International Journal of Engineering Sciences & Research Technology

(A Peer Reviewed Online Journal)
Impact Factor: 5.164

IJESRT

RESEARCHERID
THOMSON REUTERS

[Gophane * *et al.*, 8(1): January, 2019]
IC™ Value: 3.00

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

IJESRT

INTERNATIONAL JOURNAL OF ENGINEERING SCIENCES & RESEARCH TECHNOLOGY

CHEMICAL PROFILING OF BERGENIA CILIATA(HAW.) STERNB. AND INHIBITION OF KEY ENZYMES LINKED TO PRIMARY HYPEROXALURIA TYPE1 (PH1): AN IN VITROAND IN SILICO STUDY

Shweta R. Gophane[*1] & C.N.Khobragade[2]

[*1&2]School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded (MS)-431606

DOI: 10.5281/zenodo.2553038

ABSTRACT

This study sought to assess the inhibitory activities of *Bergenia ciliata* on oxalate synthesizing key enzymes glycolate oxidase and lactate dehydrogenase *in vitro and in silico*. Glycolate oxidase and lactate dehydrogenase enzymes were extracted from rat liver and purified by using Ion-Exchange chromatography(DEAE-Cellulose -52) and characterized. Ethanolic extract of *B.ciliata* was evaluated *in vitro* for its ability to inhibit the major enzyme activities of GOX and LDH through spectrophotometrically and mode of inhibition were evaluated using Lineweaver-Burk plots. GOX and LDH inhibitory activities were also figured out with molecular docking analysis. Iso-electric focusing and Sodium Dodecyl Sulfate –Polyacrylamide gel electrophoresis (SDS-PAGE) study revealed enzyme glycolate oxidase with PI value 9 with Molecular weight ~41 KDa and enzyme lactate dehydrogenase with molecular weight ~35 KDa. All extracts showed good amount of free radical scavenging activities. UV-visible analysis and FT-IR analysis confirmed presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in extracts. GC-MS analysis detected compounds might be accountable for antiurolithiac, hyperoxalouria synthesis enzymes inhibitors and antioxidant nature. Considering the results, drug formulation of investigated plant's lead compounds can be used in remedies for primary hyperoxaluria type1 (PH1) and other renal disorders.

KEYWORDS: Glycolate oxidase- Lactate dehydrogenase- primary hyperoxaluria -docking.

1. INTRODUCTION

Bergenia ciliata Sternb., commonly known as Paashaanbhed, is a well-known herb of Sikkim Himalaya for kidney stone. *Bergenia ciliata* Sternb. (family Saxifragaceae), a high value plant of the Sikkim Himalaya, has been investigated for antioxidant, antiurolithiac activity and bioactive compounds. However, scientific exploration of *B. ciliata* for phytochemicals and pharmacological properties is in infancy. With this view, the present study was undertaken to investigate *B. ciliata* rhizome ethanolic extract for antioxidant, antiurolithiac activity and bioactive compounds. Glycolate oxidase (GOX, EC 1.1.3.15) the key enzyme involved in oxalate synthesis. It was first associated with the disease primary hyperoxaluria type1 (PH1). The inhibition of GOX activity is a suitable therapeutic strategy for decreasing endogenous oxalate synthesis. The best studied GOX is that of spinach. With respect to animals, the enzyme purified from pig liver has been characterized in detail in terms of activity and inhibition; however the enzymes from rat liver are in less detail. The oxidation of Glycolate to glyoxylate is catalyzed by Glycolate oxidase [1] and the reduction of glyoxylate to Glycolate by lactate dehydrogenase (LDH) (EC 1.1.1.27) [2-4]. In normal pathway alanine glyoxylate aminotransferase (AGT) converts glyoxylate to glycine and so prevents the conversion of glyoxylate to oxalate which further form complex with calcium to lead into formation of calcium oxalate stones(Fig 1.) [5]. In case of AGT deficiency, glyoxylate gets directly converted to oxalate by enzymes GOX and LDH. Hence their inhibition proves potential in the management of urolithiasis [6, 7]. Antioxidants can help to protect cell damage caused by free radicals via inhibiting or slow down the oxidizing reactions occurring in the cells [8]. Plant phenolics have great antioxidant potential because of high redox activity [9,10]. GC-MS analysis can identify pure compounds present at less than 1gm [11]. Simple, cost-effective spectroscopic (UV-Vis, FTIR, GC-MS) methods together or separate can be used for detecting phytocomponents in this sense as well as conventional methods [12-14]. So

