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Detection and Comparative Analysis of Amylase Activity from Leaves, Seeds and Stem of Purslane (*Portulacaoleracea*)

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ABSTRACT

Purslane (Portulacaoleracea) is annual succulent plant and is good source of biologically active compounds. However biochemical characterization of hydrolytic enzymes such as amylases not fully explored. As initial step to this goal here we report detection of amylase activity from crude protein extracts of leaves, seeds and stems of purslaneby starch agar well diffusion assay. The total protein content was estimated from all three crude extracts and highest content was reported in leaves (LP) 0.53 ± 0.04 mg/ml. The crude extracts of seeds (SDP) and stem (STP) were reported to have 0.16 ± 0.02 mg/ml 0.10 mg/ml proteins respectively. Comparatively higher amylase activity was found in LP with 15 mm diameter starch clearance ring on starch agar plate. The SDP and STP showed 13 mm diameter starch clearance ring each on starch agar plate. On solution assay, LP showed substantial amylase activity i.e.1.4 mg maltose released/min/ml. The SDP and STP showed comparatively lower amylase activity.

Keywords**: **Purslane, amylase, starch agar plate, amylase activity

I. INTRODUCTION

The global life is relies on series of chemical reactions. Most of the chemical reactions proceed too slowly on their own to sustain life. The acceleration of reaction depends on catalysts. Enzymes are the biological catalysts which are key component of biological reactions [1]. Amylases are hydrolytic enzymes responsible breakage of internal 1, 4 glycosidic bonds in starch molecules. These enzymes are known to present in almost all life forms including plants microorganisms and animals [2]. The hydrolytic products mostly glucose and maltose are utilized as energy source. Amylase classified according to mode of action on glycosidic bond such as α - amylase, β - amylase and γ - amylase [1]. Amylases from different sources are used in the production of high-fructose corn syrup, as well as in alcohol Production and brewing industries. Agriculturally, amylase has been used to develop a more digestible feed for animals [2]. Amylases isolated from microorganism like fungi are used in bread making industry. The first commercially produced amylase was fungal origin which was used as a therapeutic aid to cure digestive disorder [3]. Amylases are important commercial enzymes accounting 25% of the enzyme production and are utilized for a variety of applications in food, non-food industries and clinical diagnostics worldwide [4, 5].

Amylase act on starch which is the most abundant polysaccharide on the earth. It is synthesized by plants by the process of photosynthesis and utilized in food, textile, paper, alcohol, pharmaceutical industries [4]. It is also

important source of energy for human being. Starch is deposited in plant cells in the form of granules as reserve material. Generally high level of expression of amylase is seen in germinating seeds of plants under the influence of gibberellin hormone [6]. In these tissues the content of starch found to be high and metabolized for generation of energy required for growing tissues in the process of germination. The detection, biochemical characterization and identification of amylases from various plant species is reported till date [3].

Purslane (*Portulacaoleracea*) is annual succulent plant native to many regions of world including Asia [7]. Purslaneis milky juice carrying vegetable. Purslane is adapted to poor soil and minimum water requirement crop during germination and development [8]. This plant is rich source of ω-3 fatty acid important for heart related disease [9, 10]. It possesses various biological activities including antidiabetic, antioxidant, anti-atherogenic, anti hyperlipidemic, hepatoprotective, nephro-protective and anti-Arthritic [7].The biochemically detection and characterization of physiologically and industrially important hydrolytic enzymes such as amylase from this plant species is not fully explored and is the main aim of the current investigation.

II. MATERIALS AND METHODS

2.1. Procurement of purslane sample and chemicals

The sample of purslane plant is collected from the local region of Aurangabad (MS) India. Electrophoresis system was purchased from Genei, India. Ammonium sulfate, acrylamide, bisacrylamide, tetramethylethylenediamine (TEMED), ammonium persulfate, hexane, and polyvinylpyrrolidone (PVP) were purchased from Sisco Research Laboratories (SRL), Mumbai, India. All other chemicals used in this study were of the available highest purity.

