Annals of Plant Sciences 7.4 (2018) pp. 2126-2133

ANNALS OF PLANT SCIENCES
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Research Article

# Partial purification and characterisation of cytosolic Fructose-1, 6-bisphosphatase from *Drymaria cordata*

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**Received:** 2018-03-07; **Accepted:** 2018-03-21

**Abstract**: *Drymaria cordata* is an important ethnomedicinal plant from which many important secondary metabolites have been reported. Partial purification of the enzyme, fructose 1,6-bisphosphatase was carried out following the methods of homogenization, streptomycin sulphate precipitation, ammonium sulphate cut and molecular sieve chromatography through Bio-Gel A-0.5m column. Biochemical characterization experiments were performed by standard methods with the enzyme preparation as purified from the column. Cytosolic fructose 1,6-bisphosphatase from the leaves of *Drymaria cordata* was purified to about 27-fold with 77% of recovery over homogenate fraction. The enzyme was highly specific to D-fructose-1,6-bisphosphate. With increase of protein concentration upto 300µg and incubation time upto120 minutes, the enzyme activity increased linearly. The metal ions $Mg^{2+}$ or $Mn^{2+}$ strongly stimulated the enzyme activity on the other hand $Li^{+}$, $Hg^{2+}$ and $Zn^{2+}$ were potent inhibitors. The *D. cordata* enzyme showed temperature maxima at 40°C while the optimum pH was at 8.0. The $K_m$ value of the enzyme for its substrate, Fructose 1,6-bisphosphate was 1.11µM proving its strong affinity.

**Key words:** Fructose 1,6-bisphosphatase, Fructose 1,6-bisphosphate, *Drymaria cordata,* protein purification, enzyme characterization.

## Introduction

The enzyme Frutose 1,6 bisphophatase (FBPase; EC: 3.1.3.11) is related to gluconeogenesis and photosynthesis. It catalyzes the hydrolytic cleavage of fructose-1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate in the presence of divalent cations such as magnesium, manganese or zinc (Dzugaj, 2006). The FBPase reaction is a key regulatory step in gluconeogenesis and displays high negative free energy change (Bassham and Krause 1969) and permits endogenous glucose production from amino acids, glycerol or lactate. This enzyme is ubiquitous in photosynthetic organisms such as in bacteria (Gibson *et al.,* 1990), blue- green algae (Crawford *et al.,* 1984) and higher plants (Weeden and Buchanan, 1983).

In higher plants two FBPase isoforms have been identified: one being the cytosolic FBPase which participates in the synthesis of sucrose (Kelly *et al.,* 1982) and the second enzyme is confined to the chloroplast which is essential in the photosynthethic carbon fixation into sugars (Chueca *et al.,* 2002). The regulatory property of the chloroplast enzyme is distinctly different from the cytosolic or gluconeogenic FBPase (Lazoro *et al.,* 1975).

The purification and characterization of Fructose-1, 6-bisphosphatase and its activity has been reported from plants as well as microbes. FBPase has been partially purified over 100 fold from blue green bacteria *Anacystis nidulans* (Udvardy 1982). The activity of FBPase was also detected from fermenting and non-fermenting yeast grown in a sugar medium (Gancedo and Gancedo, 1971).

Cytosolic FBPase has been purified from spinach leaves where the enzyme is found to be a tetramer with a molecular weight of 130kDa, paving a pathway for sucrose biosynthesis (Zimmermann *et al.,* 1978). The cytosolic enzyme was purified and the regulatory propertiy of the same was examined from the mesophyll of leaves of *Zea mays* (Stitt and Heldt 1984). Cytosolic FBPase has also been purified from sugarbeet (*Beta vulgaris* L.) leaves (Khayat *et al.,* 1993), endosperm of germinating castor oil seeds (Richard *et al.,* 1995). Furthermore, the enzyme has also been purified and characterized from a recombinant pea plant (Jang *et al.,* 2003), apple (*Malus dometica*) leaves (Zhou and Cheng, 2004) and the leaves of *Ginkgo biloba* (Yonzone *et al.,* 2015).

