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Research Article

ISOLATION AND CHARACTERIZATION OF POLYPHENOL OXIDASE FROM PHYLLANTHUS EMBLICA (INDIAN GOOSEBERRY)

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ABSTRACT

Polyphenol Oxidase (E C 1.10.3.1) from Phyllanthusemblica(Indian Gooseberry) was purified to homogeneity by ammonium sulphate followed by dialysis and SDS PAGE. The apparent molecular weight was 100 kD. Highly active PPO extract was obtained using 1% (W/V). Triton-X-100 and 0.1M NaCl in 0.2M phosphate buffer, pH 7.0. The optimum pH was found to be 7 and enzyme activity was stable in range of 30^0C - 40^0C. PPO showed highest activity with Catechol compared to Tyrosine. High inhibitory effects were shown by EDTA. PPO activity was enhanced by sulphates compared to chlorides. The data obtained in this study help to better understand fruit browning in Indian Gooseberry.

Key words:PPO, Purification, Characterization. Gooseberry.

INTRODUCTION

Polyphenol oxidases (PPO) EC1.10. 3. 1; are enzymes, belonging to a group of copper containing metallo proteins and are members of oxido- reductases, that catalyze the oxidation of a wide range of phenolic compounds by utilizing molecular oxygen. Multiple forms of PPO have been isolated from a wide variety of sources, including from tea leaf [1] from the pulp of banana (Musa sapientum L.)[2]From Royal Ann cherries. [3]. From leaf and fruit endosperm of coffee [4], From Raspberry fruits [5], From tobacco (Nicotianatobacum) [6].From aerial roots of a tropical orchid,Aranda `Christine 130[13] Grapes[14]. From Tea [15].Peppermint leaves (Menthapiperita). [10]Indian Gooseberry is a great herbal plant. It is being used as medicine for thousand years. The purpose of the study is to isolate and characterize of polyphenol oxidase from fruits of Phyllanthusemblica (Indian gooseberry) the results of this study would provide an understanding of the browning of the goose berry fruits and means of prolonging the shelf life.

MATERIALS AND METHODS

A. Plant material:

Phyllanthusemblica fruits (goose berries) were harvested by hand after climbing to upper branches bearing the fruits. Fresh fruits collected in Hassan city.

B. Enzyme Extraction and Partial Purification:

Ten grams of fresh berries were homogenized by grinding 50ml medium containing 0.1M phosphate buffer (pH 7), 1% (W/V) Triton-X-100 and 0.1M NaCl. The crude extract samples were centrifuged at 30,000 g for 20 min at 4ºC. Followed by Solid ammonium sulphate precipitation and dialysis (Dialysis membrame-70 LA393 from HIMEDIA), extract was used as the Gooseberry PPO enzyme source.

C. Determination of Goose Berry PPO Activity:

Goose Berry–PPO activity was determined by measuring the absorbance at 420 nm using a spectrophotometer (Pharmatech, Model ELiCO SL 150 UV-VIS- Spectrophotometry).The activity was assayed in 3 mL of reaction mixture0.2mL enzyme, 0.5mL substrate (Catechol RM6782 from HIMEDIA) “one unit of the polyphenol oxidase is defined as the enzyme which transfers 1umol Catechol to quinine per minute under defined conditions”.

D. Substrate Concentration and Specificity of Goose berry PPO:

TheGoose berry –PPO activity was determined using two different substrates namely Catechol and Tyrosine. The highest enzyme activity was obtained with 10m Mof Catechol. Therefore the concentration10m M Catechol was used as the substrate in all other experiments

E. Protein Estimation and Determination of Molecular Weight:

Protein content was estimated by the Lowry method **[17]**

F. Enzyme Kinetics:

Michaelis constant (Km) and maximum velocity (V_{max}) values of the enzyme were calculated from Lineweaver– Burk graphs.

G. Effect of pH on Goose berry–PPO Activity:

The effect of pH on Goose berry–PPO activity was determined under standard laboratory containing 3ml reaction mixture(0.2ml enzyme, 0.5ml Catechol. 2.3ml phosphate buffer of pH 4 to pH 8.)

H. Effect of Temperature on PPO Activity:

To determine the optimum temperature for Goose berry–PPO, the activity, the enzyme was measured at different temperatures (30-80°C) using 3ml reaction mixture (0.2ml enzyme, 0.5ml Catechol,2.3ml phosphate buffer**.)**

I. Effect of Inhibitors and Metallic Ions on PPO Activity:

The effects of seven metal ions ($CaCl_2$, $CuSo_4$, $MgSo_4$, KCl, $ZnSo_4$, $BaCl_2$, $NaCl_2$,), EDTA and on SDS were evaluated on Goose berry–PPO activity, using 3ml reaction mixture (0.2ml enzyme, 0.5ml catechol, 2.3ml phosphate buffer. The change in absorbance was measured spectrophotometrically at 420 nm.

RESULTS AND DISCUSSION

J. Extraction and purification of PPO:

The total protein concentrations of the dialyzed extract estimated using Lowry method [17] was 70

µg(0.2ml of enzyme) and 3.5g /100 g fresh gooseberries.