2.2. Extraction of crude amylase

The fresh purslane plant was washed with tap water. The leaves, shoots and seeds were homogenized into paste separately in distilled water containing 1 % polyvinylpyrrolidone (PVP) (ratio of sample to distilled water was 1:10 w/v) and proteins were overnight extracted at 10°C. The suspension was then centrifuged at 12,000g for 20 min at 4°C and the supernatant (crude extract) was collected. Solvent precipitation of protein was done by chilled acetone (4°C) in the ratio of 1:4, v/v supernatant to acetone. The suspension was centrifuged at 12,000g for 20 min at 4°C and precipitated proteins were collected and dissolved in minimum volume of 0.1M Tris-HCL, pH 8.0 and used as crude amylase source.

2.3. Protein quantification

Total protein content of each solvent precipitated sample (leaves, shoots and seeds) was estimated by the Folin phenol reagent using bovine serum albumin (BSA) as standard [11].

2.4. Qualitative estimation of amylase activity

Each solvent precipitated sample (leaves, shoots and seeds)was checked for amylase activity by starch agar plate method. In this method agar plate containing 1% starch was used. The combinations of crude amylase and buffer (0.1M Tris-HCL, pH 8.0) poured in the wells of the agar plate, incubated at 37°C for overnight. Plate was stained with Lugol's solution (0.1 % iodine and 1% potassium iodide) and zone of starch clearance (α-amylase activity, white zone against blue background) was observed visually.

2.5. Quantitative estimation of amylase activity

The amylase activity was determined according to method of Padul et al. [12]. The activity was determined by measuring the formation of reducing sugars when solvent precipitated samples (leaves, shoots and seeds)were incubated with starch. The standard reaction mixture was contained 1.5 ml 0.1M Tris-HCL, pH 8.0, 1 ml 1 % starch and 0.5 ml enzyme. After incubation at 37°C for 10 min, the released reducing sugars were estimated using DNSA (1% 3, 5-Dinitrosalisylic acid, 30% Sodium potassium tartarate, 0.2M NaOH) reagent. The tubes were kept in a boiling water bath for 5 min, cooled under tap water and the color of reducing sugars liberated was measured at 540 nm.

Statistical analysis

All experiments were conducted and analyzed in triplicate. Means and standard deviations were calculated and compared. Analysis was performed using Microsoft Excel.

III. RESULTS AND DISCUSSION

The clear supernatant obtained after homogenization of leaves, seeds and stem of purslane in distilled water containing 1% PVP and solvent precipitation by chilled acetone was screened for detection of amylase activity. Amylase is hydrolytic enzymes responsible endogenous digestion of starch in plants during various physiological events including germination [6]. Initially the total protein content from each solvent extracted sample was evaluated by method of Lowery et al. [11].

Table 1: Protein contents of leaves, seeds and stem of purslane (*Portulacaoleracea*). The results are presented as the means ± standard deviation (SD), n= 3

Plant tissue	Conc. of proteins (mg/ml)
Leaves (LP)	0.53 ± 0.04
Seeds (SDP)	0.16 ± 0.02
Stem (STP)	0.10 ± 0.01

Table no. 1 represents the protein contents of parts of purslane under investigation. The highest protein content was observed in leaves (0.53 ± 0.04 mg/ml) whereas comparable amount of protein was found in seeds and stems also.

Amylase activity detection was done on starch agar plate; it was found that starch agar plate showed strong amylase activity in all tested samples with highest activity in leaves (LP, 15 mm ring diameter) as equivalent to human salivary amylase (HSA) with 17 mm ring diameter (Figure 1, Table 2). The moderate activities were present in SDP and STP with 13 mm ring diameter for each.

