Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):890-900

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of anti-diabetic, antioxidant activity and phytochemical constituents of liverworts of Eastern Himalaya

Sumira Mukhia[1], Palash Mandal[1*], D. K. Singh[2] and Devendra Singh[2]

[1]Plant Physiology and Pharmacognosy Research Laboratory, Department of Botany, North Bengal University, Siliguri, West Bengal, India
[2]Botanical Survey of India, CGO Complex, 3rd MSO Building, Salt Lake Sector I, Kolkata, West Bengal, India

---

ABSTRACT

This work is an attempt to study the in-vitro anti-diabetic and antioxidant activity along with the analysis of phytochemical constituents of liverworts Ptychanthus striatus (Lehm. & Lindenb.) Nees (Lejeuneaceae), Pellia epiphylla (L.) Corda (Pelliaceae) and Bazzania oshimensis (Steph.) Horik. (Lepidoziaceae). Antioxidant activities were analyzed in-vitro by seven different assays: DPPH radical, metal chelating, superoxide, ABTS$^+$, reducing power, anti-lipid peroxidation and nitric oxide assay. Anti-diabetic potential was analysed by estimating α-amylase and α-glucosidase inhibitory activity of the plant. Detection and estimation of the constituent phytochemicals was done through qualitative test, quantitative test and TLC analysis. Results indicated significant anti-diabetic activity, antioxidant activity and occurrence of varied phytochemicals in studied plants. This finding paved the way for further analysis on these liverworts for obtaining phytochemicals of significant clinical and cosmetic importance.

Keywords: Anti-diabetic, antioxidant, phytochemicals, liverworts, TLC

INTRODUCTION

Diabetes mellitus (DM) is one among the major worldwide health problems of 21st century. Either incapability of pancreas to produce enough insulin (T1DM) or failure of body cell to respond to insulin (T2DM) results in diabetes [1]. Oxidative stress plays major role in both insulin secreting β-cell dysfunction and insulin resistance [2]. Multiple sources of oxidative stress are identified, viz. non-enzymatic, enzymatic and mitochondrial pathways. Non-enzymatic sources are: i) auto-oxidation of glucose generating $^{\bullet}OH$ radicals ii) glucose protein reaction during non-enzymatic glycation producing advanced glycosylation end products (AGEs) and iii) enhanced metabolism of glucose through polyol pathway resulting in $O_2^{\bullet-}$ production [3]. Generation of $O_2^{\bullet-}$ during mitochondrial respiratory chain is another non-enzymatic source of reactive species (RS) [4]. Persistent elevated hyperglycemia enhances glucose flux through glycolysis and tricarboxylic acid cycle and leads to an overdrive of mitochondrial electron transport chain and elevation of proton gradient, resulting generation of more $^{\bullet}O_2^-$ than mitochondrial antioxidant enzyme superoxide dismutase (SOD) can dismute. Enzymatic sources are enhanced activity of nitric oxide synthase (NOS), NAD(P)H oxidase and xanthin oxidase generating greater RS [3]. RS cause insulin resistance and disinfection of insulin secreting β-cells as they are low in free radical quenching enzymes like superoxide dismutase, catalase and glutathione peroxidase [5]. Antioxidants can, therefore, be considered effective in reducing increased blood sugar level [1]. Two enzymes α–amylase and α–glucosidase play key role in diabetes. By inhibiting these enzymes, the rate of glucose absorption and post-prandial blood sugar levels can be reduced.

Herbal remedies for diabetes and other oxidative stress related diseases are favoured due to least side effects [6]. Liverworts with its record of being used in several traditional medicine [7], have so far lagged behind in terms of exploration of its pharmacological activity and phytochemical constituents. Life strategies of bryophytes are considered to be the system of co-evolved adaptive qualities. They can survive extreme environmental conditions.

Most plants die when their relative water content falls below 20-50%. Only few plants, including bryophytes, can dry up to 4-13% and can still be resurrected and hence are referred as the desiccation tolerant plants [8]. During extreme dryness, they enter a stage of little intracellular water and almost no metabolic activity resulting in irreversible damage to lipids, protein and nucleic acids through production of RS. One of the important strategies shown by desiccation tolerant plants to limit damage to a repairable level is the production of antioxidants [9]. Considering that bryophytes can survive extreme climate and resurrect under favourable condition, it is assumed that this group of plant might possess strong antioxidative mechanism. Thus, in this work an attempt has been made to study the antioxidative and anti-diabetic activity along with phytochemical content of three important liverworts namely *Ptychanthus striatus, Pellia epiphylla* and *Bazzania oshimensis*. To our knowledge, present study is the first report detailing antidiabetic activity and phytochemical constituents of *P. striatus*, *P. epiphylla* and *B. oshimensis* found in the Darjeeling hills of Eastern Himalaya, India.

EXPERIMENTAL SECTION

Collection and identification

Liverwort samples were collected from Sinchel, Darjeeling in the month of July, 2013. The taxonomic identification was done by Dr. D.K. Singh, Scientist G and Dr. Devendra Singh Scientist C, Botanical Survey of India, Kolkata and voucher specimens have been deposited in the Central National Herbarium of the Botanical Survey of India, Howrah, India (CAL).

Animal material

Goat liver used for anti-lipid per oxidation assay was collected immediately after slay from slaughter house and experiment was conducted within 1 hour.