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

far reports on the systematic evaluation and scientific investigation of *Bergenia ciliata* or their phytoconstituents as glycolate oxidase and lactate dehydrogenase inhibitors are scare. Computational tool PASS (Prediction of Activity Spectra for Substances) predicted Antioxidant, urolithiasis treatment, glycolate oxidase and lactate dehydrogenase inhibitor activity for these derivatives. Therefore their GOX and LDH inhibitory and free radical scavenging effects were evaluated using *in vitro* assay and *in silico* procedures including docking studies with inhibitors. The mode of interaction of docked inhibitor, including identifying constituent compounds using GC-MS, determining IC_{50} values using inhibition kinetics analysis and determining inhibitory patterns using Lineweaver-Burk plots were well described using this approach.

2. MATERIALS AND METHODS

2.1. Plant Material

B.ciliata (rhizome) was procured from Yogesh pharma Pvt.Ltd., Nanded (MS), India. The sample was cleaned thoroughly, shade dried at room temperature and powdered. The powdered sample was macerated thrice with ethanol at room temperature. The extract was concentrated by low-pressure evaporation (<40°C) and then dried ina rotary vacuum evaporator and were subjected to phytochemical tests to investigate the presence or absence of active secondary metabolites using standard procedures [15].

1.2. Extraction of glycolate oxidase and lactate dehydrogenase from rat liver

Glycolate oxidase and Lactate dehydrogenase were isolated and purified from Wister albino rat liver using standard method [16]. The four albino rats were sacrificed and their livers were washed for 5 min in 2 l of 0.15 M KCl and after discarding the washings, it was further homogenized with 150 ml potassium phosphate buffer (pH 7.4, 0.1M). The homogenate was centrifuged at 12000 rev. /min for 40 min and the supernatant fractioned with ammonium sulphate. The fraction precipitating between 30 and 65% gave good recovery of both lactate dehydrogenase and glycolate oxidase. Fraction precipitating between 30 and 65% saturation was collected by centrifugation (12000 rev. /min for 30 min) and dissolved in 50 ml of Tris-HCl buffer (pH 8, 5mM). This solution was dialysed against 5 l of the Tris-HCl buffer (pH 8, 0.5mM), which was changed until no ammonia could be detected by Nessler's reagent. The dialyzed enzyme was applied to a DEAE-cellulose column (2.5 cm × 30 cm), previously equilibrated with Tris-HCl buffer (pH 8, 5mM). When the extract had passed through the column, the column was washed with 50 ml of the same buffer. The enzymes were eluted with a linear gradient of KCl (0-0.5 M) in 500 ml of the same buffer and 4 ml fractions were collected. Lactate dehydrogenase usually emerged as a large peak accompanied by a smaller second peak. Glycolate oxidase emerged as a single peak after the lactate dehydrogenase peak.

1.3. Protein estimation

Protein concentration was determined by the standard method using Bovine Serum albumin (BSA) as the standard [17].

1.4. Amino Acid Composition

Amino acid sequences of enzyme glycolate oxidase and lactate dehydrogenase protein was retrieved from uniprot database (http://www.uniprot.org) in FASTA format. Amino acid composition of retrieved sequences was done using BIOEDIT software.

1.5. Lactate dehydrogenase enzyme inhibition Assay

Lactate dehydrogenase activity was assayed at pH 7.4 by measuring the decrease in absorbance at 340 nm associated with NADH oxidation. Assay mixture contained 0.3mM NADH, 2.0 mM pyruvate and 100 µL enzyme in volume of 3.0 ml. The reaction was started by addition of enzyme. Lactate dehydrogenase inhibitory activity of test plant extracts was monitored spectrophotometrically following the absorbance at 340 nm under aerobic condition. The reaction mixture containing 0.3mM NADH, 100 µLenzymes in volume of 3.0 ml and a solution of test plant extracts in DMSO was incubated at room temperature for 15 min. The reaction was started by addition of 2 mM pyruvate and l-lactate formation was then followed by measure of decrease in absorbance at 340 nm. The inhibitory activity of each test compound was indicated by their IC_{50} values calculated using linear regression curve. The percent inhibition of enzyme activity was calculated using standard formula [18].

Percent of Inhibition (%)

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

$$= \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

Where control represents reaction mixture as described above excluding test compounds instead contain DMSO only whereas sample represents reaction mixture same as described in method.