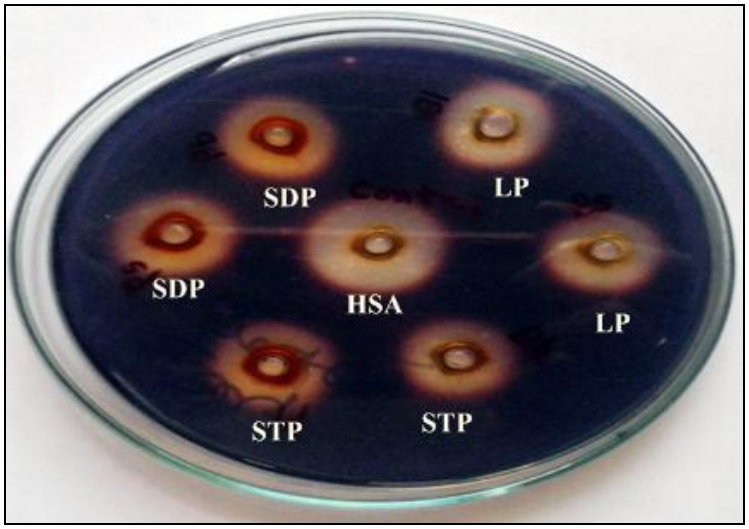

Figure 1. Detection of amylase activity in crude proteins (40 µl) purslane (*Portulacaoleracea*) by starch agar plate method. LP: Leaves of purslane; SDP: Seeds of purslane; STP: Stem of purslane; HSA: Human salivary amylase (1:10 v/v diluted).

The sample was applied to the wells in agar plate and the diameter of the resulting ring (starch clearance)around the sample well was compared to the diameters of the rings around the wells containing HSA.

Table 2: Starch agar plate data of LP, SDP, STP and HSA

Sample	Starch clearance ring diameter in mm
HSA	17
LP	15
SDP	13
STP	13

Amylase activity of LP, SDP, and STP assayed using the 3, 5 dinitrosalicylic acid (DNS) method and activity was compared with HSA (Figure 2). HSA represents highest activity (2.4 mg maltose released/min/ml) as compared to the all the samples of purslane. However substantial activity was observed in LP (1.4 mg maltose released/min/ml). This result suggests the purslane leaves could be a promising source of amylase and need further exploration.

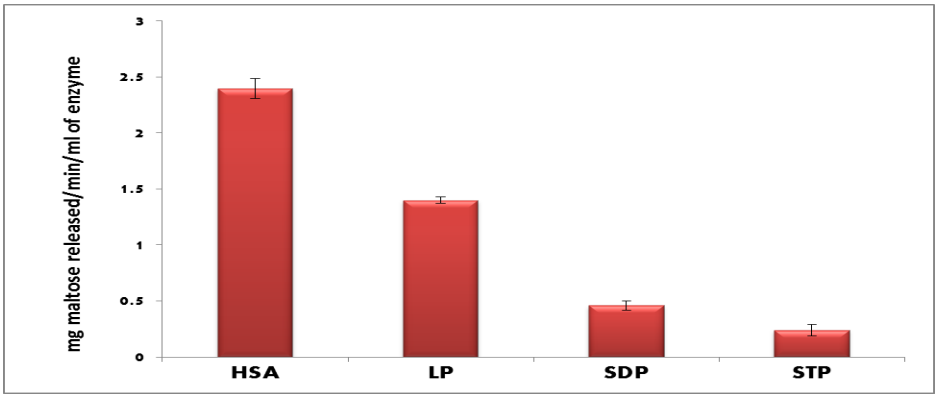

Figure 2: Amylase activity of LP, SDP, STP and HSA. LP: Leaves of purslane; SDP: Seeds of purslane; STP: Stem of purslane; HSA: Human salivary amylase (1:10 v/v diluted).The results are presented as the means ± standard deviation (SD), n= 3.

Purslane (*Portulacaoleracea*) is used both as a vegetable and as an herb for medical and therapeutic purposes. It is also explored for presence of many biologically active compounds which include flavonoids, alkaloids,Coumarins, anthraquinone glycoside and cardiac glycoside [13, 14]. The juice of leaves and other parts of this plant is known for its medicinal values [15]. Because of the limited information about the biochemical content with respective to characterization of enzymes, this study reports the substantial amylase activity from crude extracts of leaves, seeds and stem of purslane.

CONCLUSIONS

Thesubstantial amylase activity reported from crude extracts of leaves, seeds and stem of purslane in this investigation could be further characterized to gain the knowledge of nature and physiological importance of these molecules. The activity could be also explored for industrial and therapeutical application.

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