Chemicals used

Methanol (M), 2.2-diphenyl-1-picryl hydrazyl (DPPH), reduced nicotinamide adenine dinucleotide sodium salt monohydrate (NADH), nitro blue tetrazolium (NBT), sulfanilamide, phenazine methosulphate (PMS), ferrous chloride, trichloroacetic acid (TCA), ferrozine, thiobarbituric acid (TBA), glacial acetic acid, napthylethylene diamine dihydrochloride, $FeSO_4.7H_2O$, potassium dihydrogen phosphate (KH_2PO_4), potassium hydroxide (KOH), potassium ferricyanide, ethylene-diamine tetraacetic acid (EDTA), 2-deoxyribose, ferric chloride ($FeCl_3$), hydrogen peroxide (H_2O_2), sodium nitroprusside, gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), ninhydrin, lead acetate, aluminium chloride ($AlCl_3$), petroleum ether, copper acetate, chloroform, sodium hydroxide (NaOH), sulphuric acid, Dragendorff's reagent, hydrochloric acid, pyridine, α-glucosidase, α-amylase, pNPG (p-Nitrophenyl-α-D-glucopyranoside) were either purchased from Sigma Chemicals (USA) or of Merck analytical grade.

Methods of extraction

Collected liverworts were cautiously inspected to remove soil and other plant materials attached to it. As different species grow in close association with each other, sample of interest is cautiously separated from other associated taxa. It was then washed, air dried, crushed into powder and extracted with methanol.

Anti-diabetic assay

α-Glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was assessed according to the method described previously [10], with few modifications. Reaction was initiated by incubation of 2.5 ml phosphate buffer, reduced glutathione and 0.1 ml enzyme for 15 min followed by addition of 0.5 ml sample and 0.25 ml P-NPG. The mixture was then left for 15 minutes and finally reaction was stopped by adding 0.1 M Na_2CO_3. The absorbance was taken at 405 nm and the α-glucosidase inhibitory activity was calculated using formula:

$\% I = [1- (A_s-A_b) / A_c] \times 100$, where

As = absorbance of sample, Ab = absorbance of blank, Ac = absorbance of control.

α- Amylase inhibitory activity

Method of Kim *et al.* [10] with few modifications was followed to study α- amylase inhibitory activity. Aqueous extract at various concentrations, 0.02 M sodium phosphate buffer containing α- amylase and starch were mixed and incubated for 10 min. The reaction was terminated by 1 ml dinitrosalicylic acid. Absorbance was measured at 540 nm by the following formula:

$\% I = [A_{540}\, C– A_{540}\, E]/ [A_{540}\, C] \times 100$, where

A_{540} C = absorbance of control, A_{540} E = absorbance of extract

Determination antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH scavenging activity was estimated by the method of Sidduraju *et al.* [11]. To 200 µl extract 2 ml DPPH solution was added and the reduction in solution colour was measured spectrophotometrically at 517nm. Scavenging activity of the sample was calculated by using following formula:

$\% I = [(A_0-A_1) / A_0] \times 100$, where

I = inhibition, A_0 = absorbance of blank, A_1 = absorbance of test sample.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging activity was estimated following earlier method [12] with few modifications. To 2 ml sodium nitroprusside, 0.5 ml phosphate buffer and 0.5 ml extract were mixed and incubated at 25^{o}C for 150 minutes, then 3 ml Griess reagent was added and allowed to stand at room temperature for 30 minutes. The absorbance was measured at 540 nm. Nitric oxide scavenging activity was calculated by the following formula:

$\% I = [(A_0-A_1) / A_0] \times 100$, where

A_0 = absorbance of control and A_1 = absorbance of sample.

Superoxide radical (SO) scavenging assay

Superoxide scavenging activity was analysed following the method of Fu *et al.* [13] with few modifications. 1 ml sample and 1 ml nitroblue tetrazolium chloride, 1 ml nicotinamide adenine dinucleotide and 10 µl phenazine methosulphate were mixed and incubated for 30 min under fluorescent light. Absorbance was measured at 560 nm. Superoxide scavenging activity was estimated by using following formula:

$\% I = [(A_0 – A_1) / A_0 \times 100]$, where

A_0 = absorbance of control and A_1 = absorbance of sample.

$ABTS^+$ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) scavenging assay

$ABTS^+$ scavenging activity was studied by slightly modified method of Li *et al.* [14]. 1 ml sample and 2 ml $ABTS^+$ solution was incubated for 10 minutes. Scavenging activity was calculated based on percentage inhibition of absorbance at 734 nm by using formula

$\% I= [(A_0 – A_1) / A_0 \times 100]$, where

A_0 = absorbance of control and A_1 = absorbance of sample.

Metal chelating assay

Method of Dinis *et al.* [15] with slight modification was implemented for metal chelating activity estimation. 400 µl sample, 1.6 ml methanol, 40 µl of $FeCl_2$ and 80 µl Ferrozine were mixed and kept for 10 minutes before measuring the absorbance at 562 nm. Metal chelating ability was measured using formula

$\% I = (A_0 – A_1) / A_0 \times 100$, where

A_0 = absorbance of control and A_1 = absorbance of sample.

Reducing power assay

Iron reducing ability was studied by using Gulcin [16] method with few modifications. 1 ml sample, 2.5 ml phosphate buffer, 2.5 ml potassium ferricyanide were mixed and incubated for 20 min at 50° C. Then, 2.5 ml 10% trichloroacetic acid was added and centrifuged. 2.5 ml upper layer was collected, to this 2.5 ml of distilled water and 250 µl of 0.1% $FeCl_3$ was added and absorbance was measured at 700 nm.