*Drymaria cordata* (L.) Willd ex Roem. & Schult. (*D. cordata*), locally known as "Abhijalo" in Sikkim is an annual herbaceous plant belonging to family Caryophyllaceae. This plant is utilized by the local people as one of the ingredients in many indigenous poly herbal formulations (Tejavathi and Indira 2012). This plant has been used traditionally as an appetizer, depurative, emollient, febrifuge, laxative and stimulant (Saklani and Jain 1994). A number of biologically active compounds have been isolated from the leaves of the plant including cyclopeptides, flavonoid glucosides (Ding *et al.,* 1999, 2000),

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norditerpenes and norditerpene glycosides (Vargas *et al.,* 1988). An alkaloid, drymaritin which exhibits anti-HIV properties against H9 lymphocytes has been isolated from *Drymaria cordata* (Hsieh *et al.,* 2004). The present work is an attempt to investigate the FBPase enzymatic activity and its characterization from *Drymaria cordata,* since no work has so far been done on this aspect despite it being such a useful plant.

## Materials and Methods

### Plant Material

Mature leaves of *D. cordata*) were collected from fresh from around Gangtok, Sikkim (27.33˚N 88.62˚E) between 6.00 and 7.00 AM during January-February and brought to the lab for analysis. The plant was identified by the Botanical Survey of India, Eastern Regional Circle, Gangtok, India and the herbarium was deposited at the same organization. All the analytical work was carried out at between 0˚C to 4˚C.

### Partial Purification of gluconeogenic Fructose-1, 6-bisphosphatase from *D. cordata*

The separated leaves of *D. cordata* was washed several times with cold distilled water followed by chilled 50mM Tris-HCl (pH-7.0) buffer containing 0.2 mM 2-Mercaptoethanol (ME) and the partial purification of fructose-1,6-biphosphatase was done following the method outlined below:

### Homogenate

50gms of buffer washed leaf tissues obtained from *D. cordata* was homogenized with 3-volumes of 50mM Tris-HCl buffer (pH 7.0) containing 0.2 mM ME. This crude homogenate was centrifuged at 1000 RPM for 2 minutes and the supernatant was collected which was designated as the 'homogenate fraction' and the total volume was recorded.

### Low Speed Supernatant

The homogenate fraction was centrifuged at 10,000 RPM for 20 minutes in a Hermle - Z 32 HK centrifuge. The pellet was discarded and the supernatant fraction was recovered from the centrifuge tube which was marked as 'low speed supernatant.'

### Streptomycin sulphate precipitation

In order to remove contaminating nucleic acids in the form of precipitate, streptomycin sulphate powder was added gently to the low speed supernatant with constant stirring (by using a Remi magnetic stirrer) to a final concentration of 1% (w/v). After proper mixing, it was kept in ice-bucket for 20 minutes followed by a spin at 10,000 RPM for 15 minutes. The pellet was discarded and the supernatant fraction collected. This fraction was named as 'SS-fraction.'

### Ammonium sulphate fractionation

The streptomycin sulphate treated fraction obtained from the previous step was made 0-30% saturated with ammonium sulphate [$(NH_4)_2SO_4$] by adding requisite quantity (17.6g per 100ml) solid ammonium sulphate slowly with constant stirring. The mixture was kept at 0ºC for 15 minutes and centrifuged at 10,000 RPM for 20 minutes. The pellet was discarded and the supernatant fraction was made 30-70% saturated with [$(NH_4)_2SO_4$] by adding requisite quantity (27.3g per 100 ml) salt slowly with constant stirring. The crude mixture was kept at 0ºC for 15 minutes and then centrifuged at 10,000 RPM for 20 minutes. The pellet was collected which was dissolved in a minimal volume of 50 mM Tris- HCl buffer (pH 7.0) having 0.2 mM ME and dialyzed overnight against the same buffer (500 volumes). On completion of dialysis, the 30-70% [$(NH_4)_2SO_4$] fraction was recovered from the dialysis bag. This purified extract was designated as '$A_2S$-fraction.'

