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ANTIOXIDANT ACTIVITY OF LECTIN ISOLATED FROM LEAVES OF *BRYOPHYLLUM PINNATUM*

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ABSTRACT

The *Bryophyllum pinnatum* refers to the family Crassulaceae. The glycoprotein from the 0.9% of normal saline extracts of *Bryophyllum pinnatum* leaves was refined by dialysis with 50% of ammonium sulfate. Protein concentration was invented by Lowry's method. The dialyzed sample allowed for SDS-PAGE to determine the molecular weight (M_r). The antioxidant activity of crude lectin extract has been assessed by the DPPH (1, 1-Diphenyl-2-picrylhydrazyl) and ABTS methods. The present study aims to verify the antioxidant activity of lectins detached from the leaves of *Bryophyllum pinnatum*.

Keywords: Antioxidant activity; ABTS; *Bryophyllum pinnatum*; DPPH; Lectin; SDS-PAGE.

INTRODUCTION

Bryophyllum pinnatum belongs to the family Crassulaceae. Its common names as Ghayamari, air plant, love plant, life plant, Zakham-e-hyat, panfutti (Jain *et al.,* 2010). It grows worldwide and is used as folk medicine in many countries like Australia, tropical Africa, India, tropical America, and China. The plants have many therapeutic attributes (Kamboj *et al.,*2009). The chemical compounds isolated from *Bryophyllum pinnatum* are used as folk medicines against many ailments such as hypertension and kidney stones, (Lans, 2006) pulmonary infections, rheumatoid arthritis, etc(Quazi*et al.,* 2011). *Bryophyllum pinnatum* leaves are used as an antifungal (Sofowora, 1993) and anti-allergic agent (Pal *et al.,*1991). The oxidative activity was detected by DPPH and Nitric oxide free radical scavenging method. The leaf extract of *Bryophyllum pinnatum* has vital potential to scavenge the free radicals (Halliwell *et al.,* 1997).

The isolation of lectins from leaves of *Bryophyllum* and its antioxidant activity is an important step to study the nature, structural and biological properties of lectins. The study on isolation and partial characterization of a lectin with its antioxidant activity from the leaves of *Bryophyllum pinnatum* is presented here.

MATERIAL AND METHODS

Plant material

Leaves of *Bryophyllum pinnatum* were collected from the nursery at Jalandhar, Punjab, India. The plant has been validated by Dr. Sanjay Kumar Uniyal, Principal Scientist, CSIR-IHBT, Palampur, Himachal Pradesh. The specimen no. PLP15367.

Preparation of leaves extract

10ml extract of *Bryophyllum pinnatum* leaves was homogenized in 100ml of ice-chilled 0.9% of normal saline solution. The homogenized extracts were allowed to settle for 30 minutes at 4°C after which the supernatant was extracted and filtered using 3MM Whatman chromatography paper. The filtrates were centrifuged at 10,000 rpm, for 10 minutes at 4°C the obtained supernatants were stored at 4° C (Patil *et al.,* 2015).

Purification and isolation of lectin from *Bryophyllum pinnatum* leaves extract

The 50% ammonium sulfate is utilized in the precipitation of lectin from the dialyzed extract. The gradual addition of 50% ammonium sulfate in dialyzed extracts at 4°C causes the precipitation of lectins. After 100% saturation of ammonium sulfate precipitates obtained were centrifuged at 10,000 rpm for 20 minutes. Precipitates were dissolved in normal saline to free the solution from ammonium sulfate fraction again dialyzed (Patil *et al.,*2015).

Determination of Protein concentration by Lowry method

The protein concentrations of crude extract were measured by Lowry method using BSA (bovine serum albumin) as the standard protein (Lowry *et al.,* 1951).

Sodium dodecyl sulfate-Polyacrylamide gel Electrophoresis

Fig. 1: *Bryophyllum pinnatum*

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE*)* was carried out in 12% separating gel (Tris-HCl 1.5M, pH 8.8, SDS 10%, APS 10%, TEMED 10 µl) for 1.5min.Stackinggel with pH 6.8. Electrophoresis buffer (Tris HCl 25mM, Glycine 192mM, SDS 0.1% at pH 8.3). The lectin sample was prepared by heating a protein solution in a sample buffer (2% SDS, 10% glycerol, 0.02% Bromophenol, 5% 2-mercaptoethanol) at 100° C for 5 min (Laemmli et al., 1973).