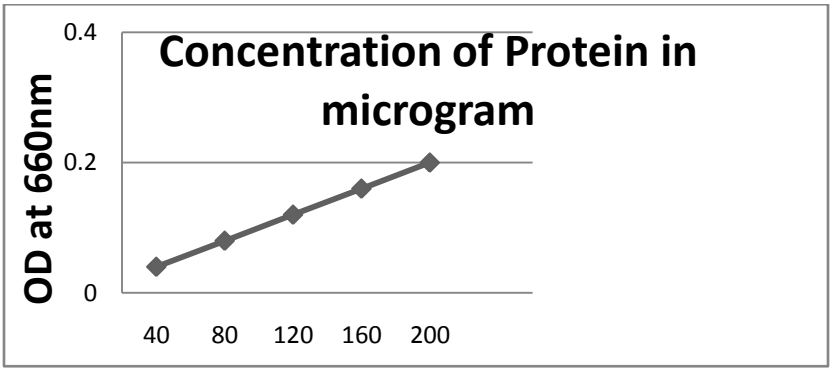

Figure 1: Concentration of Protein (µg)

K.Molecular Weight Estimation:

The analysis of SDS-PAGE gel revealed single band in purified extracts as shown in Fig-2, Corresponding to a molecular weight of 100 K Da. The molecular weight of PPO from other species has been reported as follows: Reported (Dolichoslablab) seeds PPO 120±3 kDa[7],OcimumbasilicumL.[9] 54kDa,Chinese cabbage~65kDa[11].Tea leaf 72KD[1],Rape flower 60.4kDa [8],Black pepper 60KD[12], Mango peel(Mangiferaindica) 136 KD[16]. Our results indicate that the molecular weight of Goose berry-PPO is lesser than molecular weight Mango peel (Mangiferaindica) and (Dolichoslablab) seedsbut more than those of OcimumbasilicumL., Chinese cabbage, Rape flower, Black pepper.

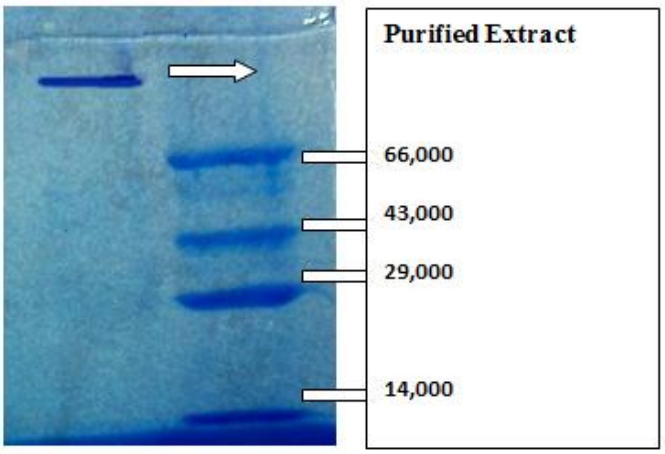

Figure 2: Electrophoretogram of PPO from extracts of fruits of goose berry. Lane; lane 1 purified extract; 2 protein marker (66,000.43,000.29,000.14,000 supplied by GENEI Bangalore)

L. Enzyme Kinetics and Substrate Specificity:

Michaelis constants (Km) and maximum reaction velocities (Vmax) and specificity (Vmax/Km) of the Gooseberry PPO was determined at optimum pH 7.0 and 40°C using Catechol km =60mM and Vmax= 0.003mM/sec (Line weaver Burk Plot)

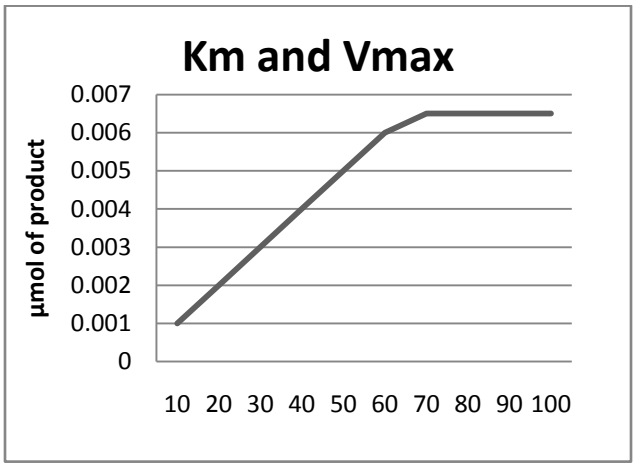

Figure 3: Substrate concentration (mMol)

M.Optimum pH:

The optimum pH exerts a strong effect on enzymatic activity; it was 7 for goose berry PPO