Lipid peroxidation assay

Lipid peroxidation inhibition activity was studied following standard method of Bouchet *et al.* [17]. Goat liver was homogenized with phosphate buffered saline. The assay mixture contained in volume 0.1 ml of $FeSO_4$, 2.8 ml 10% liver homogenate and 100 µl sample. After 30 min incubation at 37^{o}C, 1 ml reaction mixture was taken and to this, 2

ml of thiobarbituric acid-trichloroacetic acid was added and heated for 1h in water bath. Supernatant was used to measure absorbance at 535 nm. The percentage of lipid peroxidation inhibition was estimated using formula:

$\% I = [(A_F - A_1) / (A_F - A_0)] \times 100$, where

A_0 = absorbance of control, A_1= absorbance of sample and A_F = absorbance of Fe^{+2} induced oxidation.

Preliminary phytochemical analysis

Total phenol estimation

Method of Kadam *et al*. [18] with few modifications was implicated for phenol estimation. To 1 ml sample, 1 ml 95% ethanol, 5 ml distilled water and 0.5 ml 50% Folin ciocalteau reagent were added followed by addition of 1 ml 5% Na_2CO_3 after 5 min. The absorbance was measured at 725 nm. Standard curve was calibrated using different concentrations of gallic acid.

Total flavonoid estimation

Flavonoid content was estimated following method described by previous authors [19] with few modifications. 0.5 ml extract, 4 ml distilled H_2O and 0.3 ml 5% $NaNO_2$ were mixed followed by the addition of 0.3 ml of 10% $AlCl_3$ and 2 ml of 1.0 M NaOH after 5 and 6 min respectively. Finally, 2.4 ml of distilled water was added and absorbance was measured at 510 nm. Standard curve was prepared using different concentrations of quercetin.

2.7.3 Ortho-dihydric phenol estimation

Method of Mahadevan and Sridhar [20] with minor changes was followed to estimate ortho-dihydric phenol content. 0.5 ml extract, 0.5 ml of Arnow's reagent (10g each of $NaNO_2$ and sodium molybdate in 100ml H_2O), 5 ml H_2O and 1 ml of 1(N) NaOH were mixed. Absorbance was measured at 515 nm. Standard curve was prepared with different concentrations of catechol.

Qualitative phytochemical tests

Tests for steroid, tannin, triterpenoid, amino acid, resin, cardiac glycoside, alkaloid, flavonoid, reducing sugar, anthraquinones and glycosides were carried out according to the methods mentioned earlier in different literatures with few modifications [21, 22, 23, 24].

Thin layer chromatography

To screen the presence of secondary metabolites, TLC analysis (Silica gel 60 F_{254} pre-coated plates, Merck, Darmstadt, Germany) was performed using different solvent system ($CH_3COOC_2H_5$: CH_3OH : H_2O :: 100:13.5:10); ($CH_3COOC_2H_5$: CH_2O_2 : CH_3COOH : H_2O :: 100:11:11:26); ($CHCl_3$: CH_3COOH : CH_3OH : H_2O :: 64:32:12:8); (C_7H_8 : $CH_3COOC_2H_5$ :: 93:7). The developed TLC plates were then air dried and observed at 254 nm and 366 nm UV light. It was then sprayed with different spraying reagent. Spray reagents used were: KOH reagent, Berlin blue, Dragendorff's reagent, NP/PEG reagent, 10% ethanolic KOH, vanillin-sulphuric acid reagent, Fast blue salt reagent for detection of anthraglycoside, arbutin, alkaloids, flavonoid, coumarin, saponins and phenols respectively [25]. The movement of the phytochemical was determined by its retention factor (R*f*)

$$Rf = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

Statistical analysis

All statistical analysis was performed using standard software SPSS (ver. 15.0). Data was expressed as mean ± standard deviation and its difference was compared using one-way analysis of variance (ANOVA). Duncan's Multiple Range Test ($P \leq 0.05$) was also used to find out the significant difference in values. Correlation and Principal Component Analysis was done by using XLSTAT 2009 (Addinsoft) and Smith's Statistical Package.

RESULTS AND DISCUSSION

Tight control of post ingestion glucose level is important therapeutic strategy for the management of diabetes. The inhibition of carbohydrate hydrolyzing enzyme, α-amylase and α-glucosidase is an important strategy to tackle diabetes. Several synthetic α-glucosidase and α-amylase inhibitors are in clinical use but their prices are high and have many side effects [26]. All the analysed plant in this work samples showed α-amylase and α-glucosidase inhibitory activity with the highest activity shown by *B. oshimensis* in both cases. Table 1 and Table 2 represent dose dependent α-glycosidase and α-amylase inhibition potential of *P. striatus, P. epiphylla* and *B. oshimensis*. It is hypothesized that higher polyphenol content of the extract of *B. oshimensis* might be responsible for this inhibitory

activity. Similar kind of finding was also reported by Saravanam and Parimelazhagan [27]. In DM patients during persistent hyperglycemia, low density lipoprotein oxidation by the overproduction of RS contributes to oxidative protein damage and, therefore, to the pathogenesis of diabetic's complication like arteriosclerosis. All three studied liverwort extracts prevented oxidation of lipid (Figure 1). This property might be attributed to their ability to scavenge $OH^{\cdot}$ that causes peroxidation of lipid. *B. oshimensis* displayed lower IC_{50} value compared to *P. epiphylla* and *P. striatus*, which implies that the former has better lipid peroxidation inhibitory potential.