1.6. Glycolate oxidase enzyme inhibition Assay

Glycolate oxidase enzyme inhibition activity was performed in cuvette with a 1-cm light path using a UV-spectrophotometer. Each assay contained 200µM potassium phosphate (pH 7.0), 1 mg of bovine serum albumin, 3µM EDTA, 0.1µM DCIP, enzyme and water to a volume of 3 ml and a solution of test plant extracts in DMSO was incubated at room temperature for 15 min. The reaction was started by addition of 2µM Sodium glycolate was then followed by measure of decrease in absorbance at 600 nm. The inhibitory activity of each test compound was indicated by their IC_{50} values calculated using linear regression curve. The percent inhibition of enzyme activity was calculated using standard formula [18].

Percent of Inhibition (%)

$$= \frac{\text{Abs Control} - \text{Abs Sample}}{\text{Abs control}} \times 100$$

Where control represents reaction mixture as described above excluding test compounds instead contain DMSO only whereas sample represents reaction mixture same as described in method.

1.7. Molecular mass determination and Purity checking

SDS PAGE was performed according to the method of Laemmli[19]. The relative molecular mass and purity of the glycolate oxidase and lactate dehydrogenase were determined on 10% polyacrylamide gel using standard protein markers. After electrophoresis the gel was stained with Commassie Brilliant blue stain for 1 h, followed by destainingfor 30 min in distilled water and photographed.

1.8. Iso-electric focusing

Isoelectric point (PI) value of glycolate oxidase was determined by Iso-electric focusing by using 2-D PAGE with ready IPG strips (Ampholine pH gradient: 4-9) using the Bio-Rad PROTEAN IEF cell [20]. The focusing was carried out for three days i.e. Day 1 : Rehydration of IPG strips were done with glycolate oxidase for 12 h. Day 2: Enzyme focusing and Day 3: Destaining for 120 min. Glycolate oxidase focusing bands were detected by overlaying the gel with solution of Commassie Brilliant blue. The gel was observed for development of blue bands. The coordinate of active bands were recorded.

1.9. Lineweaver–Burk plots

To determine the mode of inhibition by active compounds from the plants, Lineweaver–Burk plot analysis was performed [21]. This kinetics study was carried out in the absence and presence of active compounds with varying concentrations of substrate. The initial velocity was expressed as the absorbance decrease at 340 nm for lactate dehydrogenase and 600 nm for glycolate oxidase per 10 s in the assay.

1.10. Free Radical Scavenging Activity by DPPH Assay Method

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) assay method was used for the determination of free radical scavenging activity of the extracts and ascorbic acid[22]. Different concentrations (10to200 µg/ml) of test compounds in ethanol were added separately to an equal volume of 100 µMethanolic solution of DPPH and the reaction mixture was kept at room temperature for 15 min. The absorbance of the reaction mixture was recorded at 515 nm using a UV visible spectrophotometer. The blank was prepared in a similar way without extract or ascorbic acid. Negative control was prepared by mixing 0.1 mL of ethanol with 2.9 mL of DPPH solution. Ascorbic acid was used as standard. Free radical scavenging activity was calculated using the formula % of Free radical scavenging activity.

$$= \frac{\{(\text{Control OD}) - (\text{Sample OD})\}}{(\text{Control OD})} \times 100$$

Where, control represents reaction mixture containing DPPH and ethanol excluding test compounds whereas sample represents reaction mixture as described above in the method. The concentration of sample required to

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percent inhibitions plotted against the respective concentration.

1.11. UV Spectroscopic and FTIR analysis

The extracts were examined under visible and UV light for proximate analysis. For UV spectrophotometer analysis, the extracts were centrifuged at 5000 rpm for 15 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using UV-1800 Shimadzu UV Spectrophotometer and the characteristic peaks were detected. Translucent pellet was prepared by pressing 0.001gm of the ethanolic extract mixed with 20µg of potassium bromide. The infrared spectra were performed on Thermofisher Scientific,model-Nicolet 6700 FT-IR in a scan range of 400 -4000 cm-1 and the characteristic peaks were detected [23].

1.12. Gas Chromatography-Mass Spectrometry Analysis

GC-MS Analysis of the lead organic extracts exhibiting inhibition of GOX and LDH activity were performed using a Agilent 7890 A gas chromatogram equipped and coupled to a mass detector 5975 MSD spectrometer with DB 5 MS and 30m × 0.25 µm DF of capillary column.

