ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Isolation, purification and characterization of acid phosphatase from *Scenedesmus obliquus.*

Kishore J Patil[*1], RT Mahajan[2], Hitendra Kumar Lautre[3], and Vidya A Patil[4].

[1]Department of Biotechnology, Moolji Jaitha College, Jalgaon (Maharashtra), India
[2]Department of Biotechnology, Post Graduate College of Science, Technology and Research, Jalgaon (Maharashtra), India
[3]Department of Chemistry, Government V.Y.T.P.G. Auto. College, Durg (C.G.), India
[4]Bhusawal Art's Science and P. O. Nahata Commerce College, Bhusawal, (Maharashtra), India

ABSTRACT

Algae are also very important ecologically because they are the beginning of the food chain for other animals and used in production of many economical valuable products. Whereas, ample of reports are available in morphological studies on fresh water algae throughout the globe. However, enzyme research of algal origin is ephemeral. Hydrolytic enzymes have great reasonable significance owing to their central role in biological processes. In this study, acid phosphatase enzyme characterized from *Scenedesmus obliquus* fresh water alga isolated province Waghur River located near Skegaon, district Jalgaon, Maharashtra (India). The acid phosphatase is a monomer protein purified by ion-exchange and gel filtration to 6.36 fold with an apparent molecular mass 59.41 kDa on SDS PAGE. Farther, it evidenced by LCMS analysis. It has 56.10 kDa molecular with sequence (K)IINSDNVQEAAR(E), which is partial analogous with the glucose-1-phosphate adenylyl transferase, a small subunit of *Arabidopsis thaliana*. The purified acid phosphatase has an optimum pH of 5.0, and optimum temperature for the hydrolysis of p-Nitro phenyl phosphate at 50^{o}C and the km and V_{max} 0.24 mm and 0.02 µmol $min^{-1}mg^{-1}$ respectively at the same conditions. The activation energy found to be 32.35 and Q10 value was 1.54 between 40 and 50°C and fairly stable at temperature up to 37°C. The activity of the enzyme enhanced by Triton X, Guaiacol and EDTA and heavy metals Fe^{3+}, Cu^{2+}, and Ca^{2+}. The enzyme was strongly inhibited by organic solvents, SDS, tween 80 and heavy metals Zn^{2+}, Mg^{2+}, Hg^{2+} and K^{+}. The present article reveals on bio-molecular characterization of acid phosphatase with kinetic studies.

Keywords: Acid phosphatase, kinetic studies, LCMS, *Scenedesmus obliquus.*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Algae are one of the primitive and most adaptive organisms on the earth. Nowadays, the importance of algae constantly increases in the whole world for their industrially and economically important products to fulfill the demands of increasing population. Due to the highly economically importance of algae very primordial and scanty of work are available on the algal hydrolytic enzymes. Acid phosphatase is one of them which has been isolated from *Cladophora glomerata* [1], *Chlamydomonas* [2], *Ochromonas danica* [3], *Pseudokirchneriella subcapitata* [4], *Rivularia* [5], *Scenedesmus incrassatulus* and *Synechococcus aeruginosus* [6]. Phosphatase enzyme hydrolyzes the phosphomonoesters from number of organic molecules like deoxy-ribonucleotides, ribonucleotides, proteins and phosphate esters. Phosphatase enzyme is occurred in all groups of organisms from bacteria's *Bacillus, E. Coli, Pseudomonas, Aerobactor* and *Bacillus species* [7] fungi *Aspergillus caespitosus*, *Mucor rouxii* [8], and algae *Cladophora* [1] and Chlamydomonas [2]. Plant acid phosphatases characterized from Hypericium plant's roots, tubers, bulbs, seeds, aleurone layer, leaves, maize scutellum, and suspension cells [9]. In this report, the isolation, partial purification and characterization of an acid phosphatase from fresh water alga *Scenedesmus obliquus* (SO) for the first time.

MATERIALS AND METHODS

Materials

The chemicals used of analytical grade purchased from Sigma Aldrich, USA; Himedia laboratories, Mumbai; Fischer Scientifics, India and Merck Chemicals, India.

Cultivation of SO

The micro-algae purified by strike plate technique to obtain an axenic culture. The microalgae cultivated in TAP medium [10-12] and identified by ITS sequencing [13,14].

Purification of extracellular enzyme from SO

It is cultivated in TAP medium for 24 days and centrifuged at 10000 rpm for 10 min at 4^0C. The resultant supernatant (SOCE) applied as the enzyme source for further experiments.

Protein assay

The protein concentration of soluble protein quantified by the method of Lowry *et al.* (1951) and using bovine serum albumin (BSA) as a standard substrate [15].

Phosphatase assay

The p-nitrophenyl phosphate was use for measuring acid phosphatase quantitatively. In this assay, amount of released p-nitrophenyl measured at 405 nm. The acidic phosphatase reaction mixture contains 1.5 ml 3X sodium acetate incubation buffer (1M, pH 5.0), 0.2ml 1.5X para-nitrophenyl phosphate in water. Sodium carbonate was used to stop the reaction after 15 minutes of incubation at 37^0C. Blank contained the same ingredients except the enzyme solution. The enzyme activities were expressed in unit per ml (U/ml) or µmole/min/ml.

Partial purification of acid phosphatase

The SOCE subjected to partial purification by solvent precipitation or ammonium sulfate precipitation and further dialysis. The acid phosphatase fractionated by ammonium sulfate precipitation (SOAP) [16] and solvent precipitation (SOSP) [17]. Further, both the fractions subjected, to check stability.

Purification of the acid phosphatase

Gel filtration Chromatography

The SOAP after dialysis with 20mM phosphate buffers the sample at pH 7 .0, loaded on a previously equilibrated sephadex G-75 gel column [18]. The maximum active fraction abbreviated as SOGF.

Ion-exchange chromatography

The purification of the SOGF by DEAE-cellulose column done starting by determination of buffer pH that is suitable for the ion exchange column. The experiment result showed that the enzyme was unable to modify the counter ion at pH range 5.0 – 8.0. The enzyme was able to change the counter ion above pH 8.0 that was at pH 8.6, so the Tris buffer 50 mm with pH 8.6, used as an initial buffer. While the gradient of sodium chloride and Tris 50 mm pH 8.6 buffer solution used for ablation. The protein pattern (A280) obtained from DEAE-cellulose column chromatography and the highest peaks (SOIF) subjected for phosphatase activity and the fraction ranges of highest peak are analyzed for phosphatase enzyme.