Antioxidant Activity of Lectin

DPPH Free Radical-Scavenging Activity

DPPH (1, 1-Diphenyl-2-picrylhydrazyl) can be reduced by antioxidants because it is a free radical. The different concentrations of crude lectin 4, 8, 12, 16, and 20mg/ml were used for antioxidant activities (Hatano *et al.,*1988). The radical-scavenging activity (RSA) was calculated as the percentage of DPPH. Methanol and different concentrations of plant extract was used as a blank, while DPPH solution and methanol was used as a control. IC50 values denote the concentration of the sample required to scavenge 50% of DPPH radicals.

DPPH Free Radical-Scavenging Activity % = [(Abs $_{(Cont)}$ −Abs $_{(Sample)}$/ Abs $_{(Cont)}$] ×100

DPPH has taken as control.

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging Method Principle

The oxidation of ABTS generated the pre-formed radical monocation ABTS with a blue chromogen potassium persulfate and the reduction of ABTS occurred by hydrogen donating antioxidants.

Procedure

Different substances have ability to scavenge ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) in comparison with a standard i.e. ascorbic acid. To prepare a radical cation 36mg of ABTS is dissolved in 100ml of distilled water i.e. preparation of a stock solution of 7mM ABTS mixed with 57mg of sodium persulfate in 100ml of distilled water i.e. 2.45mM potassium persulfate in an equal ratio of 1:1. Placed this mixture in dark at room temperature for 16hrs incubation until the reaction has been completed. The optical density (OD) was set at 0.7 of ABTS solution. 50 % methanol used for dilution. Lectin extracts of both plants with different concentrations were added

in every 1ml of ABTS solution. These mixtures were incubated for 30 minutes. After incubation the absorbance were taken at 745nm of samples of different concentrations(Sithisarn *et al*., 2005).

ABTS Radical Cation Scavenging Activity % = [(Abs $_{(Cont)}$ −Abs $_{(Sample)}$/ Abs $_{(Cont)}$] × 100

ABTS has taken as control.

Statistical Analysis

The statistical analyses of triplicate readings or data were expressed as mean ±SD. The data analyzed by linear regression with 95% confidence band and IC50 value of DPPH and ascorbic acid was calculated using software Graph Pad Prism 7.04.

Table 1: DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* Lectin extract

Conc. of sample (mg/ml)	O.D. of DPPH (Control)	% of Antioxidant activity
4	0.35	12.8
8	0.35	24.0
12	0.35	40.6
16	0.35	55.7
20	0.35	70.1

Table 2: ABTS Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin extract

Conc. of sample (mg/ml)	O.D. of ABTS (Control)	% of Antioxidant activity
4	0.7	8.5
8	0.7	19.3
12	0.7	36.9
16	0.7	55.8
20	0.7	74.7

RESULTS AND DISCUSSION

Determination of Protein concentration by Lowry's Method

The total protein content in the dialyzed extract of (*Bryophyllum* Lectin) BL was found to be 5.09mg/ml.

Electrophoresis (SDS-PAGE)