### Chromatography through BioGel A-0.5m column

The dialyzed $A_2S$-fraction obtained from the previous step was loaded onto a column of BioGel A-0.5m (0.6 x 8.0 cm) pre-equilibrated with 50 mM Tris-HCl buffer (pH 7.0). Proteins were eluted from the column with the same buffer in fractions of 2.0 ml at a flow rate of 10 minutes per tube. 20 such fractions were collected. Fractions containing FBPase activity were pooled together and dialyzed against 500 volume of 50 mM tris-HCl buffer (pH 7.0) containing 0.2 mm ME. This preparation (BioGel-fraction), was concentrated and used as the enzyme source for the experiments on characterization.

### Enzyme activity assay

The FBPase activity was assayed by the procedure of Udvardy *et al.,* (1982) with slight modification. The enzyme assay mixture contained $H_2O$ (620µl), 500 mM tris-HCl buffer (pH 7.0) (200µl), 50mM $MgCl_2$ (100µl), 10mM Fructose 1,6-bisphosphate (FBP) and Fructose 6 phosphate (F-6-P) [20µl], 10mM EDTA (10µl) and an appropriate aliquots of enzyme protein (50 µl) making the total volume of 1.0 ml. After incubation for 60 minutes at 37˚C, the reaction was terminated by the addition of 200µl chilled 20% trichloroacetic acid (TCA). To the deprotinized supernatant, 2.8ml of distilled $H_2O$ and 3ml of Pi-reagent was added followed by a second incubation at 37˚C for 1hr for the oxidation of FBPase reaction product, D-fructose-6-phosphate, with a subsequent release of inorganic phosphate.

The activity of the enzyme was determined by estimating the product-specific release of inorganic phosphate from D-fructose-6-phosphate by FBPase reaction. The amount of inorganic phosphate

released from the FBPase reaction product was estimated by the method of Chen *et al.,* (1956) and the protein was determined according the method of Bradford (1976) with BSA as a standard. The specific activity was defined as μmol Pi released mg protein $hr^{-1}$. As 1 mole of fructose-6-phosphate contains 1 mole of inorganic phosphate, the total mole number of inorganic phosphate released was equal to the total mole number of fructose-6-phosphate produced.

## Results and Discussion

Although many ethnobotanical and pharmacological analyses have been previously reported from this plant species, no investigation has so far been made to check the activity of FBPase in this medicinal plant. The present work is an attempt to determine the occurrence of FBPase from *D. cordata* and to biochemically characterize the enzyme.

### Purification of Fructose-1,6-bisphosphatase from *D. cordata*

The enzyme, FBPase was isolated and purified from the mature leaves of *D. cordata.* The summary on the purification of FBPase is given in table 1. The protein resolved from BioGel A-0.5m column was taken for analysis. In the present study, an overall purification of the enzyme to about 27-fold with about 77% recovery based on total activity was achieved. This recovery is quantitatively similar to that of castor-bean seedlings purified with Sephadex G-200 (Youle and Huang, 1976).