Characterization of SOIF

The SOIF characterized for optimum pH, temperature, effect of metals, detergents and solvent on enzyme activity. Molecular weight was determined by SDS-PAGE.

Effect of temperature on SOIF activity

The optimal temperature for SOIF action was determined by incubating the assay mixture described above at different temperatures between 4^0C and 85^0C for 15 minute at 50^0 C. The relationship between Energy of activation (Ea) and temperature, formulated empirically by an integrated form of the Arrhenius equation [19].

Effect of pH on SOIF activity

In order to find the pH profiling of SIGF, sodium acetate buffer (pH 3.0-5.0), potassium phosphate buffer (pH 5.0-7.0), Tris-HCl buffer (pH 7.0-9.0) and glycine-NaOH buffer (pH 9.0-12.0) selected. The concentration of all buffers is 50 mM. The assay mixture (1.5ml each buffer, 0.2ml 50 mM substrate and 0.1ml of enzyme solution) was incubated at optimum temperature for 15 min in different pH values of above buffers and the enzyme activity was determined by spectrophotometric enzyme assay.

Effect of substrate on SOIF activity

Determination of SOIF activity in different substrate concentration from 5 to 60 mM p-nitrophenyl phosphate, studied. The kinetic parameters like Km and Vmax of enzyme preparation were determined using pNPP substrate. The values are determined by using the Michaelis-Menten plot and Lineweaver-Burk plot [20].

Effect of solvent on SOIF activity

To determine the effect of various solvents as possible activators or inhibitors on the partially purified acid phosphatase, the enzyme solution were pre-incubated with 100 µl solvent at 37 ^{0}C for 5 min with the compounds and then the activity was assayed. The substrate added to the medium and incubated at standard conditions.

Effect of detergents on SOIF activity

To determine the effect of various detergents as possible activators or inhibitors or the purified acid phosphatase, the enzyme solution pre-incubated, with 100 µl detergents (1mM) at 37^0C for 5 min with the compounds and then the activity determine. The substrate added to the medium and incubated at standard conditions.

Effect of metals on SOIF activity

To determine the effect of various metals as possible activators or inhibitors or the purified acid phosphatase, the enzyme solution pre-incubated with 100 µl of 1 mM metal solution at 37 ^{0}C for 5 min with the compounds and then analyzed for enzyme activity. The substrate added to the medium and incubated at standard conditions.

Thermal inactivation of SOGF

The thermal inactivation of the enzyme was determined at 37 and 55^{0}C for a period of 10 to 120 min. the enzyme incubated in 100 mM buffer at optimum pH. Aliquots removed at intervals and immediately quantified for enzyme activity.

Molecular weight determination by Polyacrylamide gel electrophoresis (PAGE)

Electrophoresis carried out by the method of Laemmli (1970) [21] on (10% w/v) acrylamide gels under denaturating and non-denaturating conditions. In denaturating conditions, a sample of SOIF incubated for 5 min at 100^{0}C with SDS PAGE sample loading buffer containing 2-mercaptoethanol. For non-denaturing conditions, samples had been mixed but before running with sample buffer without 2 mercaptoethanol and SDS. Gels were stained with Coomassie brilliant blue R 250.The molecular mass standard markers GeNe^{TM} SDS PAGE kit containing 66.0 kDa., 43.0 kDa., 29.0 kDa., and 14.3 kDa were used.

Characterization of SOIF by LCMS

The SOIF subjected to LCMS analysis at IIT, Powai, Mumbai (India).

RESULTS

Identification of *Scenedesmus obliquus*

The isolated strains identified by ITS1 and ITS4 region sequencing, identified as *Scenedesmus obliquus*.

Partial purification of acid phosphatase

Precipitation of SOCE by salt precipitation and solvent precipitation

Fractional precipitation of SOCE with ammonium sulfate

The SOCE shows the presence of two iso enzymes since, 20% ammonium sulfate precipitation shows higher specific activity than 40%. Although the biggest portion of acid phosphatase enzyme precipitated at 80%, saturation had 260.86% higher specific activity compared to the crude enzyme with 33.91% recovery. For further purification 80%, saturated acid phosphatase (SOAP) used.

The result of solvent precipitation indicated that as the solvent ratio increases the extracted protein content and enzyme activity also increased (table 3) and the precipitated acid phosphatase (SOSP).

Table 3 Enzyme activity of SOCE with SOSP

	SO acid phosphatase		
Solvent and enzyme ratio	EA (U/ml)	PC µg/ml	SA (U/mg)
SOCE	3.46	1.15	3.01
SOSP1 (1:1)	1.59	0.31	5.12
SOSP2 (1:2)	5.36	0.54	9.93

EA= Enzyme activity, PC= protein concentration, SA = Specific activity

ISSN: 0975-8585

Stability of SOAP and SOSP2

The outcomes of comparative stability in between SOAP1 and SOSP2 offered in Table 4. The loss of enzyme activity occurred during storage of precipitates at 4°C for a week. The loss of SO acid phosphatase 13.98% and 93.02% of acid phosphatase activity were lost in SOSP2 and SOAP respectively.

Table 4 Stability of SOAP and SOSP2

Technique	Acid phosphatase activity (units/min)			Half life
	Initial activity	Activity after a week	Loss of activity (%)	
SOAP1	19.3	16.6	13.8	32.19
SOSP2	15.2	1.06	93.02	1.82

The enzyme extracted with ammonium sulfate precipitation showed maximum activity and storage stability as compared with acetone-precipitated fraction of acid phosphatase; hence, the ammonium sulfate precipitated fraction of enzymes subjected to chromatographic separation of acid phosphatase. It can thus be stated that the solvent precipitation is suitable for enzyme precipitation for characterization of enzyme in terms of molecular weight determination, but not suitable for studying enzyme application and enzyme kinetics.

Purification of acid phosphatase

Gel filtration Chromatography

The dialyzed and SOAP 22.24 mg loaded on sephadex G-100 column (20 X 1.5 cm). The SOCE high protein content observed in nine fractions, 2, 8, 16, 22, 26, 30, 36, 38 and 41. The protein rich fractions subjected to phosphatase assay, where in, 15 – 17 fraction of SO showed acidic phosphatase activity and abbreviated as SOGF. The results also indicated that there might be the presence of more than one acid phosphatase in SO. Further, SOGF subjected to ion exchange chromatography.

Ion-exchange chromatography

The phosphatase activity was in the fraction numbers 36-42 fraction of SOCE. The total enzyme activity of fractioned samples from 39 to 40 is 16.33 units. After ion exchange, the SO acid phosphatase named SOIF.