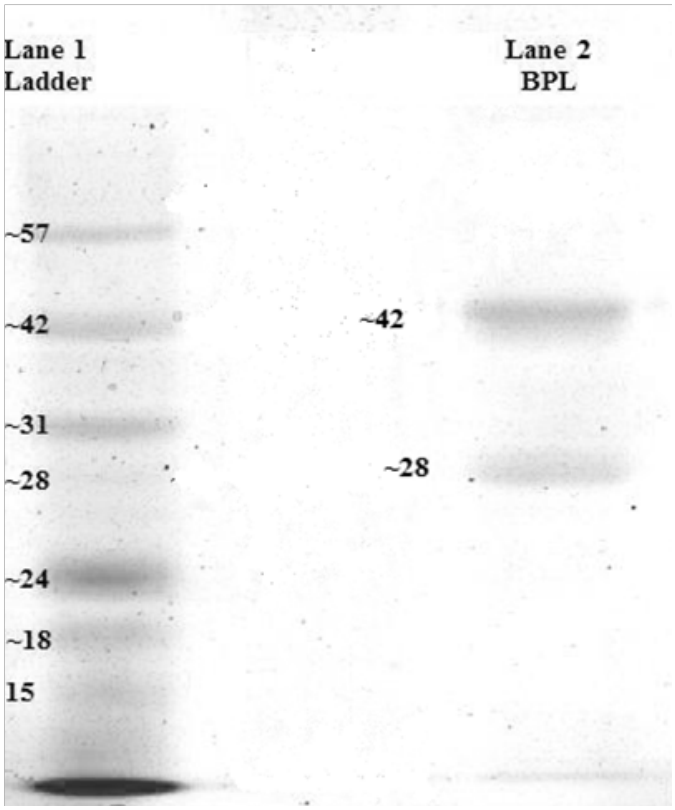


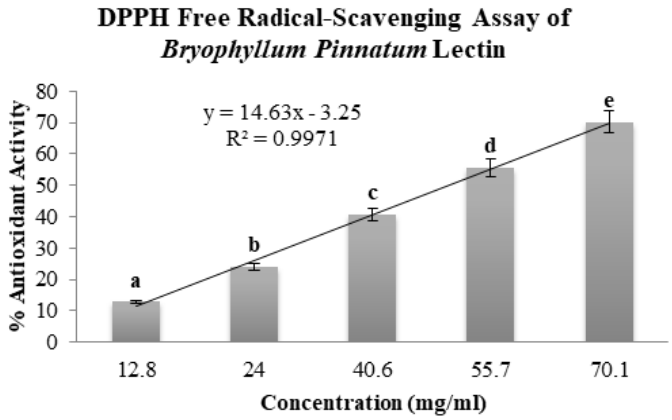
Fig. 2: Band pattern of *Bryophyllum* lectin on SDS-PAGE

SDS-PAGE resulted into the appearance of band with molecular weight of 28kDa and 42kDa in the purified lectin of *Bryophyllum pinnatum.*

In this study, the different concentrations of crude lectin 4, 8, 12, 16, and 20 mg/ml were taken. The scavenging effect of crude lectin on DPPH was significantly increased with the increase in the concentration of crude lectin. The antioxidant activity of *Bryophyllum* lectin with DPPH assay has shown in Table.1 and Graph. 1. The IC50 value of crude lectin is **14.3 mg/ml**. Ascorbic acid is used as standard at a concentration of 4, 8, 12, 16, 20 mg/ml. IC50 value of Ascorbic acid show its 50% activity at very low concentration i.e. **11.5mg/ml.**

ABTS [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] Radical Cation Scavenging assay for *Bryophyllum pinnatum* Lectin extract

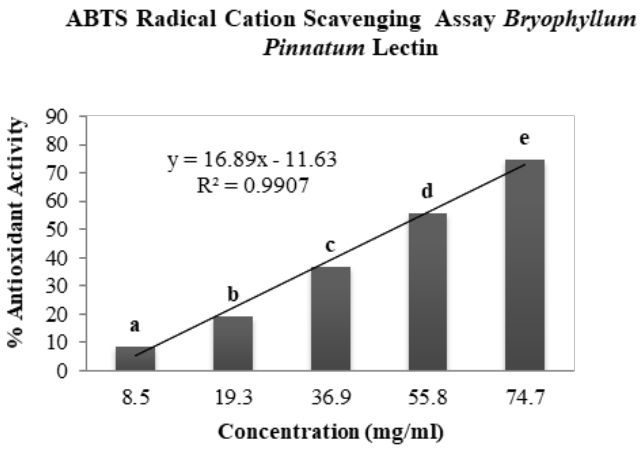
The different concentrations of crude lectin 4, 8, 12, 16 and 20 mg/ml were taken. The scavenging effect of crude lectin on ABTS was increased as the concentration of



Graph 1: DPPH Free Radical-Scavenging Assay of *Bryophyllum pinnatum* crude lectin extract. Antioxidant activity of *Bryophyllum* lectin (BL) at different concentration (mean ± SD, n=3) with One way analysis of variance (ANOVA) P- value < 0.05 by Tukey's test so it is statistically significant.

lectin was increased. The antioxidant activity of *Bryophyllum* lectin with ABTS assay has shown in Table .2

and Graph. 2. The IC50 value of crude lectin is 14.6mg/ml. Ascorbic acid is used as standard at concentrations of 4, 8, 12, 16 and 20 mg/ml. IC50 value of Ascorbic acid show its 50% activity at very low concentration i.e. 9.6mg/ml.