Ultra-high purity helium was the carrier gas with flow rate of 1 mL/min, the injector mode- split (1:30), the injection volume 1 µL, the temperature program used is as follows: 60°C (hold for 2 min), then increased to 320°C at 3°C/min, held at 280°C (10 min) and temperature scan, m/z 30-600 amu. Appropriate solvent controls were also run. The identification of the components was based on the comparison of their mass spectra with those of AMDIS and NIST Version-Year 2011 was used MS data library.

1.13. Docking

The crystal structure of Human glycolate oxidase (GOX) in complex with CDST and crystal structure of Lactate dehydrogenase A in complex with inhibitor compound 23 and zinc were obtained from protein data bank (PDB entry 2RDT and 5W8I) respectively. The structures were refined using MM3 in the BioMedCache (version 6.1) software package. The active site was located in the refined model using automatic sequence alignment mode in BioMedCache workspace by selecting bound ligand embedded in 5A° shell of residue water [24]. 2D structure of compounds were drawn in CHEM DRAW (version 11.0) and subjected to energy minimization in the MOPAC module, using the AMI procedure for closed shell systems, implemented in the CS chem 3D ultra[25]. Ligands were docked into the active site of glycolate oxidase (GOX) and Lactate dehydrogenase (LDH) using BioMedCache (version 6.1). Cache automates the docking of ligands into active site by potential mean force (PMF). Potential mean force extracts pair wise atomic potentials from structure information of known protein ligand complexes contained in protein data bank. It has been also demonstrated to show a significant correlation between experimental binding affinities and its computed score for diverse protein ligand complexes [26].

1.14. Data Analysis

The results are expressed as mean ± SD. Student's *t*-test and one way ANOVA were applicable and used to analyze level of statistical significance between groups. $P<0.05$ were considered statistically significant. Linear regression analysis was used to calculate the IC_{50} values.OriginPro 8 (Ver 8E) and Microsoft Excel 2007 (Roselle, IL, USA) were used for the statistical and graphical evaluations.

3. RESULTS

Phytochemical screening was performed for the ethanol extract of *Bergenia ciliata*(rhizome) that resulted in the presence and absence of various phytochemicals(Table 1).Qualitative phytochemical analysis of plants confirms the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponin, flavonoids, steroid and phenol. Quantitative analysis demonstrated high amount of phenolic, flavonoids and moderate amount of coumarin and procanthocyanidins contents in ethanolic extract of *Bergenia ciliata* as shown in (Table 2supplementary data). The two enzyme activities glycolate oxidase and lactate dehydrogenase were readily separated on DEAE-cellulose (Fig.2). A partial degree of purification was achieved by dialysis and DEAE cellulose chromatography (Fig. 3; Table3). The Km and Vmax values as determined by double reciprocal Lineweaver-Burk plot for the enzyme glycolate oxidase 0.645 mM/ml and 0.033 IU/ml respectively (Fig. 4 Supplementary Data). The Km of Lactate dehydrogenase for substrate pyruvate was found to be 1.72 mM and