Purification scheme of SOIF from *Scenedesmus obliquus*

The method employed for purification of acid phosphatase was salt precipitation, dialysis, gel filtration and ion exchange chromatography. The table 5 shows recovery and fold of purification of acid phosphatase enzyme. The crude extract of SO contained, total 346.82 units of enzyme, out of which 237.32 units (12 ml) of the enzyme could be recovered by ammonium sulfate and dialysis. The 182.16 units (8 ml) of enzyme subjected to gel filtration out of which 40.05 units (6 ml) of enzyme obtained. Further 33.35 units (5 ml) of enzyme were loaded on the ion exchange column out of which only 16.33 units of enzyme recovered with 6.36 fold purification and 4.71% of the recovery. The specific activity of SOIF is 19.14 units/mg.

Table 5 Purification scheme of SOCE

Technique	Total enzyme (U)	Total protein concentration (mg)	Specific activity (U/mg)	Purification folds	Yield (%)
SOCE	346.82	115.2	3.01	1	100
SOAP	237.32	33.36	8.19	2.72	68.42
SOGF	40.05	2.24	17.84	5.93	11.55
SOIF	16.33	0.85	19.14	6.36	4.71

ISSN: 0975-8585

Effect of Temperature on SOIF

The SOIF is most active at a temperature between 30°C and 60°C. It retained more than 44 % activity till 60°C, where in the activity dropped rapidly below 30°C and above 60°C and showed maximum activity at 50°C. Above 50°C, the enzyme activity declined rapidly as the temperature was increased, only the enzyme was not completely inactivated at 80°C in SO (figure 5). The effect of temperature on the SOIF showed that it is stable up to 80°C with temperature optima at 50°C. The activation energy (Ea) found to be 32.35 KJ mole^{-1} and Q10 value was 1.54 between 40 and 50°C (optimum temperature) for SOGF.

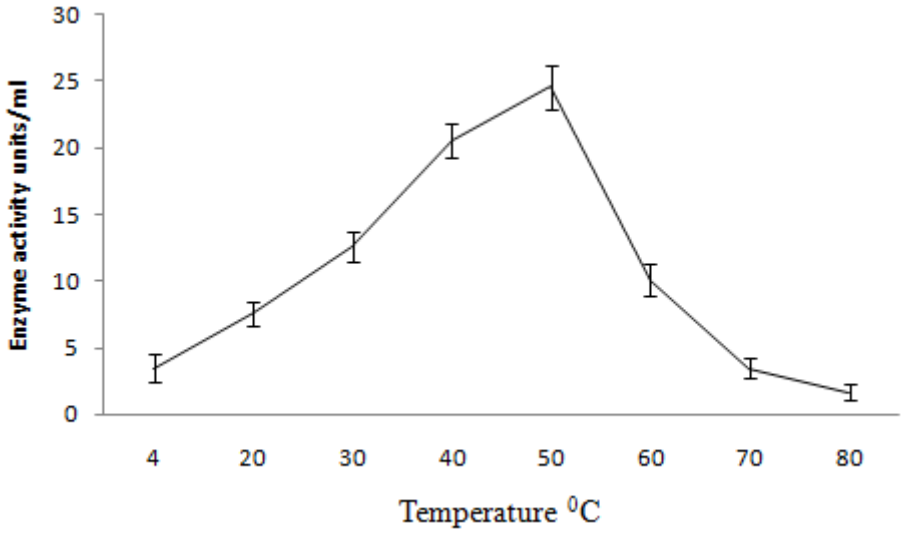

Figure 5 Effect of Temperature on SOIF

Effect of pH on SOIF

The SOIF optimum pH observed at 5.0 with enzyme activity 4.85 U/ml, most stability in the pH range 4.0 – 7.0, and its activity decreased sharply above pH 5.0 retaining only 12% of enzyme activity. The effect of pH on the SOIF showed that it is stable in a range of 4.0-7.0 with close pH optima of 5.0 (figure 6).

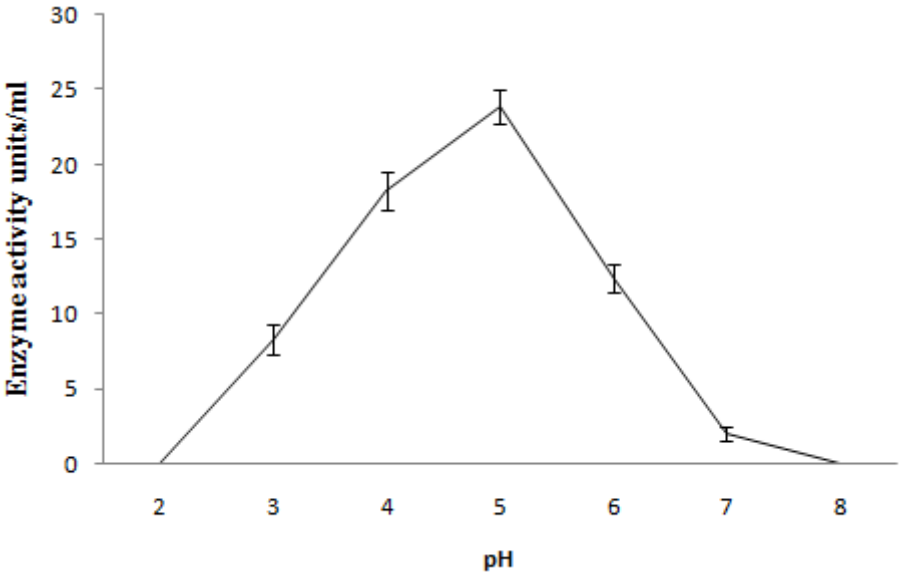

Figure 6 Effect of pH on SIGF

ISSN: 0975-8585

Effect of substrate on SOIF activity

The K_m value for pNPP obtained, form the acid phosphatase of both algae was within the same magnitude. When the activity of the enzyme measured at various concentrations of *p*NPP substrate, a double-reciprocal plot gave a straight line (Figure 7) that allowed the determination of K_m and V_{max} values of 0.24 mM and 0.020 µmol min^{-1} mg^{-1} for SOIF.