**Table 1.** Summary of partial purification of cytosolic Fructose-1,6- bisphosphatase from *D. cordata* leaves

| Purification step | Protein Content (mg/ml) | Specific activity [μ mol F-6-P produced ($mg^{-1}$) protein $h^{-1}$] | Total activity [μ mol F-6-P produced ($mg^{-1}$) protein $h^{-1}$] | Recovery (%) | Purification (fold) |
|---|---|---|---|---|---|
| Homogenate | 1.4 ± 0.2 | 10.7 ± 0.6 | 2250 ±68.3 | 100 ± 2.64 | 1.0 ± 0.01 |
| 10K-supernatant | 1.3 ± 0.16 | 11.0 ± 0.8 | 2059 ±49.2 | 91.5 ± 2.0 | 1.03 ± 0.3 |
| SS-fraction | 1.2 ± 0.12 | 13.3 ± 0.9 | 2138 ±58.4 | 95.0 ± 1.9 | 1.2 ± 0.1 |
| $A_2S$-fraction | 1.1 ± 0.1 | 63.2 ± 0.5 | 695 ± 13.3 | 30.89±1.5 | 5.9 ± 0.2 |
| BioGel-fraction | 0.9 ± 0.02 | 297.4 ± 4.8 | 1739 ±39.6 | 77.2 ± 2.3 | 27.35 ± 0.3 |

(Values are Mean ± SE, n = 3)

### Characterization of the partially purified enzyme

The BioGel purified fraction of the enzyme was taken and the enzymatic characterization of cytosolic Fructose-1,6-bisphosphatase from *Drymaria cordata* was performed for the following parameters:

### Stability

An important property of the *D. cordata* FBPase is the low stability of its catalytic activity. Stability of *the enzyme* varied with the enzyme preparation at different stages of purification. The low speed supernatant fraction remained active for 12-14 days when stored at -20°C. The $(NH_4)_2SO_4$ purified fraction recovered from dialysis bag maintained only about 70% of its activity upto 3-4 days when stored at identical temperature. Addition of an enzyme stabilizer 2-mercaptoethanol (ME) clearly increased the stability of the enzyme by 4-5 days. Similarly, the same enzyme purified from *Selenastrum minutum* is only stable for 2-5 days (Botha and Turpin, 1990). The stability of cytosolic *D. cordata* FBPase was very short in contrast to the same enzyme from other higher plants. The cytosolic Enzyme purified from spinach leaves did not lose its activity upto 2 months of storage in 50% glycerol at -20°C (Herzog *et al.,* 1984). Similarly, at -20°C, the *Anacystis nidulans* FBPase could be stored, while maintaining its activity for several weeks (Udvardy *et al.,* 1982).

### Substrate specificity

The enzyme activity could be remarkably detected in the leaves of *D. cordata* and the cytosolic FBPase enzyme was highly specific for its substrate FBP. The partially purified *D. cordata* FBPase has been found to preferentially utilize FBP as substrate even in presence of other phosphorylated intermediates such as, Fructose-6-phosphate, D-glucose-6-phosphate (G-6-P) and D-glucose-1-phosphate (G-1-P) at identical concentrations (10mM). When the specific substrate of the enzyme, FBP was not added to the reaction mixture, enzymatic synthesis of F-6-P could not be detected. However, this enzyme FBPase from *D.a cordata* could slightly utilize D-fructose-6-phosphate with about 17.14% activity in comparison to the other phosphates as shown in table 2. This may be a unique feature of *D.cordata* FBPase.

**Table 2.** Effect of some substrate isomers on cytosolic Fructose-1,6- bisphosphatase activity from *D. cordata* leaves.

| Compound | Concentration (mM) | Specific activity [μ mol F-6-P produced ($mg^{-1}$) protein $h^{-1}$] | Percent activity |
|---|---|---|---|
| D-fructose-1,6-bisphosphate | 10 | 319.65 ±5.3 | 100.00 ± 2.0 |
| D-fructose-6-phosphate | 10 | 54.70 ±0.4 | 17.14 ± 0.8 |
| D-glucose-1-phosphate | 10 | 20.51 ±0.2 | 6.42 ± 0.2 |
| D-glucose-6-phosphate | 10 | 32.47 ±0.3 | 10.17 ± 0.6 |

(Values are Mean ± SE, n = 3)

