins. The supernatant was filtered using Whatman filter paper and labelled as crude extract. To inactivate any catalase present in the extract it was heated to 65^0C [10-12].

Partial Purification: The crude extract was further subjected to partial purification by precipitation and dialysis.The protein precipitation was done by ammonium sulphate precipitation method. 80% ammonium sulphate

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precipitation was carried out by adding 8.26g of the salt into the crude extract and this was stirred until the salt solubilized using a magnetic stirrer to prevent the formation of foam. This was followed by centrifugation at 8,000 rpm for 10mins. The precipitate was dissolved in buffer,dialyzed at 4^0C overnightand was used for further steps [13].

Preparation of Plant leaf extracts: The leaves are air dried and crushed into fine powder using motor and pestle. This powder is stored in airtight containers for further biochemical analysis. This fresh sample (1 gram) is homogenized in 20ml of 3% aqueous sulfosalicylic acid and centrifuged at 9000 rpm for 15 minutes. Supernatant is collected for further analysis [14].

Estimation of Enzyme Activity in the Presence of Plant leaf extract: 0.1mL of the enzyme was incubated with varied volumes of the plant leaf extracts for 10 min .After 10 mins, 4-Amino Antipyrine(1.4mL) and H_2O_2 (1.5mL) were added to these test tubes and the absorbance was read colorimetrically at 510nm using solution without Plant leaf extracts as blank.The activity of the enzyme was calculated using the formula:

$$IU/mL = \frac{\Delta A * V * D}{6.58 * d * v}$$

Where,
ΔA = Absorbance at 510nm
V = Total volume of reaction mixture.
D = Enzyme dilution factor
6.58 = mM extinction coefficient of Quinonimine dye ($L.mmol^{-1}.cm^{-1}$)
D = Light path length (1cm)
v = Volume of enzyme sample (0.10mL)

Estimation of Proline by Ninhydrin method[15]: Proline content was measured using ninhydrin reagent. During extraction with sulfosalicylic acid proteins are precipitated as complex. Extraction process involves the homogenization of dried leaves of medicinal plants in 10ml of 3% sulfosalicylic acid &centrifugation at 9000rpmfor 15 min. The obtained supernatant was mixed with acetic acid ninhydrin reagent and incubated for 1 hr. at 100^0C. The reaction was terminated in ice bath. The product was extracted with 4ml toluene. In this process, the extracted proline was made to react with ninhydrin in acidic conditions to form the chromophore (red color)& absorbance was measured at 520nm.

Estimation of Methionine in leaf: Methionine is one of the essential, Sulphur containing amino acid. In this process the sample is 1sthydrolyzed under acidic condition. The liberated methionine gives a yellow color with nitroprusside solution to under alkaline condition and turns red on acidification. Glycine was added to the reaction mixture in order to inhibit color formation with other amino acids. **[15]**

III. RESULTS AND DISCUSSION

Effect of plant leaf extracts on peroxidase activity:
The enzyme was treated with different volumes of the plant leaf extracts and the activity of the enzyme was tested. The results (as shown in Fig 1) showed the reduction in the enzyme activity. The enzyme at blank (0 mL of extract) showed equal amount of activity with all the extracts (1.036 IU/min/568ug). Plant leaf extracts caused different levels of reduction in the activity of the enzyme. Maximum reduction was seen by the neem and tulsi leaf extracts with enzyme activity of 0.212 IU/min/568ug with 1 mL of the extract and 10mins of incubation. Whereas, the curry leaf extracts caused lesser reduction in the enzyme activity with the value of 0.612 IU/min/568ug with same volume of plant leaf extract andtime of incubation

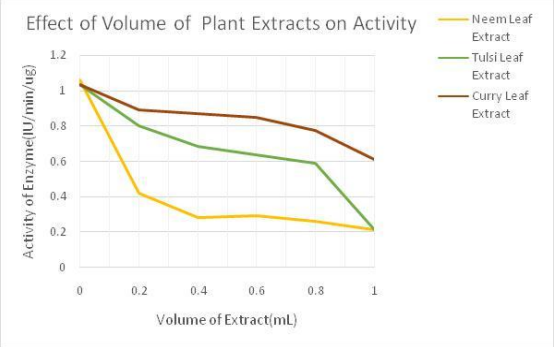

Figure 1: Effect of plant leaf extracts on activity of enzyme

As the extracts caused inhibition of enzyme activity and the available reports on the inhibition of peroxidase by sulfur containing aminoacids, biochemical analysis of the extracts was carried out.[16].

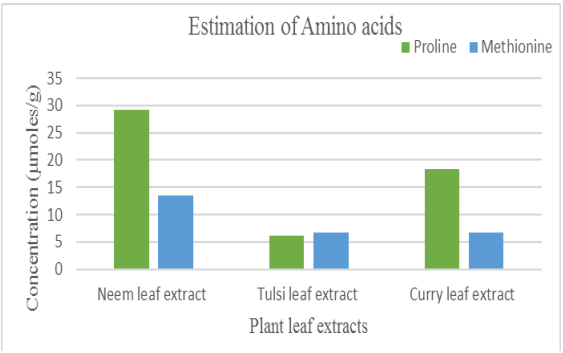

Figure 2.Estimation of aminoacids in medicinal plant leaf extract

The Plant leaf extracts were analysed for two amino acids, Proline and methionine. Fig 2 represents the proline and methionine content in the three leaf extracts and the concentration of methionine and proline was seen highest in Neem (A. indica) leaf extracts, 13.5 umoles/g and 29.25 umoles/g and less in Tulsi leaf extracts 6.7umoles/g and 6.06 umoles/g respectively.