Table 6 Kinetics of SOIF

Calculation method	Michaelis-Menten	Lineweaver-Burk	Hofstee	Eadie
V_{max}	0.0204	0.0208	0.0257	0.0218
K_m	0.24	0.253	0.25	0.272

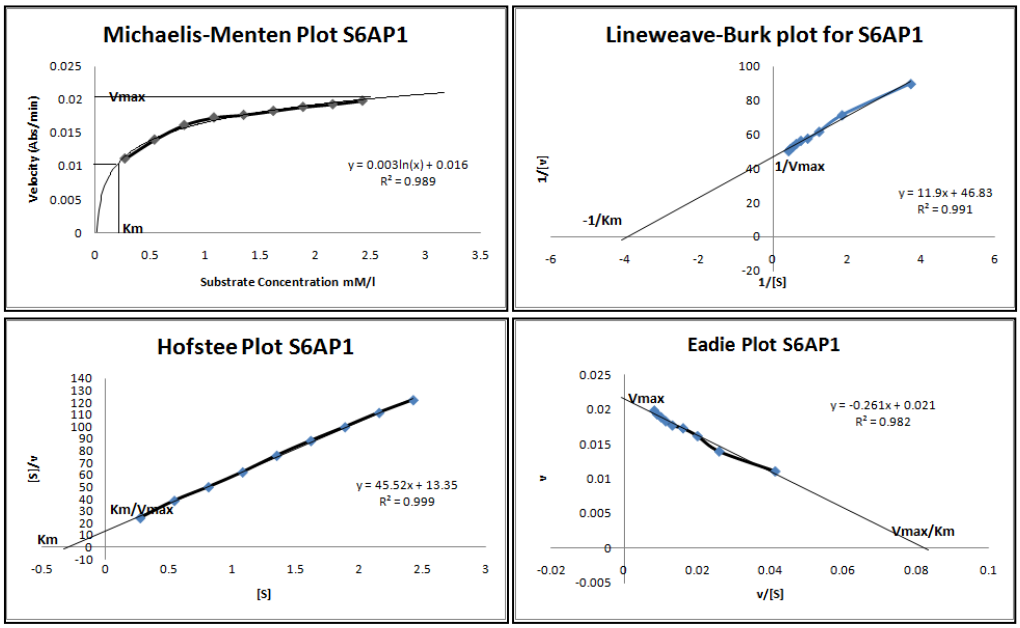

Figure 7 K_m and V_{max} of SOIF, M-M plot, L-B plot, Hofstee Plot and Eadie Plot

Effect of solvent on SOIF activity

The effect of solvent on SOIF determined after incubation in various 100 µl organic solvents with enzyme solution and reaction mixture. The SOIF inhibited by all the tested solvents and the activity reduced by 81.37% by isoamyl alcohol and 27.84% in case of chloroform.

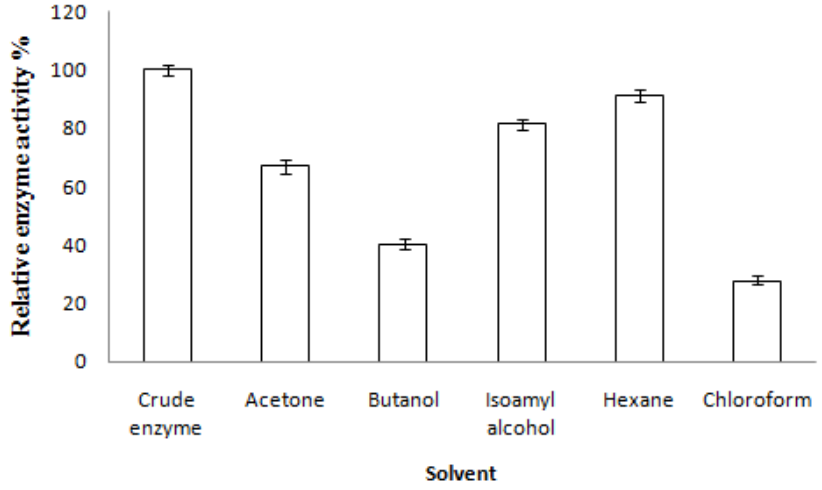

Figure 8 Effect of organic solvents on SOIF

ISSN: 0975-8585

Effect of detergents and chelator on SOIF

The influence of various detergents on the SOIF activities studied (Figure 9). The SOIF activity inhibited by SDS and Tween and stimulated by Triton X, Guaiacol and EDTA.

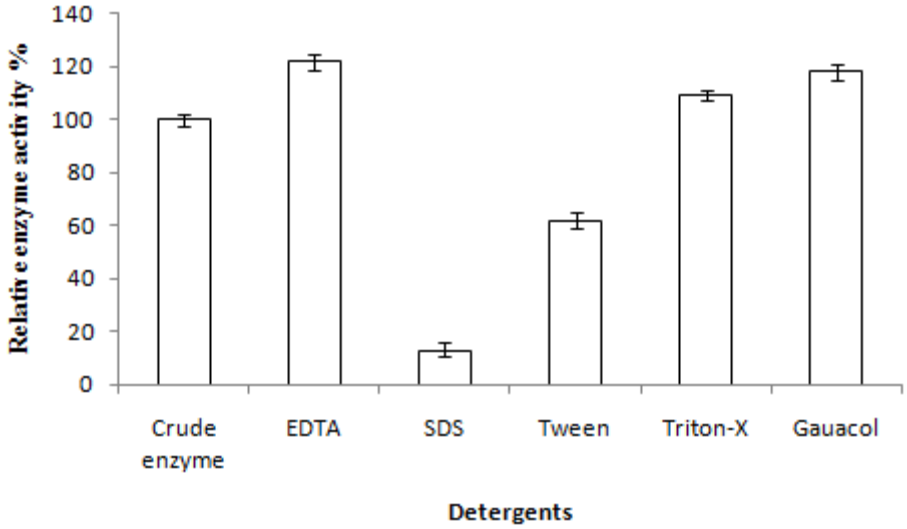

Figure 9 Effect of detergents and chelator on SOIF

Effect of metals on SOIF activity

The purified SOIF, sensitive to ions at various degrees, depending on ion nature and isoenzymes. SOIF, activity enhanced by Fe^{3+}, Cu^{2+}, and Ca^{2+} and inhibited by Zn^{2+}, Mg^{2+}, Hg^{2+} and K^{+} (figure 10). Indeed, for two enzymes Fe^{3+}, Ca^{2+}, Cu^{2+} and Hg^{2+} found to be common activator ions and Zn^{2+} found to common inhibitor.