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Vmax 2.27 IU/ml (Fig.7 Supplementary Data).The enzyme glycolate oxidase showed optimum activity at pH 8 and 30^{0}C temperature as well as broad stability over a wide range of pH (7-8.5) and temperature (20^{0}C-40^{0}C) (Fig. 5 and 6 Supplementary Data)while enzyme lactate dehydrogenase showed optimum activity at pH 7 and 40^{0}C temperature as well as broad stability over a wide range of pH (6-7.5) and temperature (20^{0}C -60^{0}C) (Fig. 8 and 9 Supplementary Data).The enzyme glycolate oxidase is ~43 KDa molecular weight and having PI value 9.0 (Fig. 10 and 11) whereas lactate dehydrogenase enzyme showed ~35 KDa molecular weight (Fig. 12).The inhibition assay was carried out at five different concentrations of extract ranging from 10-100 µg/ml. The crude extracts possessing GOX inhibitory activity with IC_{50} 61.77 µg/mland LDH inhibitory activity with IC_{50}61.32 µg/ml. (Fig.13; Table4 and 5). The primary sequence of the enzyme glycolate oxidase and lactate dehydrogenase were retrieved from uniprot database (http://www.uniprot.org) in FASTA format. The molecular weight obtained from BIOEDIT data and the amino acid composition was mentioned in (Table 6 and 7**).** The data shows, both the enzyme were rich in leucine and valine (Fig. 14 and 15). DPPH is a stable free radical which is used to estimate antioxidant activity of the extracts. The identification of positive results is dependent on the color reduction property of DPPH from purple to yellow and even colourless if plant extract has much of free radical scavenging activity. The result showed increase in the effect of radical scavenging with increase in concentration. The ethanol extract of *Bergenia ciliata*has showed 98.8% of DPPH radical scavenging activity with IC_{50} values 43.48 µg/ml(Fig16a) which is significant with standard ascorbic acid IC_{50} values 14.82 µg/ml (Fig 16b). UV-VIS spectrum profile (wavelength and absorbance) were represented in (Table 8; Fig 17). The qualitative UV-VIS spectrum profile of *Bergenia ciliata* ethanolic extract was selected at wavelength from 200 to 1000 nm due to sharpness of the peaks and proper baseline. The profile showed the peaks at 380,321, 218, 369 and 283 nm with the absorption of 3.136, 3.835, 3.953, 2.828 and 3.125 respectively. The FTIR spectrum was used to identify the functional group at the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in Table 9. The FTIR spectrum profile was illustrated in the (Fig18). The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in different plant species. UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition [27].GC-MS chromatogram of the ethanolic extract showed different peaks indicating the presence of phytochemical constituents (Fig19). All the constituents were characterized and identified by comparison of the mass spectra of the constituents with the NIST library. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 10).GC–MS analysis of the *Bergenia ciliata*ethanol extracts revealed the presence of various bioactive compounds with their biological activity (Table 11; Fig 24).The ethanolic plant extracts demonstrated glycolate oxidase and lactate dehydrogenase inhibitory activity at 10-100 µg/ml. In case of glycolate oxidase and lactate dehydrogenase inhibition, *B.ciliata* were found to be non-competitive inhibitors as Km values were constant while Vmax consequently decreased with increased inhibitor concentration (Fig 20 and 21).These finding suggest that inhibition of glycolate oxidase and lactate dehydrogenase activity, leading to retardation of oxalate synthesis is one of the mechanism through which the plant tested in this study could be exhibiting their antiurolithiac effect. Modulation of glycolate oxidase and lactate dehydrogenase activity by compounds in these extracts would thus eventually lead to a lowering of oxalate content. Presence of glycolate oxidase and lactate dehydrogenase inhibitor blocks the normal pathway of conversion of glyoxylate to oxalate which further form complex with calcium to lead into the formation of calcium oxalate stones. Thus, oxalate synthesis can be blocked in hyperoxaluria conditions with glycolate oxidase and lactate dehydrogenase inhibitors. This glycolate oxidase and lactate dehydrogenase inhibition could occur in a concentration dependent or independent manner depending upon the bioactive compounds.A docking study could propose more insight into understanding the enzyme-inhibitor interactions and the structural features of active site of enzyme [28] Molecular docking on glycolate oxidase (PDB entry 2RDT) and lactate dehydrogenase (PDB entry SW8I) were performed using BioMedCache (Version 6.1) software. Docking includes vander walls and electrostatic interactions between active site and ligand. The highest scoring orientations for each ligand proposed a feasible binding mode of the inhibitor in the active site of enzyme. Docking of sorbopyranose,1,2,3,4,5- pentakis-o-(trimethylsilyl)-,L- and Myo-Inositol,1,2,3,4,5,- hexakis-o-(trimethylsilyl)- into active site of glycolate oxidase and lactate dehydrogenase were carried out and results were mentioned in (Table 12) and (Fig. 22 and 23).Amino acid residues were observed involved in binding of compounds we can conclude that regardless of larger structure,

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

compounds occupy a position in active site of glycolate oxidase and lactate dehydrogenase that favours its binding and inhibition at that site.

