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Isolation, partial purification, product formation and characterization of β- glucosidase from spikes of *Hordeum vulgare*

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Abstract
β-glucosidase (EC 3.2.1.21) was extracted from spikes of *Hordeum vulgare* and was purified using ammonium sulphate fractional precipitation and Sephadex G-25 chromatography. The molecular weight of enzyme was found in the range of 13-85KDa .The enzyme β-glucosidase has optimum pH 5.0 and the optimum temperature was found at 60ºC. Bioethanol was produced from spikes of *Hordeum vulgare.*

Keywords: *Hordeum vulgare*, β-Glucosidase, SDS PAGE, Enzyme activity, etc.

Introduction
In plants, β-glucosidase activity is involved in processes such as the compartmentalization and activity of phytohormones, defense mechanisms against microbes, insects, or parasitic activity (Bell, 1981) [1]. Floral development and pigmentation (Harborne and Mabry, 1982) [10] and they are also thought to have roles in lignification and cell wall decomposition. Beta-glucosidases are able to cleave the beta-glucosidic linkages in di- and oligo glucosaccharides and several other glycoconjugates. These enzymes are widely distributed and have important roles in many biological processes. In cellulytic microorganisms beta-glucosidase is involved in cellulose induction and hydrolysis (Bisara and Mishra, 1989; Tomme *et al.,* 1995) [3, 16]. In plants the enzyme is involved in beta-glucan synthesis during cell wall development, pigment metabolism, fruit ripening and defence mechanism (Easen, 1993) [7]. These enzymes can be classified on the basis of substrate activity and nucleotide sequence identity (Henrissat and Davies, 1997; Bhatia *et al.,* 2002) [11, 2]. Based on their substrate specificity, they are divided into three classes; class I which contain aryl β–glucosidases, class II contains true cellobiases and class III contains β–glucosidases having broad substrate specificity. Most of the β–glucosidases belongs to class III which can cleave β 1, 4; β 1, 6; β 1, 2 and α 1, 3; α 1, 4; α 1, 6 glycosidic bonds (Riou *et al.,* 1998; Bhatia *et al.,* 2002) [15, 2]. In the present study the enzyme was extracted from *Hordeum vulgare* spikes.

Materials and Methods
Collection of root sample
The spikes of *Hordeum vulgare* was collected for the isolation of β- glycosidase enzyme. The roots were ground to fine powder in a chilled mortar and pestle using liquid nitrogen and extraction buffer (1 ml buffer/1g tissue) which was mixed with it. After that the extract was centrifuged at 12000 rpm 4ºC/30 minutes. Then the supernatant was ultra-filtered to obtain crude enzyme solution.

Precipitation of Enzyme
Precipitation by ammonium sulphate:
The crude extract was precipitated by adding ammonium sulfate at different saturation levels (30%, 50% and 70%) and kept overnight in refrigerator. Then centrifugation was done at 12000 rpm for 10 min at 4ºC. Thereafter the pellet was collected and dissolved in minimum volume of citrate buffer for enzyme activity determination.

Determination of β –glucosidase activity: The β-glucosidase activity was determined against p-nitrophenyl β–D glucopyranoside (p-NPG) as its substrate in citrate buffer at room temperature and the activity was estimated using double beam spectrophotometer at wavelength 405 nm.

Partial purification and SDS-PAGE
The isolated enzyme was partially purified by gel filtration chromatography viz., sephadex G-25 and the molecular weight was determined through SDS-PAGE technique.

Characterization of purified β-glucosidase
The effect of different pH (3,4,5,6,7,8 and 9), temperatures ((20ºC, 30 ºC, 40ºC, 50 ºC, 60ºC, 70ºC and 80ºC) and varying concentration of substrate (p-nitro phenyl β-D-glucopyranoside in the range of 0.5- 3.0mM) was studied on the activity of β-glucosidase enzyme.