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IV.CONCLUSIONS

The results obtained in the present study indicate that the activity of partially purified peroxidase was inhibited rapidly by neem leaf extract as compared to the inhibition by Tulasi and curry leaf extracts. These results assume significance in view of the earlier reports on the inhibition of cabbage peroxidase activity by sulfur containing aminoacid,cysteine. In the present study, biochemical analysis of leaf extracts also demonstrated that the neem leaf extract contained more of sulfur containing aminoacid ,methionine,than the other two leaf extracts. The peroxidase activity also showed a rapid decrease when the enzyme extract was incubated with neem leaf extract. Thus, the present study concludes that the biomolecules present in medicinal plants could influence the activity of the enzymes like peroxidases.

REFERENCES

[1]. Veda P Pandey, Manika Awasthi, Swati Singh, Sameeksha Tiwari and Upendra N Dwivedi *A Comprehensive Review on Function and Application of PlantPeroxidases*. Biochem Anal Biochem, 6:1, **2017**

[2]. Passardi F, Penel C, Dunand C. *Performing the paradoxical: how plantperoxidases modify the cell wall*. Trends Plant Sci, 9: 534-540,**2004**

[3]. Barceló AR, Pomer.F.*Oxidation of cinnamyl alcohols and aldehydes bya basic peroxidase from lignifying Zinnia elegans hypocotyls.* Phytochem, 57:1105-1113**, 2001**.

[4]. Bernards MA, Fleming WD, Llewellyn DB, Priefer R, Yang X, et al. *Biochemical characterization of the suberization-associated anionic peroxidase of potato*, **I**nformation Systems Division, National Agricultural Library **1999**

[5]. Allison SD, Schultz *J Differential activity of peroxidase isozymes inresponse to wounding, gypsy moth and plant hormones in northern red oak*. JChemEcol 30: 1363-1379.**2004**

[6]. Liszkay A, Kenk B, Schopfer P *Evidence for the involvement of cell wallperoxidase in the generation of hydroxyl radicals mediating extension growth*.Planta 217: 658-667, **2003**

[7]. Mc Innis SM, Desikan R, Hancock JT, Hiscock SJ Production of reactiveoxygen species and reactive nitrogen species by angiosperm stigmas andpollen: potential signalling crosstalk? New Phytologist 172: 221-228, **2006**

[8]. Abdur Rauf and Noor Jehan Natural Products as a Potential Enzyme Inhibitors from Medicinal Plants, Enzyme Inhibitors and Activators, Murat Senturk, IntechOpen, DOI: 10.5772/67376. Available from: https://www.intechopen.com/books/enzyme-inhibitors-and-activators/natural-products-as-a-potential-enzyme-inhibitors-from-medicinal-plants, March 29th 2017).

[9]. Varsha Keshavan, Sarika Puri and BedadyutiMohanty; *Determination of kinetics of peroxidase enzyme isolated from Brassica oleracea* Insights in Enzyme Research ISSN 2573-4466 Vol.2 No.2:4,**2018**

[10]. Krestnikov IL, Ledentsov NN, Hoffman A, Bimberg D, Sakharov, et al. *Quantum dot origin of luminescence in InGaN-GaN structures.* Phys Rev B 66: 155-310, **2002**

[11]. Koksal E, Gulcin I (Antioxidant *Activity of Cauliflower (Brassica oleracea L)*. Turk J Agric For 32: 65-78,**2008**

[12]. Singh BK, Sharma SR, Singh B Antioxidant enzymes in cabbage: *Variability and inheritance of superoxide dismutase, peroxidase and catalase*. Scientia Horticulturae 124: 9-13,**2010**

[13]. Senthilkumar Rathnamsamy, Raveesh Singh, Rufus Auxilia, B.N. Vedhahari. Extraction of peroxidase from various plant sources and its biodegradation studies on phenolic compounds. Downstream Processing Laboratory, School of Chemical and Biotechnology, Sastra University, Thanjavur.

[14]. Sushree Priyanka Dash, Sangita Dixit and SoubhagyalaxmiSahoo. *Phytochemical and Biochemical Characterizations from Leaf Extracts from Azadirachta Indica: An Important Medicinal Plant*, OMICS International, 10.4172/2161-1009.1000323,**2017**

[15]. S. Sadasivam, Ph. D, A. Manickam, Ph.D. Centre for plant molecular biology Tamil NaduAgricultural University Coimbatore ISBN:81-224-0976-8

[16]. Shodhganga.inflibnet.ac.in>bitstream. Shodhganga chapter V 129-148.

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