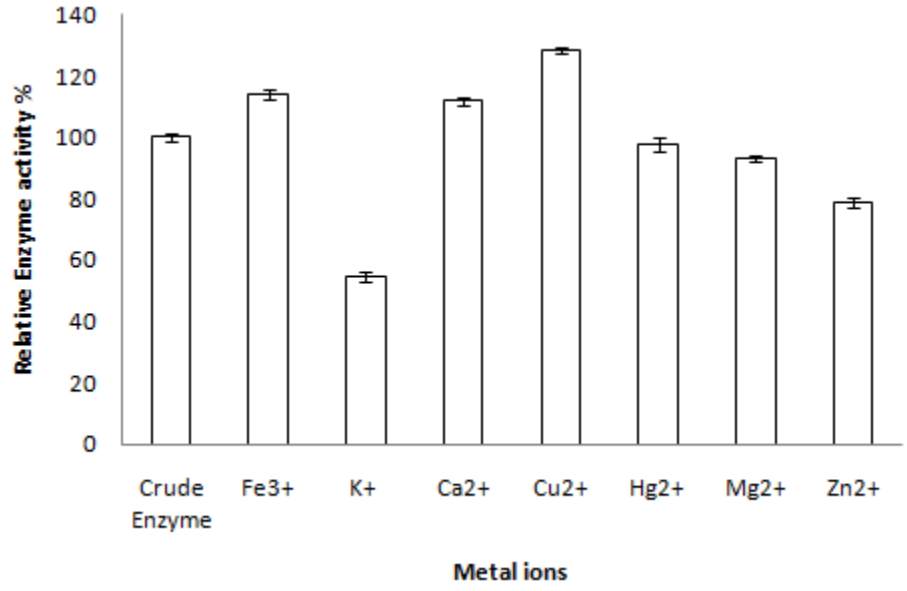

Figure 10 Effect of metal ions on SOIF

Thermal inactivation of SOGF

The thermal inactivation studies indicated that SOGF remained completely stable for 120 min at 4 °C (figure 11). However, the SOGF were less stable and lost 29% of their hydrolytic activity after 120 min of incubation, respectively at their temperature optima. The enzymes were fairly stable at temperature up to 37°C. Above 50°C, their activities declined rapidly as the temperature was increased. This conduct appears to be common for plant acid phosphatase. Although, acid phosphatase from soybean seeds showed maximum catalytic activity at 60°C. This enzyme lost its complete activity at 68°C after 10 min **[22]**.

ISSN: 0975-8585

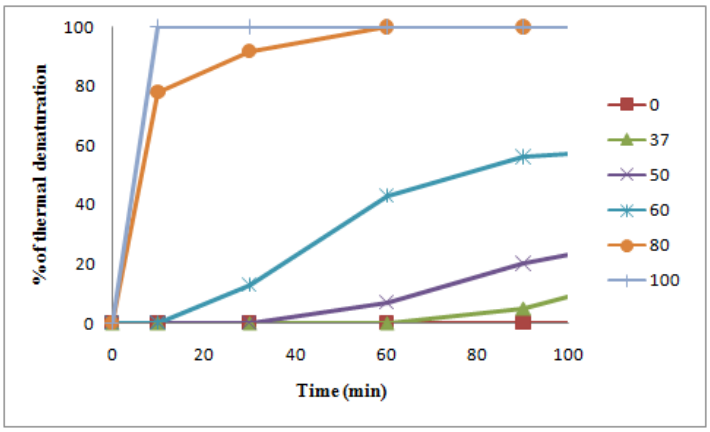

Figure 11 Percentage of thermal inactivation of SOGF

Molecular weight determination by Polyacrylamide gel electrophoresis (PAGE)

After SDS-PAGE analysis under reducing conditions, single bands were observed for acid phosphatase SOIF (Figure 12 and table 7) and their apparent molecular weight were estimated to be 59 kDa.

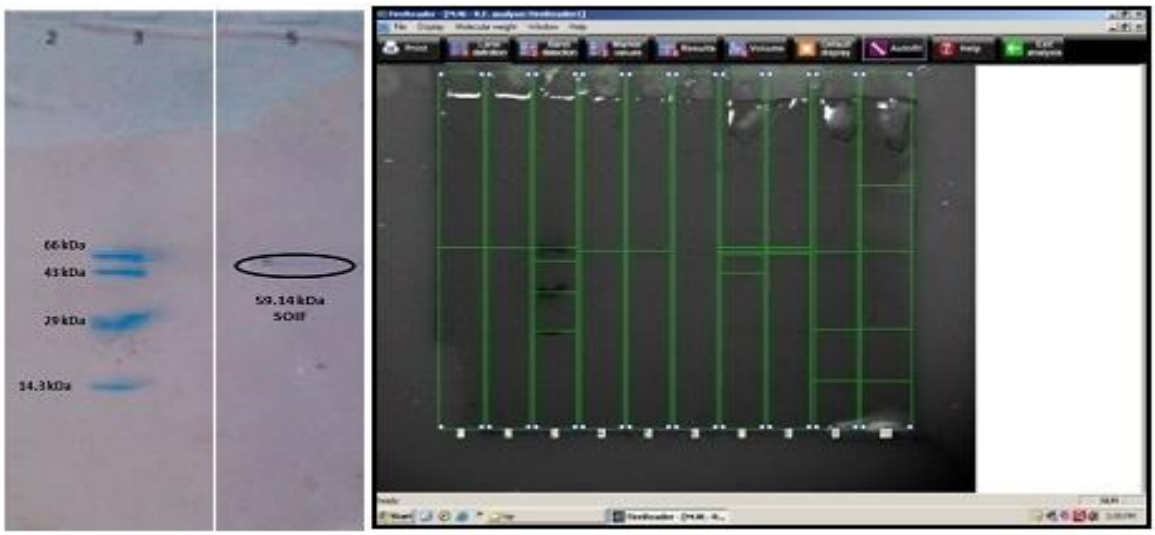

Figure 12 SDS-PAGE of SOAP, SOGF, SOIF
Lane 3- Marker Proteins, Lane 5 – SOIF, Lane 10 – SOAP and Lane 8- SOGF

Table 7 Molecular weight determination of SOIF by SDS PAGE

Molecular weight (kDa)	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
Band 1	67.48	66.00	66.00	59.41	59.41		67.48	67.48	57.79	177.29
Band 2			43.00				57.79	59.41	14.93	59.41
Band 3			29.00				53.10	53.86		15.14
Band 4			14.30				32.36			

Note: B= Band, L = Lane number and kDa = Kilo dalton

Characterization of SOIF by LCMS

The SOIF subjected to LCMS at IIT, Powai, Mumbai. The answers disclosed that (figure 13), the partially purified extract contained 10 polypeptides of amino acids from which a polypeptide having a

ISSN: 0975-8585

molecular weight 56991.9 DA with sequence (K) IINSDNVQEAAR (E) matched with the glucose-1-phosphate adenylyl transferase small subunit of Arabidopsis thaliana (figure 14).