Bioethanol production from *Hordeum vulgare spikes*
Spikes of *Hordeum vulgare* were used for the bioethanol production. The bioethanol produced was poured into a Petri dish and lighten with matchstick. After that its ethanol content was compared to the lab grade ethanol.

Results and Discussion
Isolation and activity determination of β -glucosidase
The enzyme was isolated from the spikes of *Hordeum vulgare,* precipitated by ammonium sulphate to get the partially purified extract of enzyme (without cell debries). After precipitation the enzymes activity was determined using p-nitrophenyl β-Dglucopyranoside as substrate at 405nm which was found to be 0.007u/ml. The purity of β -glucosidase was studied by purification table and found to be 24.8% as shown in Table 1.

Table 1: Fold Purification table of β-glucosidase via. Barley spikes

Volume (cm^3)	Concentration	Total protein	Activity	Total activity	Specific activity	Purification fold	Overall yield
$15cm^3$	10.45	156.75	0.036	1.005	0.0064	1.00	100%
$7cm^3$	10.05	70.35	0.040	0.469	0.0066	1.03	46.6%
$4cm^3$	9.50	38.0	0.044	0.25	0.0066	1.03	24.8%

Characterization of purified β- glucosidase activity
Effect of pH
The β- glucosidase extracted from *Hordeum vulgare* spikes was optimized at different pH (3, 4, 5, 6, 7, 8 and 9) range using sodium hydrogen phosphate-citrate buffer. The maximum activity was observed at pH 5 (0.389U/ml±0.02) (Figure 1). The optimum pH for catalyzing β- glucosidase action of different plants ranged as strawberry 4.0 (Bothast and Saha, 1997) [4]. *Lodgepole Pine* - 5 to 6 in variety of substrate (Dharmawardhana *et al.,* 1995) [6].

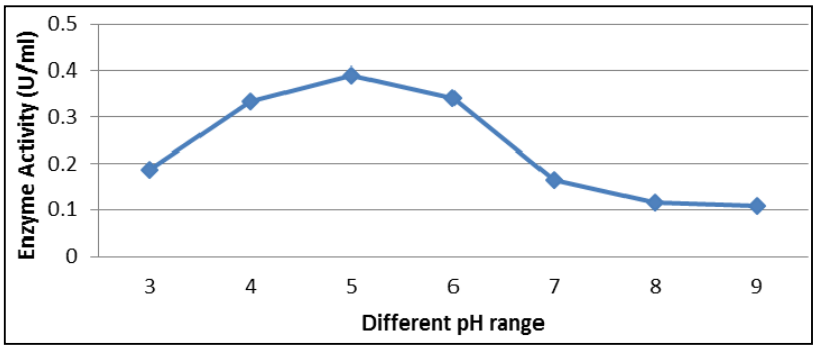

Fig 1: Effect of pH on β- glucosidase activity from Hordeum vulgare (a) Maximum activity was observed at pH 5 (b) Minimum activity was observed at pH 9

Effect of temperature
The β-glucosidase extracted from *Hordeum vulgare* spikes was optimized at different temperature ((20ºC, 30 ºC, 40ºC, 50 ºC, 60ºC, 70ºC and 80ºC) range using sodium hydrogen phosphate-citrate buffer. The maximum activity was observed at 60ºC (1.058U/ml±0.17) (Figure 2).The optimum temperatures in different plants were as Strawberry 60ºC (Poulton and Li, 1994) [14]., *Leuconostoc mesenteroides* 50ºC (Cicek and Esen, 1999) [5].