4. DISCUSSION

Occurrence of urolithiasis is increasing worldwide due to dietary habits like intake of food rich in purine, oxalate and calcium. Synthetic drugs are commonly employed for the treatment of urolithiasis which includes oxalate synthesis inhibitory mechanism of action. However, it has serious side effects. Thus, new alternatives with increased therapeutic activity and lesser side effects are desired. Our program looks for glycolate oxidase and lactate dehydrogenase inhibitors of phytochemical origin from ethanolic extract of *B.ciliata* (rhizome). The rhizome of *B.ciliata* is being frequently used in ayurveda for treatment of kidney stone and related disorders. *B.ciliata* crude ethanolic extract inhibited glycolate oxidase and lactate dehydrogenase in a concentration dependant manner. The *in vitro* and *in silico* inhibition of glycolate oxidase and lactate dehydrogenase by ethanolic crude extract of *B.ciliata* is moderate. However, at higher doses both the enzymes would be significantly inhibited. Presence of phenolic and flavonoids content in the extract may have contributed towards inhibition of enzymes. Since ethanol extract of *Bergenia ciliata*was showing greater enzyme inhibitor potential and DPPH radical scavenging activity. DPPH radical scavenging assay revealed that the extracts might prevent reactive radical species from damaging biomolecules such as lipoproteins, polyunsaturated fatty acids (PUFA), DNA, amino acids, proteins and sugars in susceptible biological and food systems [29].

5. CONCLUSION

We have extracted, purified and characterized glycolate oxidase and lactate dehydrogenase for inhibitory study. Chemical profiling of *B.ciliata* was done with UV-FTIR and GC-MS analysis. Ethanolic extract of *B.ciliata* showed enzyme inhibitory activity and free radical scavenging activity with respect to standard ascorbic acid. Docking study were performed that revealed the binding position of sorbopyranose,1,2,3,4,5-pentakis-o-(trimethylsilyl)-,L- and Myo-Inositol,1,2,3,4,5,- hexakis-o-(trimethylsilyl)-into active site of enzyme and confirmed the potential of its as glycolate oxidase and lactate dehydrogenase inhibitors.

REFERENCES

[1] K. E. Richardson, N. E. Tolbert, “Oxidation of glyoxylic acid to oxalic acid by glycolic acid oxidase”, Journal of Biological Chemistry, 236,1280-1284, 1961.
[2] S. Sawaki, N. Hattori, K. Yamada, “Oxidation and reduction of glyoxylate by lactate dehydrogenase”. JVitaminol, 13, 93-97,1967.
[3] M. Romano, M. Serra, “The action of crystalline lactate dehydrogenase from rabbit muscle on glyoxylate”,BiochemBiophys Acta,177(3), 421-426, 1969.
[4] N.Sugiyama, N. Taniguchi, “Evaluation of the role of Lactate dehydrogenase in oxalate synthesis”. Phytochemistry, 571-574, 1997.
[5] J.Behnam, Emma L. Williams, Susanne Brink, Gill Rumsby, Christopher J. Danpure, “Reconstruction of human hepatocyte glyoxylate metabolic pathways in stably transformed Chinese-hamster ovary cells”,Biochemical Journal. 394(2)409- 416, 2006.
[6] B. Marion, Coulter-Mackie, T. W. Colin, M. H. Robert, “Primary Hyperoxaluria Type 1”. Gene reviews, 1-18, 2009.
[7] M. Hatch, R. W. Freel, “Intestinal transport of an obdurate anion: oxalate”, Urol research,33, 1-6, 2005.
[8] B. Halliwell, Gutteridge, “Free radicals in biology and medicine”, 4th. Oxford: Oxford University Press. 2007.
[9] Y. Lu , Y. L. Foo, “Polyphenolics of salvia—a review”. Phytochem, 75:197–202, 2002.
[10] Y. Peter and D. Wong, “Studies on the dual antioxidant and antibacterial properties of parsley (*Petroselinum crispum*) and cilantro (*Coriandrum sativum*) extracts”, Food Chem,97:505–515, 2006.
[11] D. Liebler, J. Burr, L. Philips and A. Ham, “Gas chromatography-mass spectrometry analysis of vitamin E and its oxidation products”, AnalBiochem, 236, 27-34, 1996.
[12] P. Aysal, A. Ambrus, S. Lehotay and A. Cannavan, “Validation of an efficient method for the determination of pesticide residues in fruits and vegetables using ethyl acetate for extraction”, J. Environ. Sci. Heal., 42, 481-490, 2007.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