Table 1: The percent inhibition of yeast alpha-glucosidase by methanolic extracts of *P. striatus, P. epiphylla* and *B. oshimensis* at varying concentration

Concentration (mg/ml)	% Inhibition by *P. striatus*	IC_{50} mg/ml	% Inhibition by *P. epiphylla*	IC_{50} mg/ml	% Inhibition by *B. oshimensis*	IC_{50} mg/ml
1	45.89		42.89		43.20	
2	50.17		46.89		51.03	
4	54.32	2.18	67.91	1.88	65.23	1.74
7	65.88		83.46		78.95	
10	71.21		90.83		88.96	

Table 2: The percent inhibition of yeast alpha-amylase by methanolic extracts of *P. striatus, P. epiphylla* and *B. oshimensis* at varying concentration

Concentration (mg/ml)	% Inhibition by *P. striatus*	IC_{50} mg/ml	% Inhibition by *P. epiphylla*	IC_{50} mg/ml	% Inhibition by *B. oshimensis*	IC_{50} mg/ml
1	49.49		43.89		38.26	
2	51.65		48.19		58.39	
4	56.16	1.72	71.94	1.58	66.32	1.53
7	65.88		86.93		86.93	
10	71.21		95.21		92.13	

As studies suggest that oxidative stress imposed by hyperglycemia induced RS play major role in the pathogenesis of T2DM, antioxidative therapies reducing oxidative stress can be effective in controlling diabetic complications [28]. Figure 1and 2 represents the potential of *P. striatus, P. epiphylla* and *B. oshimensis* to scavenge different free radicals that might be generated in the living system. On examination of antioxidant activity, studied plant extracts potentially scavenged DPPH radical. *B. oshimensis* extract showed high scavenging activity of DPPH radical, which might be attributed to their ability to donate electron or hydrogen radical to $DPPH^{\bullet}$ and stabilize them. Similarly, ABTS radical scavenging activity is also greater in case of *B. oshimensis* than other two. The result suggests that plant extracts produce sufficient donor hydrogen molecules that reduce $DPPH^{\bullet}$ and $ABTS^{+}$ radicals. Similar quantum of activity was also shown by moss *Polytrichastrum alpinum* [29].

Free ferrous ions are the most powerful pro-oxidants and thus its reduction is important for protection against oxidative damage and lipid peroxidation by Fenton reaction [27]. The metal chelating assay shows that *P. epiphylla* has higher chelating activity. Fe^{2+} and ferrozine forms complexes to generate hydroxyl radical, in this assay plant extract may have interfered in the complex formation resulting in above mentioned chelating effect. Superoxide is considered one among strongest radicals, as it acts as a precursor for other RS like H_2O_2, O_2^{-} and $OH^{\bullet}$ that are extremely reactive and capable of damaging bio-molecules of living system. Three liverwort sample scavenged superoxide in the following order *P. striatus* > *B. oshimensis* > *P. epiphylla*.

Reducing power is considered as an effective assay for assessment of antioxidant reducing potential. It is the ability to reduce Fe^{3+}-ferricyanide complex to Fe^{2+}. It is assumed that reductive ability of plant extract might be due to the presence of compounds that are electron donors having the capability to reduce oxidized Fe^{3+} to Fe^{2+} [30]. *B. oshimensis* have the highest capability to reduce oxidized Fe^{3+} to Fe^{2+} than other two plant samples. Nitric oxide (NO) is an important bio-molecule, but its sustained level is toxic to tissue. NO generates spontaneously from sodium nitroprusside in aqueous solution and reacts with oxygen to form nitrite (NO_2^{-}) anion. Scavengers of NO compete with oxygen to inhibit the formation of nitrite [31]. Present study demonstrated that the methanolic extract of *B. oshimensis* has better NO scavenging activity than *P. striatus* and *P. epiphylla*.

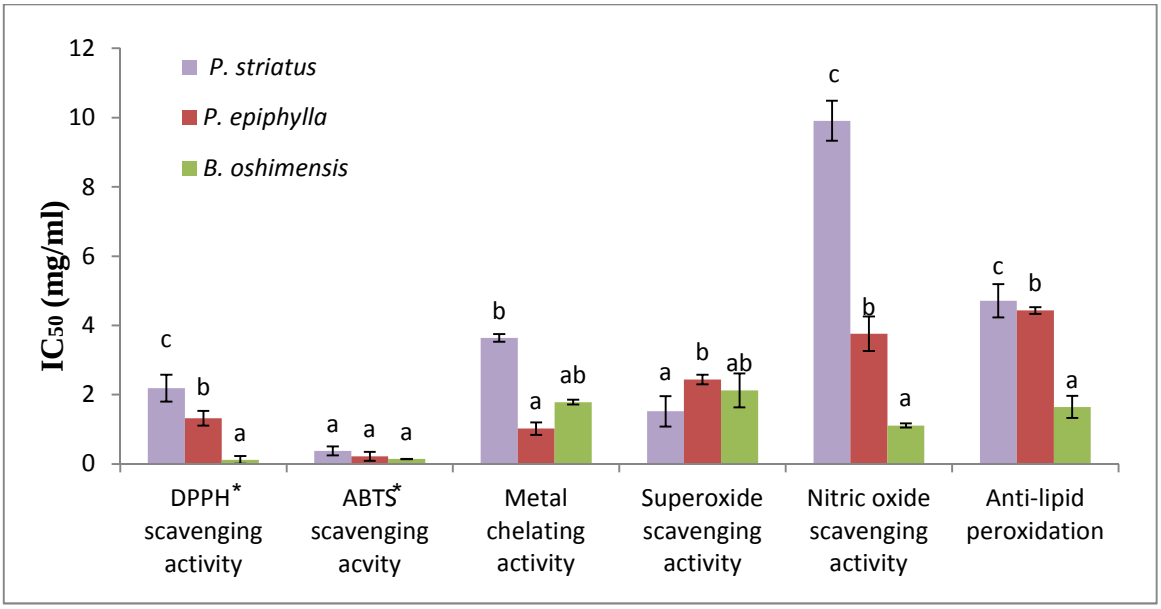


Figure 1: Radical scavenging activity of crude extracts of *P. striatu, P. epiphylla* and *B. oshimensis*
Values with different letters (a, b, c) are significantly ($p < 0.05$) different from each other by Duncan's multiple range test (DMRT)