**Requirement for *Drymaria cordata* FBPase activity**

*D. cordata* FBPase when assayed in presence of 500mM Tris-HCl buffer (pH 7.5), 50mM MgCl2, 10mM FBP, 10mM F-6-P, 10mM EDTA with an appropriate protein aliquot was called the complete set and this reaction mixture recorded the maximum activity. The absence of the specific substrate i.e., FBP resulted in strong inhibition of the *Drymaria cordata* FBPase activity. About 76.82% activity was lost when Tris-HCl buffer was omitted from the complete reaction mixture. Deduction of $MgCl_2$ and EDTA caused the reduction of enzyme activity by 34.4% and 47.41% respectively. Similarly the heat killed enzyme showed no such enzyme activity (Table 3). In contrast, the cytosolic FBPase from *Ginkgo biloba* leaves showed a reduction of enzyme activity by 58%, 68% and 50% by the deduction of tris buffer, $MgCl_2$ and EDTA respectively from the reaction mixture (Yonzone *et al.,* 2015).

**Progress of FBPase reaction with incubation time**

Incubation of *Drymaria cordata* FBPase was carried out for different time periods between the ranges of 0 min and 150 mins at an interval of 30 minutes under standard assay conditions (Table 4). It was observed that the reaction proceeds linearly upto 120 min with a slight increase in FBPase activity in each stage. However, *Ginkgo biloba* FBPase showed time linearity only upto 60 minutes under identical conditions Yonzone *et al.,* 2015).

**Table 3**. Effect of composition of incubation medium on cytosolic Fructose-1,6- bisphosphatase activity from *D. cordata* leaves

| Condition | Specific activity [µ mol F-6-P produced ($mg^{-1}$) protein $h^{-1}$] | Percent activity |
|---|---|---|
| Complete set | 302.25 ±5.2 | 100.00 ± 2.0 |
| Without substrate (FBP) | 0.5 ±0.00 | 0.00 |
| Without buffer (Tris-HCl) | 198.29 ±3.9 | 65.60 ± 1.7 |
| Without $MgCl_2$ | 70.08 ±0.5 | 23.18 ± 1.0 |
| Without EDTA | 158.97 ±3.6 | 52.59 ± 1.2 |
| Heat killed enzyme | 0.5 ±0.00 | 0.00 |

(Values are Mean ± SE, n = 3)

**Table 4.** Effect of different incubation time on cytosolic Fructose-1,6- bisphosphatase activity from *D. cordata* leaves

| Sl. No. | Reaction incubation time (minutes) | FBPase activity (µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$) |
|---|---|---|
| 1. | 0 | 220.51 ±4.2 |
| 2. | 30 | 307.69 ±5.2 |
| 3. | 60 | 312.65 ±5.3 |
| 4. | 90 | 357.26 ±5.7 |
| 5. | 120 | 379.48 ±5.9 |
| 6. | 150 | 366.25 ± 3.6 |

(Values are Mean ± SE, n = 3)

**Table 5.** Effect of varied enzyme concentration on Fructose-1,6- bisphosphatase activity from *D. cordata* leaves

| Sl. No. | Enzyme protein concentration in the reaction mixture (µg) | Specific activity [µ mol F-6-P produced ($mg^{-1}$) protein $h^{-1}$] |
|---|---|---|
| 1 | 0 | 59.82 ±0.4 |
| 2 | 50 | 220.51 ±4.2 |
| 3 | 100 | 263.24 ±4.5 |
| 4 | 150 | 302.24 ±5.2 |
| 5 | 200 | 319.65 ±5.3 |
| 6 | 250 | 329.91 ±5.5 |
| 7 | 300 | 384.61 ±6.0 |
| 8 | 350 | 357.26 ±5.7 |
| 9 | 400 | 369.23 ±5.8 |

(Values are Mean ± SE, n = 3)

**Progress of FBPase reaction with respect to protein concentration**

Using increasing concentration of *Drymaria cordata* enzyme protein from from 0-400µg, Fructose-1,6-bisphosphatase assay was carried out under optimal conditions. It has been revealed that the *Drymaria cordata* FBPase activity has increased linearly with the increase of protein concentration up to 300µg (Table 5), whereas the activity of *Ginkgo biloba* enzyme) increased upto a concentration of 400µg (Yonzone *et al.,* 2015) while a related enzyme, inositol synthase from *Lunularia cruciata* showed similar value i.e., increased upto a concentration of 300µg (Chhetri *et al.,* 2009).