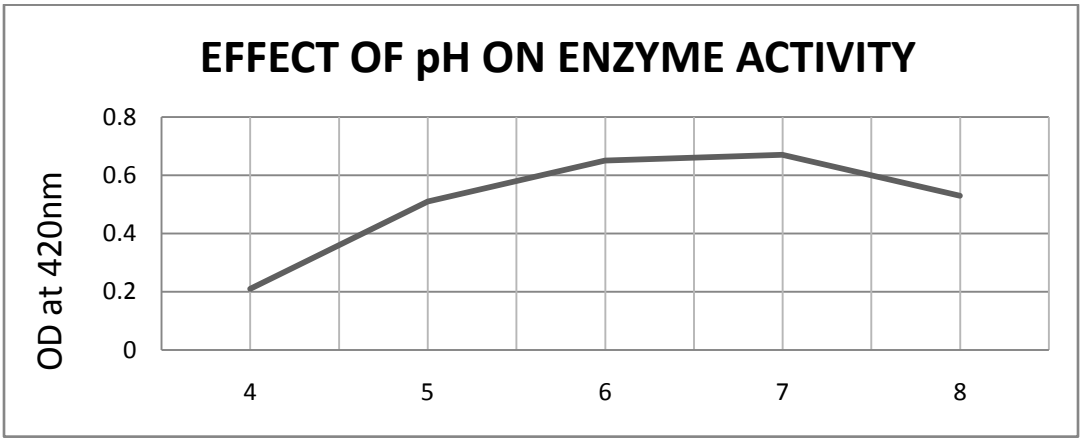

Figure 4:pH

N. Effect of Temperature:

Effects of temperatures were assayed using Catechol as a substrate. Over a temperature range of 30-80 °C at the optimum pH.

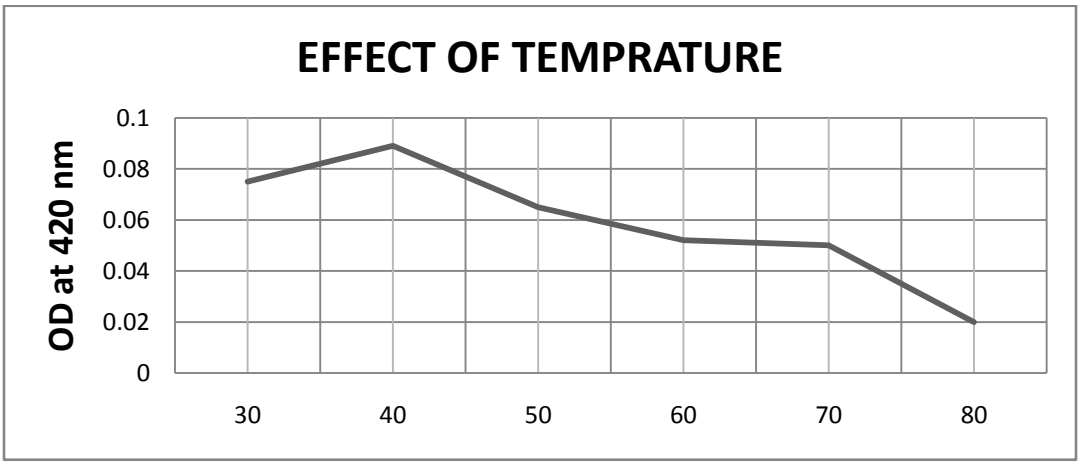

Figure 5: Temperature (°C)

O. Effect of Inhibitors and Metallic compounds:

We studied and evaluated effects of seven metal ions ($CaCl_2$, $CuSo_4$, $MgSo_4$, KCl, $ZnSo_4$, $BaCl_2$, $NaCl_2$,) SDS and EDTA. The results indicates that Gooseberry PPO is a copper containing enzyme, copper sulphate and zinc sulphate (10mM)serves as an activator for its activity. SDS and EDTA (10mM) showed inhibitory effects on the activity of PPO.

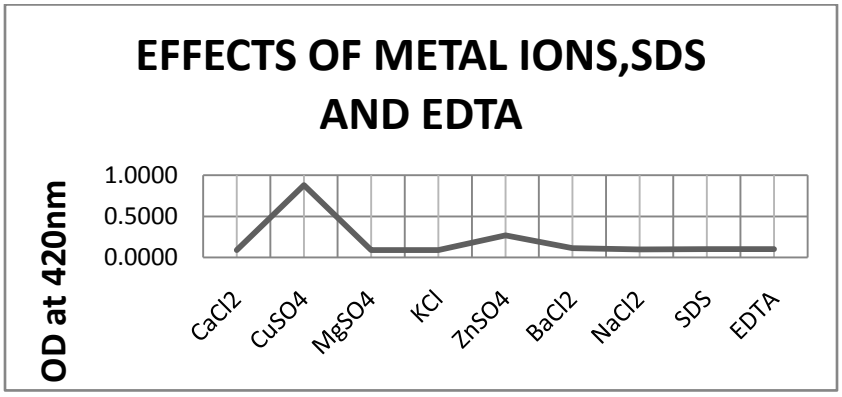

Figure 6: Metal ions, SDS and EDTA

CONCLUSION

This is the first report on the studies of PPO from the extract of Gooseberry, in particular Phyllanthusemblica. Ammonium sulphate precipitated fraction of the aqueous extract of Phyllanthusemblica berries and dialyzed fractions demonstrated polyphenol oxidase activity and its characteristic physicochemical properties. This study is helpful to understand browning properties of Gooseberries

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