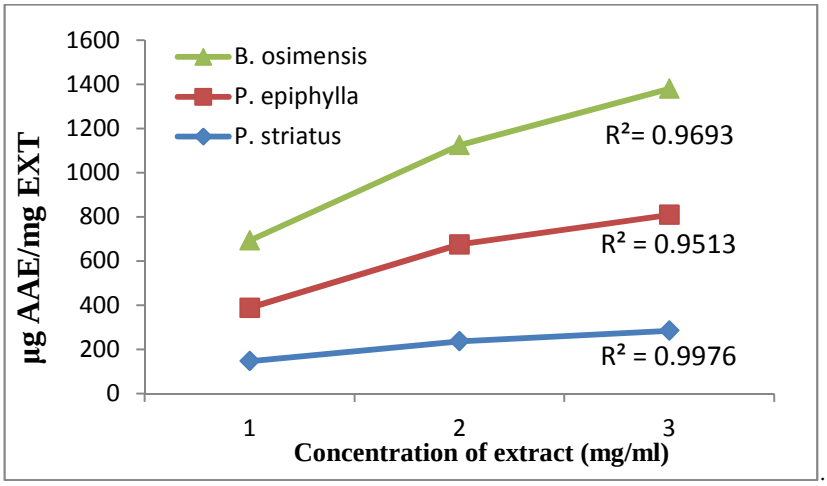


Figure 2: Reducing potential of crude extract of *P. striatus, P. epiphylla* and *B. oshimensis*

Table 3: Preliminary phytochemical analysis of *P. striatus, P. epiphylla* and *B. oshimensis*

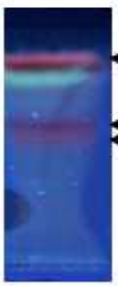
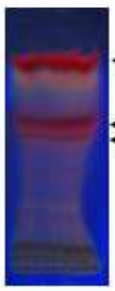
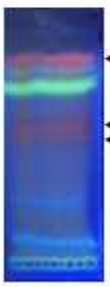
	Ptychanthus striatus	*Pellia epiphylla*	*Bazzania oshimensis*
Steroid	+++	++	+
Tannin	++	+++	+
Triterpenoids	+++	+++	+
Amino acid	++	+++	+
Resin	++	++	-
Cardiac glycoside	+++	++	++
Flavonoids	++	++	++
Alkaloid	-	-	-
Reducing sugar	++	+	+
Anthraquinones	+	+	+
Glycosides	-	-	-

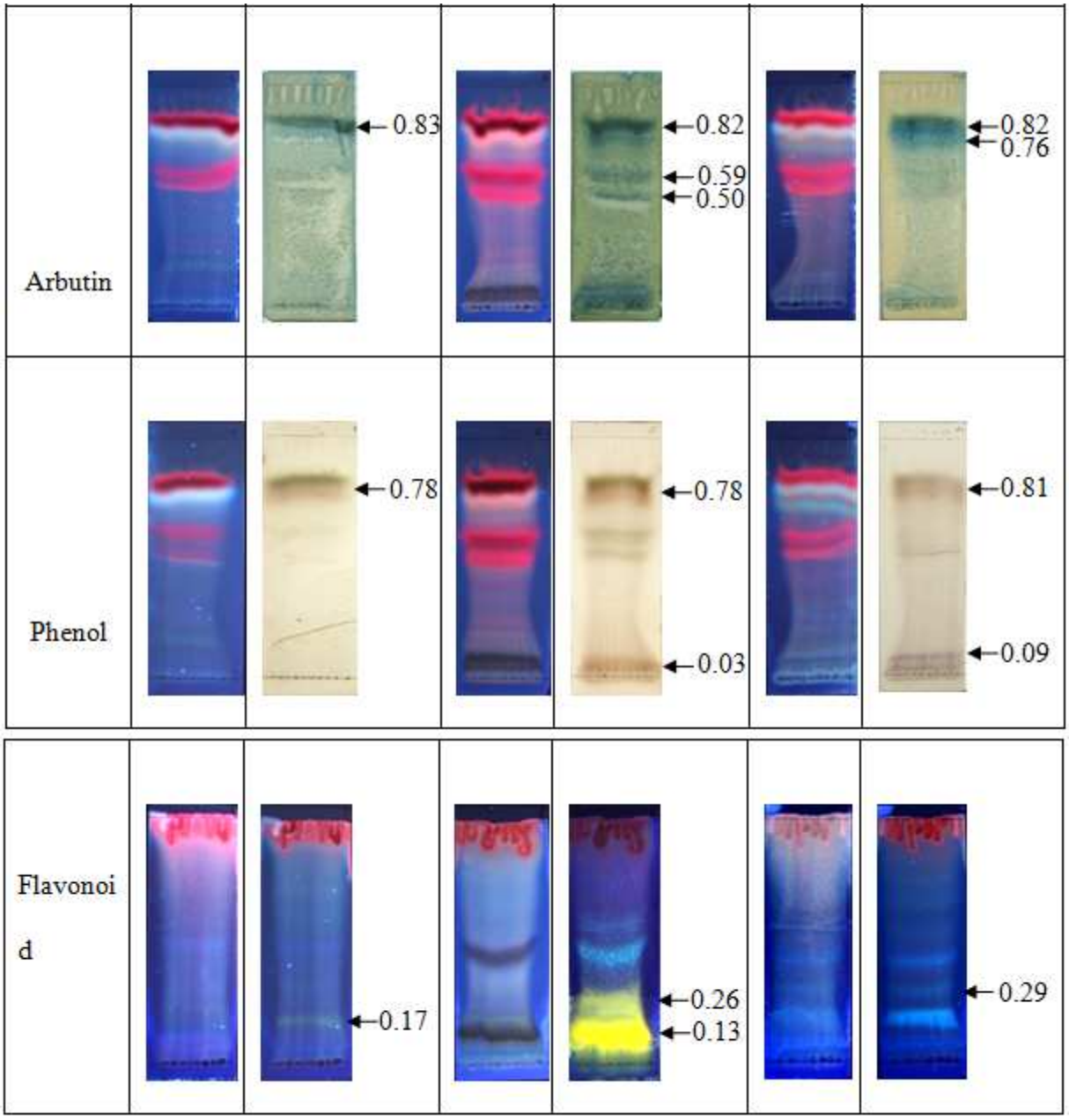
(+++) appreciable amount; (++) moderate; (+) trace amount; (-) completely absent.