**FBPase activity at different incubation temperature**

In order to find out the relative enzyme activity as a function of incubation temperature, FBPase from *D. cordata* was incubated separately for one hour at

temperature between the ranges of 0˚C and 60˚C at an interval of ten degrees each in presence of the standard assay mixture. Results plotted in fig.1 show that the activity of the enzyme was least at 0˚C, 10˚C and 60˚C. However, the enzyme was remarkably active between the temperature ranges of 20˚C and 50˚C with the maximum activity encountered at 40˚C. However, the cytosolic enzyme from spinach and pea leaves showed a low linear activity when stored at 0˚C-120˚C (Weeden and Buchanan, 1983). Cytosolic FBPase from higher plants generally showed temperature maxima near about 30˚C.

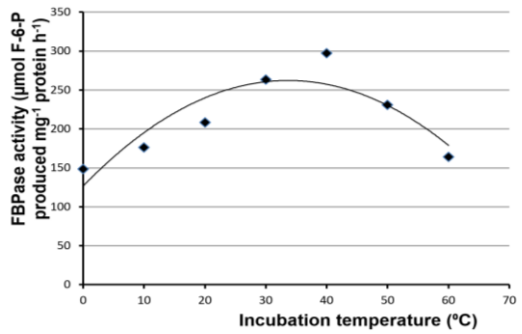


**Figure 1.** Effect of different incubation temperatures on Fructose-1,6-bisphosphatase activity from *D. cordata* leaves (enzyme activity defined as µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$)

**FBPase enzyme activity at different pH**

From the data represented in fig.2, it becomes clear that the FBPase enzyme activity was appreciably increased at a narrow pH range from 6.5 to 8.5. The *D. cordata* FBPase exhibited optimum activity at a pH range of 7.0-8.0 with maximum at pH 8.0. The castor bean seedling FBPase also showed optimal activity between pH 7.0 and 8.5, but the activity was 50 times greater in pH 8.5 as compared to that at pH 7.0 (Youle and Huang, 1976). Similar pH optimum of 8.0 was found in the enzyme from *Selenastrum minutum* which changes to 8.5 when DTT was removed from the reaction mixture (Botha and Turpin, 1990).

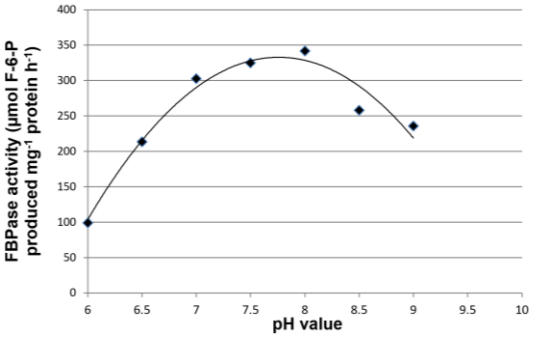


**Figure-2:** Effect of different pH on **c**ytosolic Fructose-1,6-bisphosphatase activity from *D. cordata* leaves (enzyme activity defined as µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$)

The pH maxima of 8.0 for *D. cordata* FBPase activity was quite similar to the pH optima towards the alkaline range shown by the same enzyme in other plants viz., pH 8.0 in spinach leaves (Zimmermann *et al.,* 1978) and *S. cerevisiae* (Funyama *et al.,* 1979). Similarly, *G. biloba* showed a pH maximum of 7.5-8.0 (Yonzone *et al.,* 2015). In contrast, the pH optimum was found to be towards neutral in FBPase extracted from different fatty seeds (Youle and Huang, 1976) while the same was found to be 7.5 in apple leaves (Zhou and Cheng 2004).