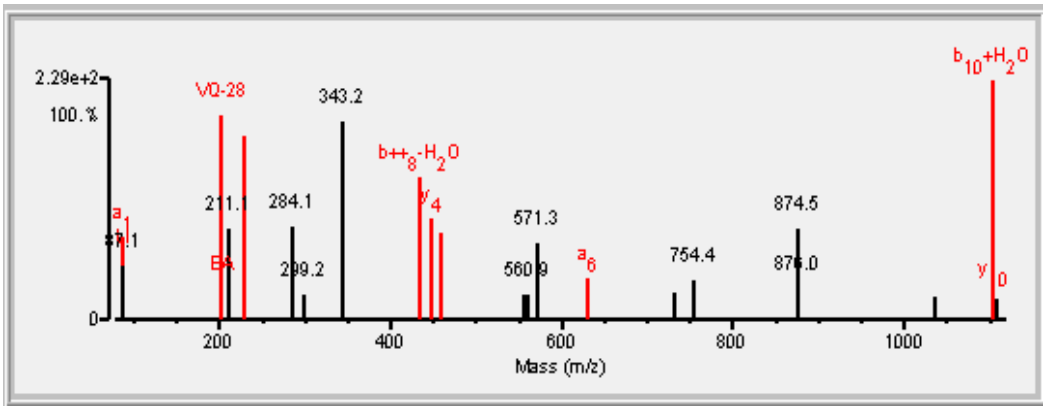

Figure 13 LCMS analysis of a SOIF protein peptide matches with acid phosphatase

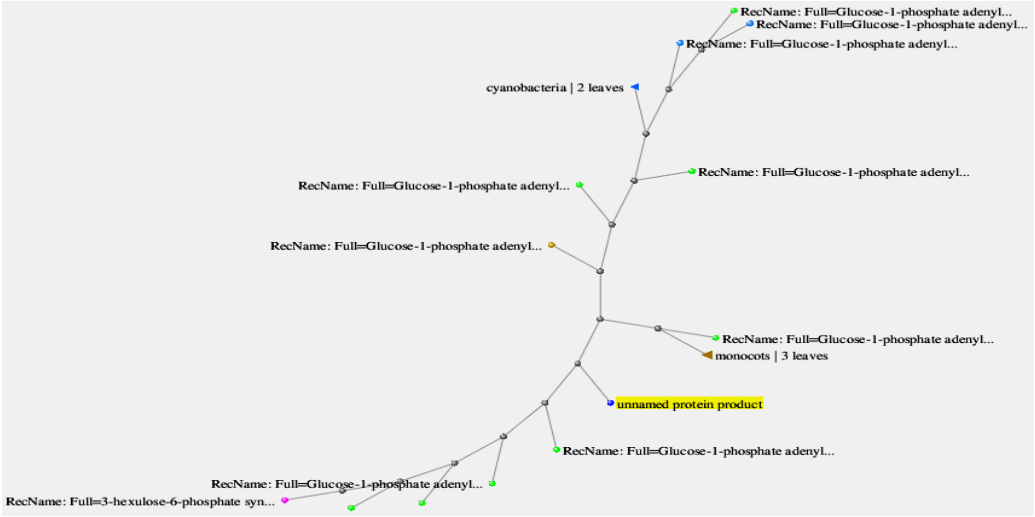

Figure 14 Tree diagram obtained from Protein Blast (NCBI)

DISCUSSION

The micro-algal strain collected and isolated from Jalgaon district, Maharashtra (India). The isolated strains identified by ITS1 and ITS4 region sequencing, identified as *Scenedesmus obliquus.* The isolated enzyme screened for the presence of acid phosphatase. The method employed for purification of acid phosphatase was salt precipitation, dialysis, gel filtration and ion exchange chromatography. The result of salt precipitation, gel filtration and SDS-PAGE shows that SOE contains isoenzymes of acid phosphates, having molecular weight 59.41 kDa and 13 kDa (Not reported in this paper). The purification technique of acid phosphatase showed that the specific activity 19.14 units/mg for SOIF. This value is higher than reported for *Vigna aconitifolia* [23], *Trichorderma harianum* [24] and *Vigna mungao* [18]. Nevertheless, these values are more depressed than those reported for the wheat germ acid phosphatase [25]. Such information on algae acid phosphatase is not available in collecting references. The optimum temperature for SOGF is 50^0C, which is beneficial compromise for mesophilic reaction. The temperature optima determined for hydrolysis for these acid phosphatase were largely consistent with other temperature optima 55°C of *Arachis hypogaea* isoenzymes [26], 55°C of *Vigna mungo* [18]. This value is higher than reported for 47°C of diabetic patient and 37°C of normal patient [27], 30°C of *Vigna aconitifolia* [23]. The activation energy (Ea) found to be 32.35 and Q10 value was 1.54 between 40 and 50°C (optimum temperature) for SOIF. The obtained values were similar to those described (37.94 kJ mol^{-1}) *Pseudokirchneriella subcapitata* [4], phosphatase extracted from the green algae *C. reinhardtii* (44.73 kJ mol^{-1}) [28] and for the acid phosphatase from other organisms, like, bovine kidney (45.44 kJ mol^{-1}) [29]. The activation energy value obtained for the acid phosphatase in this work is also close to the values reported for