Graph 2: ABTS Radical Cation Scavenging Assay of *Bryophyllum pinnatum* lectin extract. Antioxidant activity of *Bryophyllum* lectin at different concentration (mean ± SD, n=3) with One way analysis of variance (ANOVA) P- value < 0.05 by Tukey's test so it is statistically significant.

CONCLUSION

Antioxidant can neutralize and remove the free radicals from blood stream. The scavenging of free radicals occur by two mechanism i.e. reduction of ROS (reactive oxygen species) or by antioxidant defense mechanism (Faruq *et al.,* 2017). The lectin extract of *Bryophyllum pinnatum* has antioxidant activity (Saha*et al.,* 2014). The IC50 values of DPPH assay of Ascorbic acid i.e. 11.5mg/ml, *Bryophyllum pinnatum* i.e. 14.5mg/ml.Lectins has the anti-oxidant property. However, further studies are

required to analyze the therapeutic effects of lectins in cancer related studies. There is a great deal of expectation for this neglected region of translational exploration to additional utilization in cancer-related ailments.

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REFERENCES

Faruq, S.; A. Sehgal. (2017). Evaluation of antioxidant and antigenotoxic effect of kahwa. *Ind. J. Trad. Knowl* 116(2): 277-28.

Halliwell, B.; JMC, Gutteridge. (1997). Free radical in Biology and Medicine, Nitric oxide scavenging by curcuminoids. *J. pharma.Pharmacol* 49(1):105-7.

Hatano, T.; H, Kagawa.; T, Yasuhara.; T, Okuda. (1988). Two new flavanoids and other constituents in licorice root: their relative astringency and radical scavenging effects. *ChemPharma Bull* 36(6): 2009-2097.

Jain, V.C.; N.M, Patel.; D.P, Shah.; P.K, Patel.; B.H, Joshi. (2010). Antioxidant and antimicrobial activities of Bryophyllum calycinumsalisb leaf. *Pharmacol* 1:393-405.

Kamboj, A.; A. Saluja (2009).*Bryophyllum Pinnatum*(Lam.) Kurz.: phytochemical and pharmacological profile: a review. *Pharmacognosy Reviews* 1; 3(6):364.

Laemmli, U.K.; M, Favre.(1973). Gel electrophoresis of proteins. *J MolBiol* 80: 575-599.

Lans, C. A. (2006). Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. *J EthnobioEthnomed* 2(1):45.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. (1951). Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem* 193(1): 265-275.

Pal, S.; T. Sen.; A.K.N, Chaudhuri.(1999). Neuropsychopharmacological profile of the methanolic fraction of *Bryophyllum Pinnatum* leaf extract. *J Pham Pharmacol* 51(3): 312-318.

Patil, M.B.; Deshpande, K.V.; (2015).Isolation and Characterization of lectins from leaves of DregeaVolubilis. *J Glob Biosci* 4 (6), 2496-2503.

Quazi, M.A.; A.U, Tatiya,; M, Khurshid,; S, Nazim,; S, Siraj. (2011). The miracle plant (Kalanchoepinnata); a phytochemical and pharmacological review. Int Res Ayurveda Pharm 2(5): 1478-1482.

Saha, R.K.; Tuhin, S.H.M.; Jahan, N.; Roy, A.; Roy, P. (2014). Antibacterial and antioxidant activities of a food lectin isolated from the seeds of Lablab purpureous. American J Ethnomed 1(1): 008-017.

Sithisarn, P.; R, Supabphol.; W, Gritsanapan. (2005). Antioxidant activity of Siamese neem tree. *J Ethno Pharmcol 99*(1): 109-112.

Sofowora, E.A. (1993). Phytochemical assays. *Med Plant Trad Med Africa* 93: 150-153.