**Effect of monovalent and divalent cations**

Effect of some monovalent cations and divalent cations on *D. cordata* FBPase activity was studied using variable concentration (0-10mM) of chloride salts of $K^+$, $Na^+$, $NH^{4+}$, $Li^+$, $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$. The partially purified of *D. cordata* FBPase was incubated in presence of the variable concentration of individual metal ions mentioned, to the usual assay components and keeping one control set without adding any such cation. Results of such experiments have been shown in table 6. Among the monovalent cations tested, $K^+$, $Na^+$ and $NH_4^+$ slightly enhanced FBPase activity at least upto 10mM, on the contrary $Li^+$ was a very effective inhibitor of this enzyme. Earlier studies on *Purococcus furiosus* had shown that monovalent cations $K^+$ showed a slight stimulating effect on the enzyme reaction, whereas $Li^+$ inhibited the activity (Verhees *et al.,* 2002).

Of the divalent cations, cytosolic FBPase from *D. cordata* leaves has shown an absolute requirement for $Mg^{2+}$ or $Mn^{2+}$which has a strong stimulatory effect to the enzyme activity. On the other hand, $Zn^+$, $Hg^+$ and $Cd^+$ salts were found to act as potent inhibitors of *D. cordata* FBPase activity. Similarly, in *Magnifera indica* FBPase activity was inhibited by $Zn^{+2}$ and no activity was detected in the absence of $Mg^{+2}$ (Kumar and Selvaraj 1990). In apple leaves, $Na^{2+}$, $Ca^{2+}$, $Cu^{2+}$, $Zn^{2+}$ and $Hg^{2+}$ inhibited the enzyme activity while $Mn^{2+}$ synergsistically stimulated the activity of the FBPase enzyme (Zhou and Cheng, 2004). *Gingko biloba* FBPase showed a similar characteristic where the divalent cations, $Mg^{+2}$ and $Mn^{+2}$ were strongly stimulatory while $Zn^{+2}$ and $Li^{+1}$ were strongly inhibitory to the FBPase activity (Yonzone *et al.,* 2015). The strong inhibition due to heavy metals suggests that one or more free sulphydryl groups are present within the active site of the enzyme (Chhetri *et al.,* 2006).

**Table 6.** Effect of monovalent and divalent cations on cytosolic Fructose-1,6-bisphosphatase activity from *D. cordata* leaves

| Cation concentration (mM) | Specific activity (µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | $K^+$ | $Na^+$ | $Li^+$ | $NH_4^+$ | $Mg^{2+}$ | $Mn^{2+}$ | $Zn^{2+}$ | $Hg^{2+}$ |
| 0 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 | 70 ±1.5 |
| 2 | 92 ±0.7 | 50 ±0.3 | 67 ±1.0 | 82 ±0.6 | 191 ±1.9 | 99 ±0.8 | 64 ±1.5 | 64 ±2.0 |
| 4 | 109 ±1.0 | 104 ±0.9 | 42 ±0.5 | 99 ±0.8 | 213 ±2.2 | 109 ±1.0 | 54 ±1.0 | 47 ±1.1 |
| 6 | 114 ±1.1 | 126 ±1.2 | 37 ±0.7 | 105 ±0.9 | 225 ±2.6 | 186 ±1.8 | 49 ±0.2 | 32 ±0.8 |
| 8 | 126 ±1.2 | 120 ±1.2 | 32 ±0.2 | 109 ±1.0 | 235 ±2.9 | 186 ±1.8 | 40 ±0.3 | 28 ±0.7 |
| 10 | 160 ±1.6 | 126 ±1.2 | 26 ±0.4 | 131 ±1.3 | 241 ±3.1 | 198 ±1.9 | 29 ±0.1 | 14 ±0.5 |

Values are Mean ± SE, n = 3)