other algae enzymes, such as hydrogenase [30]. The effect of pH on the SOIF showed that it is stable in a range of 4.0 -6.0 and 4.0-7.0 with close pH optima of 4.0 and 5.0 (figure 6). This stability is a good compromise for hydrolysis of natural substrates and biosynthesis reaction that need to performed for a long time by the enzymes [25]. Most of the algal phosphatase reported to have pH optima on the acidic side. The same observation is obtained in case of acid phosphatase from *Pseudokirchneriella subcapitata* [4], pH optima 4.5 of *Cladophora glomerata* [1], 5.6 and 5.0 of *Arachis hypogaea* isoenzymes [25], 5.4 of *Vigna aconitifolia* [23], 5.0 of *Vigna mungo* [18]. The apparent Km and V_{max} values for pNPP (0.24 mM and 0.020 µmol $min^{-1}mg^{-1}$ for SOIF) obtained for the acid phosphatase from SO strain were within the same magnitude as those observed for acid phosphatase from other algae species:0.27 mM and 0.037 µmol $min^{-1}mg^{-1}$ for *P. subcapitata* [4], 0.29 mM for *Euglena gracilis* [31], 0.5 mM for *O. danica* [3], 0.19 mM for the unicellular green algae *Chlamydomonas reinhardtii* [28]. Similar values also noted for the Km values for acid phosphatases extracted from fungi [32] and from plants such as 0.03 mM and 9.3 µmole/L for *Hypericum perforatum* [19]. In contrast to our work higher K_m values are reported for 24 mM and 14 mM for *Cladophora glomerata* acid phosphatase [1]. The SOIF inhibited by all the tested solvents and the activity reduced by 81.37% by isoamyl alcohol and 27.84% in case of chloroform. Somewhat, similar results is known for Chicken liver acid phosphatase, activity which is inhibited by solvents like methanol, ethanol and acetone [33]. The SOIF activity inhibited by SDS and Tween and stimulated by Triton X, Guaiacol and EDTA. Somewhat similar results are known in *A. hypogaea* isoenzymes, the stimulatory effect being showed by anionic and non ionic detergents make the latter useful when extracting acid phosphatase by giving better stability [25]. Similar to our studies, Triton X-100 known as an activator for the acid phosphatases from developing peanut cotyledons [34]. However, in contrast to our results few reports, have shown, EDTA as an inhibitor of *C. reinhardi* [18]. Potato acid phosphatase activity completely inhibited by SDS at 3 mM concentration [35]. The demand of metal ions for acid phosphatase activity also changed according to plant species, development stages and isoenzymes heterogeneity [25]. SOIF, activity enhanced by Fe^{3+}, Cu^{2+}, and Ca^{2+} and inhibited by Zn^{2+}, Mg^{2+}, Hg^{2+} and K^{+} (figure 10). Indeed, for two enzymes Fe^{3+}, Ca^{2+}, Cu^{2+} and Hg^{2+} found to be common activator ions and Zn^{2+} found to common inhibitor. Our results bear out with the consequences of other studies, which report the in vivo acid phosphatase activity alteration by metals in unicellular green algae. For example, the specific activity in *S. capricornutum* decreased by about 70% by 4.6 µM Zn^{2+} [36]. In *Scenedesmus obliquus*, this inhibition was approximately 40% by 0.12 mM Zn^{2+}, while near 140% specific activity increase in *Scenedesmus quadricauda* exposed to this same condition observed [37]. The thermal inactivation studies indicated that SOGF remained completely stable for 120 min at 4 °C. However, the SOGF were less stable and lost 29% of their hydrolytic activity after 120 min of incubation at optimum temperature. Although, acid phosphatase from soybean seeds has maximum catalytic activity at 60°C. This enzyme lost its complete activity at 68°C after 10 min [22]. The molecular weight of acid phosphatase SOIF estimated to be around 59 -66 kDa. These results indicated that the purified enzyme was a monomer protein. A similar observation observed in wheat germ 55.00 ± 5.0 (Verjee, 1969), 68.00 kDa Euphorbia characias latex purple acid phosphatase [38], 60.40 kDa *Arabidopsis* purple acid phosphatase with phytase activity [39], 58.00 and 60.00 kDa purple acid phosphatase with phytase activity, in *Arabidopsis* [40]. In comparison to other were lower than those from potato tuber (100 kDa) [41]. Nevertheless, these acid phosphatase has higher molecular weights than the purified acid phosphatase from 18.00 kDa from sheep liver [42], wherein more than two isoenzymes are isolated, having different molecular weight enzymes 55.00, 60.00, 120.00 kDa purple acid phosphatase isozymes secreted by phosphate-starved *Arabidopsis thaliana* [43], 57.00, 84.00 and 122.00 kDa purple acid phosphatase isozymes in tomato (*Lycopersicon esculentum*) suspension cells and seedlings [44]. The LCMC data of SOIF reveals that, the polypeptide having molecular weight 56991.9 Da with sequence (K) IINSDNVQEAAR (E) matched with the glucose-1-phosphate adenylyl transferase small subunit of *Arabidopsis thaliana*. Although, the sequence of algal acid phosphatase is not available in library, since, compared with glucose-1-phosphate adenylyl transferase of *Arabidopsis thaliana*. Literature suggest that the acid phosphatase has being isolated from *Cladophora glomerata* [1], *Chlamydomonas* [2-3], *Pseudokirchneriella subcapitata* [4], *Rivularia* [5], *Scenedesmus incrassatulus* and *Synechococcus aeruginosus* [6]. However yet not characterized to such extent, this is our first attempt to characterized algal acid phosphatase.

ISSN: 0975-8585

Table Comparison of biochemical properties of isolated acid phosphatase and referred acid phosphatase

Organism name	pH	Temperature	MW (kDa)	Km (mM)	Vmax (µmol/min/mg)	Activators	Inhibitors	References
Scenedesmus obliquus	5.0	50	59.41	0.24	0.020	Hg^{2+}, Fe^{3+}, Cu^{2+}, and Ca^{2+}	Zn^{2+}, Mg^{2+}, Hg^{2+} and K^{+}.	
Pseudokirchneriella subcapitata	5.0	47	--	0.27	0.037	--	Hg^{2+}	4
Rohu Fish Liver	5.0	40	50	0.25	1.1	--	P, F, Va, Mo and Ta	45
Hypericum perforatum L.	5.0	15	57	0.03	9.3	Cu^{2+}	Mo	33
Artemisia vulgaris pollen	5.4	--	73-76	0.16		Tween 20 and Triton X-100	P, Py, F, Hg^{2+} ,Cu^{2+} and Zn^{2+}	46
Cladophora glomerata	4.5	--	--	0.27	0.037	--	P, Mo, Zn^{2+} and I	1
Ochromonas danica	4.8	--	--	0.33	--	EDTA	Co^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+}, Ar, and Ta	3
Aspergillus niger ITCC 7782.10	4.0	60	33 and 67	0.28 and 0.72	1.21 and 1.35	SDS, Ta and Cu^{2+}	UV, Hg^{2+}, Co^{2+} and Fe^{3+}	47
Vigna radiataSeeds	5.5	50	29	0.3	1.33	--	P, Va	48

Ar = arsenate, P = phosphates, Py = Pyrophosphate, F= fluoride, Va = vanadate, Mo = molybdate and Ta = tartarat