Study claims that phytochemicals especially phenols to be accountable for oxidative stress reducing ability [32]. An insight into chemical nature can present rich data in understanding correlation between phenolic compound and stress reducing ability. By qualitative analysis, the phytochemicals like steroid, tannin, triterpenoids, amino acids, resin, cardiac glycoside, flavonoids, reducing sugar and anthraquinones are proven to occur in all studied hepatics (Table 3). These secondary metabolites present in the plant are accountable for the displayed antioxidative [33] and α-glucosidase inhibitory [28] activity by such plants. TLC profiling provides impressive information regarding the

existence of different phytochemicals. TLC analysis of *P. striatus*, *P. epiphylla* and *B. oshimensis* further confirmed the presence of phenolic compounds like coumarin, alkaloid, anthraglycoside, arbutin, phenol and flavonoids (Table 4). Different phyto-compounds encompass unique R_f values in different solvent system providing an important clue in understanding their polarity.

Table 4: Determination of chemical constituents of *P. striatus, P. epiphylla* and *B. oshimensis* by TLC

	Ptychanthus striatus		*Pellia epiphylla*		*Bezzania oshimensis*	
	Pre Detection	Post Detection	Pre Detection	Post Detection	Pre Detection	Post Detection
Coumarin						
Alkaloid						
Anthraglycosides						



The phytochemical screening indicated that the phenolic compounds to be major components of the liverwort extract. The quantitative estimation of crude chemical compounds present in the studied hepatics is summarized in Table 5. Quantification of total phenol, flavonoid and ortho-dihydric phenol demonstrated that *B. oshimensis* extract has higher phenol, flavonoid and ortho-dihydric phenol content compared to *P. striatus* and *P. epiphylla*. High phenolic compound content of *B. oshimensis* extract justifies the higher antioxidant activity showed by the plant. Flavonoids are considered as good inhibitors of α-glucosidase and also the regulators of oxidative stress induced diabetic complications [34]. Result from our work also supported this fact, where *B. oshimensis* with higher flavonoid content showed higher α-glucosidase inhibitory activity.

Table 5: Total phenol, flavonoid and orthodihydric phenol content of crude methanolic extract of *P. striatus, P. epiphylla* and *B. oshimensis*

	TPC (mg gallic acid eqv / g EWT)	TFC (mg quercetin eqv / g EWT)	OPC (mg catechol eqv / g EWT)
P. striatus	32.27±0.21	9.78±0.06	2.37±0.06
P. epiphylla	41.29±0.18	13.56±0.05	6.066±0.06
B. oshimensis	63.33±0.19	17.88±0.05	6.18±0.07

Abbreviation used: Extractive weight (EWT), Total phenol (TPC), flavonoid (TFC) and ortho-dihydric phenol content (OPC)

Data in the literature are sometimes contradictory regarding correlation between antioxidant activity of the plants and their polyphenol content. Strong correlation was observed between the two by some authors [28][35] while others observed no such correlation [36][37] or very weaker one only. From the Pearson correlation test (Table 6), significant positive correlation was absorbed between alpha glucosidase inhibitory activity and ABTS radical scavenging activity. While, flavonoid content and DPPH$^{\bullet}$ scavenging activity; superoxide scavenging and metal chelating activity; reducing power and ABTS radical scavenging activity; reducing power and nitric oxide scavenging activity were found to be negatively correlated with each other. It is difficult to explain relationship between antioxidant activity and phenolic content using statistical tools because antioxidant potential of single compound within a group can differ extremely and thus an equal amount of phenolics doesn't always show similar radical scavenging activity. Moreover different antioxidant assay used as well as synergism of antioxidant compounds with each other may also affect antioxidant activity significantly [38].