### Effect of EDTA

The catalytic activity of the FBPase enzyme of *D. cordata* was measured by using different concentration of EDTA in the ranges of 0-100mM. As evident from the result described in fig. 3 EDTA activated the enzyme activity up to concentration 40mM and then as the concentration increased (60-100mM) it inhibited the activity of FBPase. Similar effect was observed in *Gingko biloba* where EDTA was stimulatory to cytosolic FBPase activity upto 30mM, beyond which it seemed to be toxic. Inhibition by EDTA was also a characteristic feature of FBPase from *Acer pseudoplantanus* (Scala and Semersky, 1971).

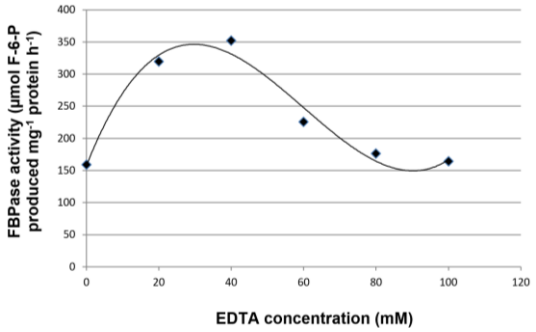


**Figure 3.** Effect of varied EDTA concentration on **c**ytosolic Fructose-1,6-bisphosphatase activity from *D. cordata* (enzyme activity defined as µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$)

### Determination of $K_m$ value:

The activity of ammonium sulphate purified *D. cordata* FBPase was found to increase linearly with respect to the increase in the concentration of FBP ranging from 0-10mM with an interval of 2mM each. The enzyme activity increased linearly upto 10mM. The average $K_m$ value for *D. cordata* cytosolic FBPase for its substrate FBP was 1.11µM which was nearer to that of the cytoplasmic FBPase from *Gingko biloba* leaves having a $K_m$ value of 1.86µM (Yonzone *et al.,* 2015) as determined in accordance with the rate equation of Michaelis-Menten (**Fig. 4**). In contrast, the $K_m$ value was quite high for the same substrate for the enzyme obtained from other species e.g., 3µM from spinach leaves (Habron *et al.,* 1981); 3.1µM from apple leaf (Zhou and Cheng, 2004) and 16.7µM from *Vigna radiata* (Lal *et al.,* 2005).

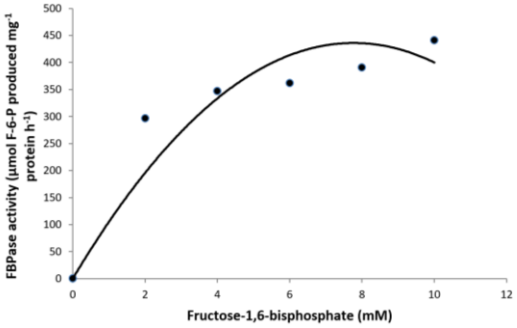


**Figure 4**. Effect of varied substrate (FBP) concentration on **c**ytosolic Fructose-1,6-bisphosphatase activity from *D. cordata* (enzyme activity defined as µmol F-6-P produced $mg^{-1}$ protein $h^{-1}$)

## Conclusion

The present work describes the occurrence of Fructose 1,6-bisphosphatase, in *Drymaria cordata*, its partial purification and biochemical characterization. This study is important since it provided an understanding of the metabolic regulation and characterization of the enzyme that may pave the way for its application in science and industry in the days to come.

### Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article

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**Cite this article as:**

Niki Doma Sherpa, Raksha Mukhia, Dhani Raj Chhetri. Partial purification and characterisation of cytosolic Fructose-1, 6-bisphosphatase from *Drymaria cordata*. *Annals of Plant Sciences 7.4 (2018) pp. 2126-2133*.

http://dx.doi.org/10.21746/aps.2018.7.4.9

**Source of support:** Nil
**Conflict of interest:** Nil