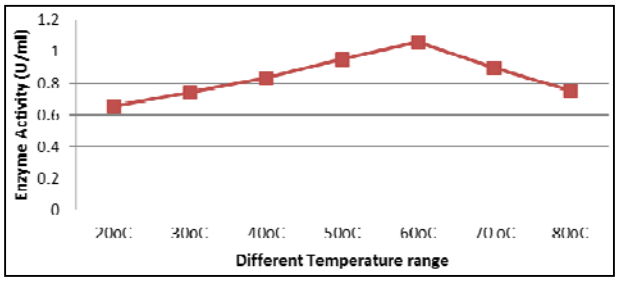

Fig 2: Effect of temperature on β- glucosidase activity from Hordeum vulgare spikes (a) Maximum activity was observed at 60ºC (b) Minimum activity was observed at 20ºC

Effect of substrate concentration
The effect of different concentration of substrate was studied on activity of β-glucosidase from *Hordeum vulgare* spikes. The maximum activity was observed at 1.5mM (Figure 4).The effect of mono/disaccharides (1 mg/ml) on β–glucosidase activity was studied using pNPG as a substrate (Kaur *et al.,* 2007) [12].

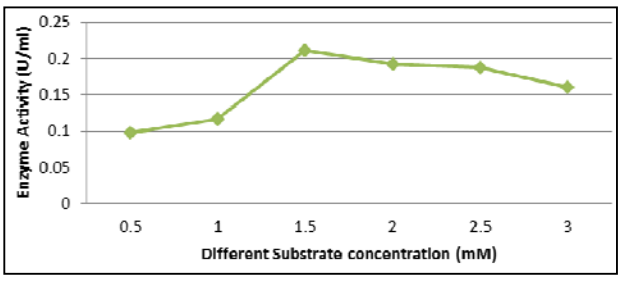

Fig 3: Effect of substrate concentration on β- glucosidase activity from Hordeum vulgare spikes (a) Maximum activity was observed in 1.5mM (b) Minimum activity was observed in 0.5mM

Molecular weight determination of enzyme
The molecular weight of enzyme isolated from *Hordeum*

vulgare spikes was determined by SDS-PAGE using specific markers of known molecular weight. The molecular weight of β-glucosidase from *Hordeum vulgare* spikes was found to be 13-85kDa (Table 2) (Figure 4). The molecular weight of different bands in *Rauvolfia serpentine* was found in the range of 19.48 KDa to 92.257 KDa (Verma *et al.,* 2011) [17].

Table 2: Molecular weight from SDS (Coomassie staining) gel of *Hordeum vulgare spikes* β- glucosidase

S. No.	M.W. of marker (kDa)	Distance of marker (cm)	Distance of protein band (cm)	M.W. of protein band (kDa)
1.	100kd	0.5cm	1.6cm	85kd
2.	75kd	1.1cm	2.5cm	69kd
3.	65kd	1.7cm	3.0cm	55kd
4.	50kd	2.4cm	4.9cm	48kd
5.	45kd	3.0cm	5.5cm	23kd
6.	25kd	4.4cm	5.8cm	15kd
7.	15kd	5.4cm	6.2cm	13kd

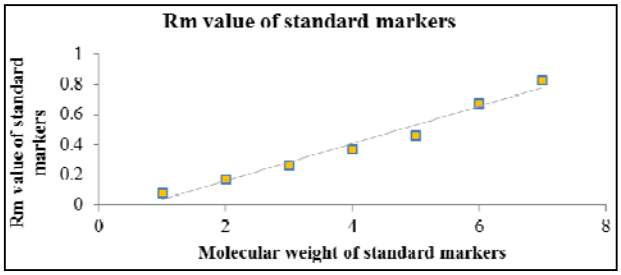

Fig 4: Determining the molecular weight of protein by SDS-PAGE

Bioethanol production from *Hordeum vulgare* spikes

Substrate screened for bioethanol production was *Hordeum vulgare* spikes. At first malt was prepared and then mash was prepared. Malt and mash were mixed in Erlenmeyer flask and inoculated with inoculum *S. cerevisiae* and were kept for fermentation in anaerobic condition for 15 days at room temperature. After fermentation distillation of fermented product was done with distillation apparatus. By comparing the alcohol content of bioethanol produced from the spikes of *Hordeum vulgare* was found to be lower than absolute alcohol.

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