Table 6: Correlation between antioxidant content and radical scavenging assay of three liverwort samples

	DPPH$^{\bullet}$	ABTS^{+}	NO	MC	SO	RP	LP	α-G	TPC	TFC
ABTS^{+}	0.961									
NO	0.931	0.996								
MC	0.617	0.812	0.862							
SO	-0.569	-0.776	-0.830	**-0.998***						
RP	-0.944	**-0.998***	**-0.999***	-0.843	0.810					
LP	0.949	0.824	0.768	0.338	-0.281	-0.791				
α-G	0.969	**0.999***	0.992	0.792	-0.754	-0.996	0.843			
TPC	-0.989	-0.910	-0.868	-0.497	0.444	0.885	-0.985	-0.924		
TFC	**-0.999***	-0.974	-0.949	-0.658	0.612	0.960	-0.931	-0.981	0.980	
OPC	-0.802	-0.936	-0.965	-0.965	0.948	0.954	-0.572	-0.924	0.706	0.832

**. Correlation is significant at the 0.05 level (2-tailed).*

Abbreviation used: superoxide (SO), nitric oxide (NO), metal chelating (MC), Antilipid peroxidation (ALP), α-glucosidase (αG), α-amylase (αA)

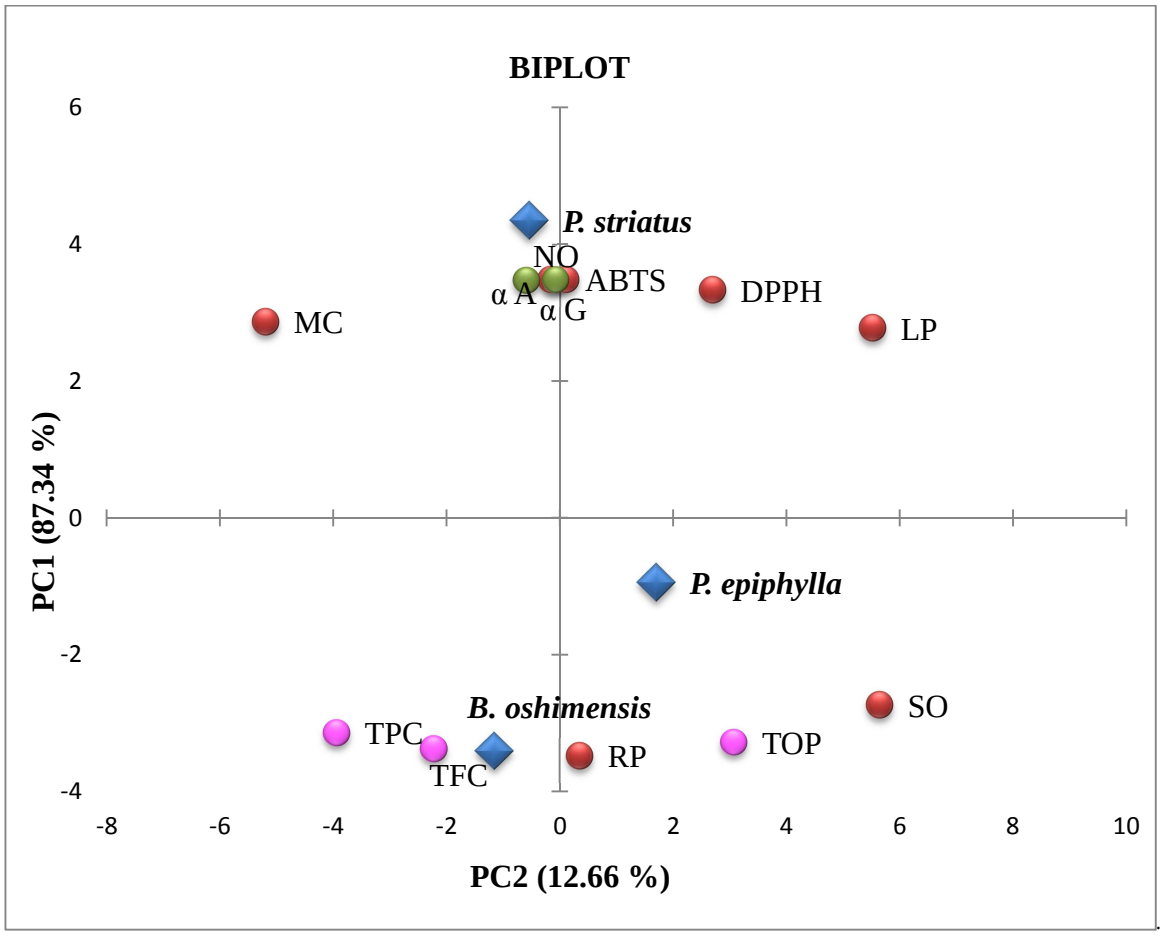


Figure 3: Principal component analysis of radical scavenging activity, anti-diabetic activity and phenolic compounds

For better understanding of relationship among the analyzed variables, Principal Component Analysis (PCA) was applied. First two principal components (PC1 and PC2) obtained after statistical analysis accounted for 87.34 % and 12.66 % of the variance respectively. The loading plots of first and second principle components are shown in figure 3. The loading of PC1 had a strong correlation with *in vitro* anti-diabetic and radical scavenging but not with the phenolic compounds indicating these antioxidant and antidiabetic activity to be controlled by compounds other than phenolics. Whereas the Principal Component 2 (PC2) had a high correlation with reducing power and super oxide scavenging activity as well as total phenolic compounds.

CONCLUSION

Till date, higher group of plants like angiosperm have been mostly investigated for the drug development, but studies suggest that plants belonging to same group have similar phytochemical constituents. Thus for the discovery of new therapeutic substances, there's a constant need to search for novel resources within unexplored group of plants like liverworts (bryophytes). At present, only about 5% percent of liverworts are chemically studied worldwide [39]. Similarly, in India only recently, studies have been initiated to screen the bioactivities as well as phytochemicals of liverworts. This work confirms the *in vitro* anti-diabetic and free-radical scavenging potential as well as the existence of versatile groups of bioactive phytochemicals in three liverworts namely *P. striatus, P. epiphylla* and *B. oshimensis* collected from Eastern Himalaya.

Acknowledgement
We are highly indebted to "University Grant Commission" for funding this work under "Maulana Aazad National Fellowship" scheme and the Director, Botanical Survey of India, Kolkata for facilitating the identification of specimens.