ISSN: 0975-8585

REFERENCES

[1] El-Shahed AM, Ibrahim H, Abd-Elnaeim M, Pakistan journal of Biological Sciences 2006; 9(13): 2456-2461.
[2] Loppes R, Matagne RF, Genetics 1973; 75: 593-604.
[3] Patni NJ, Aaronson S, Journal of general microbiology 1974; 83(1): 9-20.
[4] Claudio MJ, Hiroshi A, Scientia Agricola (Piracicaba, Brazil) 2009; 66(5): 634-642.
[5] Banerjee M, John J, Applied Ecology and Environmental Research 2005; 3(1): 55-60.
[6] Reddy TRK, Lakshminarayana R, Proceeding Indian Academy. Science (Plant Science) 1988; 98(6):475-482.
[7] Mahesh M, Guleria N, Rajesh TS, Somashekhar R, Puttaiah ET, International Journal of Applied Biology and Pharmaceutical Technology 2010;1(1):21-33.
[8] Luis HS, Guimaraes SC, Peixoto N, Michele M, Ana CS, Rizzatti VC, Sandrim FF, Zanoelo AC, Aquino MM, Altino BJ, Maria-de L, Polizeli TM, Braz. J. Microbiol. 2006;37 (4) Sao Paulo Oct./Dec. http://dx.doi.org/10.1590/S1517-83822006000400014
[9] Demir Y, Azize A, Safinur Y, Demir N, International Journal Of Agriculture & Biology, 2004; 1560–8530: 1089–1092.
[10] Lucia HP, Carvalho JC, Sunaosato M, Electronic Journal of Biotechnology 2002; 5(3): 51-257.
[11] Abbas R, Bahman R, Faezeh G, Pakistan Journal of Biological Sciences 2006; 9:777-782.
[12] Umarah M, Wajahat H, Ikram-ul-H, Malik KA, Current Research Journal of Biological Sciences 2011;3(3):240-245.
[13] Soylu EN, Gonulol A, Turk Journal of Biol 2012;36: 247-254.
[14] Kovacevic G, Franjevic D, Jelencic B, Kalafatic M, Folia biologica (Karakow) 2010; 58(1-2): 135-143.
[15] Lowry OH, Rosebrough, NJ, Farr AL, Randall RJ, Journal of Biological Chemistry 1951; 193; 265.
[16] Sadasivam S, Manickam A, Biochemical Methods, New Age International (2010).
[17] Amlabu E, Nok AJ, Sallau AB, Parasitology International 2009; 58:238–242.
[18] Asaduzzaman AKM, Habibur RM, Tanziama Y, Archives of Biological Science Belgrade 2011; 63(3): 747-756.
[19] Segel IH, Biochemical Calculations: How to Solve Mathematical Problems in General Biochemistry, 2nd Edition, Wiley, ISBN: 978-0-471-77421-1, (1976).
[20] Lin MF, Clinton GM, Biochem Journal 1984`; 235: 351-7.
[21] Laemmli UK, Nature (London) 1970; 227: 680-685.
[22] Ferreira CV, Granjeiro JM, Taga EM, Aoyama H, Plant Physiol Biochem 1998; 36: 487- 494.
[23] Mohammed AA, American Journal of plant physiology 2011; 1-5.
[24] Leitao VO, Lima RCM, Vainstein MH, Ulhoa CJ, Biotechnology Letters 2010; 32:1083–1088.
[25] Waumack PP, Robert LVE, Archives of Biochemistry and Biophysics 1991; 288 (2): 621-633.
[26] Gonnety JT, Iranina Journal of Biotechnology 2006; 4(1): 26 -35.
[27] Bushra FH, International Journal Of Advance Biological Research 2012;2(2):241-245.
[28] Lien T, Knutsen G, Physiologia Plantarum 1973;28: 291-298.
[29] Granjeiro JM, Ferreira CV, Juca MB, Taga, EM, Aoyama H, Biochemistry and Molecular Biology International 1997; 41: 1201-1208.
[30] Schnackenberg J, Schulz R, Senger H, FEBS Letters 1993; 327: 21-24.
[31] Bennun A, Blum JJ, Biochimica et Biophysica Acta, 1966; 128: 106-123.
[32] Tsekova K, Galabova D, Todorova K, Iiieva S, Process biochemistry 2002; 37:753-758.
[33] Saeed A, Khan AR, Naz R, Hassan M, Pakistan J. Sci. Ind. Res.1998; 5: 264–270.
[34] Shekar S, Tumaney AW, Rao STJV, Rajasekharan R, Plant physiology 2002;128:988-996.
[35] Lalitha J, Mulimani VH, International Union of Biochemistry and Molecular Biology Life 1997; 41:797–803.
[36] Kong FX, Chen Y, Bulletin of Environmental Contamination and Toxicology 1995;55: 759-765.
[37] Omar HH, International Biodeterioration and Biodegradation 2002; 50; 95-100.
[38] Pintus F, Spano D, Corongiu S, Floris G, Medda R, Biochemistry 2011;76(6):694-701.
[39] Zhang W, Gruszewshi HA, Chevone BI, Nessler CL, Plant Physiology 2008;146(2): 431-40.
[40] Kuang R, Chan KH, Yeung E, Lim BL, Plant Physiology 2009; 151(1): 199-209.
[41] Gellatly KS, Moorehead GBG, Duff SMG, Lefebvre DD, Plaxton W, Plant physiology 1994; 106: 223-232.
[42] Khan AR, Rubina N, Mukhtiar H, Saeed A, Journal chemistry Society Pakistan 1997; 19(1): 60-69.
[43] Tran HT, Qian W, Hurley BA, She YM, Wang D, Plaxton WC, Plant cell Environment 2010; 33(11): 1789-803.

[44] Bozzo GG, Dunn EL, Plaxton WC, Plant, Cell & Environment 2006; 29(2): 303-13.
[45] Siddiqua, A, Saeed A, Naz R, Sherazi M, Abbas, S, Saeed A, International Journal of Agriculture and Biology 2012; 14: 223-228.
[46] Cirkovic T, Allergy: 2002; 57: 1013–1020.
[47] Jain R, Garg V, Dangwal K, Lily MK, Turkish Journal of Biochemistry–Turk J Biochem. 2013; 38 (4): 396–402.
[48] Nadir S, Saeed A, Naz R, Siddiqua A, Sherazi M, Wazir SM, Saeed A, J. Chem. Soc. Pak., 2012; 34:3.