REFERENCES

[1] NMH Da Costa Mousinho; JJ Van Tonder and V Steenkamp. *Nat Prod Res*., **2013**, 8, 1279-1284.
[2] G Drews; P Krippeit-Drews and M Dufer. *Pflugers Arch*., **2010**, 460, 703-718.
[3] JS Johansen; AK Harris; JR David and A Ergul. *Cardiovasc Diabetol*., **2005**, 4, 5.
[4] AY Andreyev; YE Kushnareva and AA Starkov. *Biochem (Mosc)*., **2005**, 70, 200-214.
[5] M Tiedge; S Lortz; J Drinkgern and S Lenzen. *Diabetes*., **1997**, 46, 1733-1742.
[6] P Mandal; Mitali G; MT Kumar and DP Prasad. *Int J. Drug Res*., **2010**, 2, 830-843.
[7] S Mukhia; P Mandal; DK Singh; D Singh and D Choudhury. *Int J. Pharm Sci Res*., **2014**, 5, 4552-4561.
[8] P Scott. *Ann Bot*., **2000**, 85, 159-166.
[9] I Kranner; RP Beckett; S Wornik; M Zorn and HW Pfeifhofer. *Plant J*., **2002**, 31, 13-24.
[10] YM Kim; YK Jeong; MH Wang; W Lee and H Rhee. *Nutrition*., **2005**, 21, 756-761.
[11] P Sidduraju; P Mohan and K Becker. *Food Chem*., **2002**, 79, 61-67.
[12] L Marcocci; L Packer; MT Droy-Lefaix; A Sekaki and M Grades-Albert. *Methods Enzymol*., **1994**, 234, 462-475.
[13] W Fu; J Chen; Y Cai; Y Lei; Chen L; Pei L, *et al*. *J. Ethnopharmacol*., **2010**, 130, 521-528.
[14] XC Li; XT Wu and L Huang. *Molecules*., **2009**, 14, 5349-5361.
[15] TCP Dinis; VM Madeira and LM Almeida. *Arch Biochem Biophys*., **1994**, 315, 161-169.
[16] I Gulcin. *Chem Biol Interact*., **2009**, 179, 71-80.
[17] N Bouchet; L Barrier and B Fameoneau. *Phytother Res*., **1998**, 12, 159-162.
[18] VB Kadam; RK Momin; Fatima S and UB Kadam. *Int J. Pharm Res Biosci*., **2013**, 2, 202-206.
[19] M Atanassova; S Georgieva and K Ivancheva. *J. Chem Technol Metall*., **2011**, 46, 81-88.
[20] A Mahadevan and R.Sridhar. Methods in physiological plant pathology. Sivakami Publication, Chennai, **1986**.
[21] A Kumar; R Ilavarasan; T Jayachandan; M Decaraman; P Aravindhan; N. Padmanabhan and MRV Krishnan. *Pak J. Nutr*., **2009**, 8, 83-85.
[22] J Ngbede; RA Yakubu and DA Nyam. *Res J. Biol Sci*., **2008**, 3, 1076-1078.
[23] HA Ibrahim and H Ibrahim. *Int J. Pure Appl Sci Technol*., **2009**, 3, 39-43.
[24] G Trease and WC Evans. Ed Textbook of Pharmacognosy, 12th Edition, Balliere Publishers, **1983**.
[25] H Wagner and S Bladt. Plant Drug Analysis: A Thin Layer Chromatography Alas. 2nd Edition, Spinger-Verlag. Berlin, Heidelberg, **1996**, 349-362.
[26] D Gomathi; M Kalaiselvi and C Uma. *Int Res J. Pharm*., **2012**, 3, 226-229.
[27] S Saravanam and T Parimelazhagan. *Food Sci Hum Wellness*., **2014**, 3, 56-64.
[28] M Ghosal and P Mandal. *Int J. Pharma Biol Sci*., **2013**, 4, 671-684.
[29] HD Bhattarai; B Paudel; HL Lee; H Oh and HY Joung. *Z Naturforsch C*., **2009**; 64: 197-200.
[30] P Jayanthi and P Lalitha. *Int J. Pharm Pharm Sci*., **2011**, 3, 126-128.
[31] P Mandal and M Ghosal. *Int J. Pharm Sci Rev Res*., **2012**, 13, 39-47.

[32] A Subba and P Mandal. *Free Rad Antiox*., **2015**, 5, 6-12.
[33] S Sahoo; G Ghosh; D Das and S Nayak. *Asian Pac J. Trop Biomed*., **2013**, 3, 871-876.
[34] CV Montefusco-Pereira; MJ de Carvalho; AP de Araujo Boleti; LS Teixeira; HR Matos and ES Lima. *Appl Biochem Biotechnol*., **2013**, 170, 1367-1378.
[35] C Coman; OD Rugina and C Socaciu. *Not Bot Horti Agrobo*., **2012**, 40, 1842-4309.
[36] E Souri; G Amin; H Farsam and M Tehrani. *DARU J. Pharm Sci*., **2008**, 16, 83-87.
[37] SY Qusti, AN Abo-khatwa and MAB Lahwa.. *Eur J. Biol Sci* **2010**, 2, 40-51.
[38] MP Kahkonen; AI Hopia and H Marina. *J. Agric Food Chem*., **2001**, 49, 4076-4082.
[39] Y Asakawa. Maechantiophyta: a good source of biologically active compounds, In: H. Mohamed, B. B. Baki, A.N. Boyce & P.K.Y. Lee (Eds), Bryology in the New Millennium, University of Malaya, Kuala Lampur, **2008**, 367.