[13] M. Ibrahim and M. Abd-El-Aal , "Spectroscopic study of Heavy Metals Interaction with Organic Acid", Int. J. Environment and Pollution, 35(1), 99-110,2008.
[14] M. Ibrahim, A. Hameed and A. Jalbout, "Molecular Spectroscopic Study of River Nile Sediment in the Greater Cairo Region", Applied Spectroscopy, 62(3), 306-311, 2008.
[15] E, Trease, W. Evans,"Textbook of pharmacognosy", 3 rd ed. London: Bailliere Tindal, 81-90, 1989.
[16] Hassan asker and David davies, "Purification of rat liver enzymes involved in the oxidation of glyoxylate", BiochimBiophys Acta, 761,103-108,1983.
[17] N. Lowry andRosebrough, "Protein measurement with Folin phenol reagent." Journal of Biological.Chemistry, 193,265-275, 1951.
[18] L. Kong,Y. Zhang, X. Pan, R. Tan, C. Cheng, "Inhibition of xanthine oxidase by liquiritigenin and isoliquiritigenin isolated *Sinofranchetiachinensis"*, Cell Mol Life Sci, 57:500-505, 2000.
[19] Laemmli, "Cleavage of structural proteins during the assembly of the head of bacteriophage T4". Nature, 27, 680-185, 1970.
[20] P. Righetii, "Iso-electric focusing: Theory, methodology and applications", Elsevier, Amsterdam, 1990.
[21] Segel, Biochemical calculations. 2nd edition. New York: John Wiley & Sons. 1976; SBN:978-0-471-77421-1.
[22] I. Koleva, T. Van Beek, J. Linssen, de Groot A and L.Evstatieva, "Screening of plant extracts for antioxidant activity: a comparative study on three testing methods". Phytochemical Analysis, 8-17, 2002.
[23] C. Chander, T. Lohitnath, Mukesh Kumar and P.Kalaichelvan, "Production and characterization of biosurfactant from Bacillus subtilis MTCC441 and its evaluation to use as bioemulsifier for food bio-preservative", Adv Appl Sci Res. 3: 1827-1831, 2012.
[24] C. Khobragade, S.Beedkar, R. Bodhade andA.Vinchurkar, "Comparative structural modelling and docking studies of oxalate oxidase: possible implication in enzyme supplementation therapy for urolithiasis". International J of Biological Macromolecules, 48: 466-473, 2011.
[25] G. Mandawad, BhaskarDawane, SupriyaBeedkar, C. Khobragade, Omprakash Yemul, Trisubstituted thiophene analogues of 1-thiazolyl-2-pyrazoline, super oxidase inhibitors and free radical scavengers. Bioorganic and medicinal chemistry. 21(1):365-72, 2013.
[26] Sippl,"Recognition of errors in three-dimensional structures of proteins",Proteins, 17, 355-362, 1993.
[27] Komal Kumar and Devi Prasad A, "Identification and comparison of biomolecules in medicinal plants of *Tephrosiatinctoria*and *Atylosia albicans* by using FTIR", Romanian J. Biophys, 21(1):63-71, 2011.
[28] R. Bodhade, S. Beedkar, A. Manwar and C. Khobragade, "Homology modeling and docking study of xanthine oxidase of Arthrobacter sp. XL26", Int. J. Biol. Macromolecules. 47, 298–303, 2010.
[29] B. Halliwell, R. Aeschbach, J. Loliger and O.I. Aruoma. "The characterization of antioxidants". Food Chem Toxicol, 33, 601-617, 1995

TABLES

Table 1: Preliminary phytochemical screenings of Berginiaciliata (pashanbheda) rhizome

Phytoconstituents	Test/Reagents	*Berginiaciliata*(ethanolic extract)
Alkoids	Dragendorff's Test	+
	Hager's Test	+
	Wagner's Test	+
Proteins	Biuret Test	+
	Ninhydrin's Test	+
	Millon's Test	+
Tannins	Lead acetate	+
	Ferric chloride	+

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Steroids	Salkowski test	+
Carbohydrate	Fehling's Test	+
	Benedict's Test	+
Flavonoids	Shinoda test	+
	Lead Acetate Test	+
	Sodium Hydroxide Test	+
Glycoside	Keller-killani Test	+
Saponins	Foam Test	+

Table 2: Quantitative estimation of total phenolics, flavonoids, flavonols, Proanthocyanidins and coumarin (n=3)

Plants	**Extracts**	**Total phenolics (GAE mg/g)**	**Total flavonoids (RE mg/g)**	**Total flavonols (QE mg/g)**	**Total Proanthocyanidins (Catechin mg/g)**	**Total coumarin (coumarin mg/g)**
Bergenia ciliata	Ethanolic	440.8±0.60	90.29±0.30	83.9±0.29	0.9114±0.01	2.675±0.5

Values are expressed in mean±SD ; *: $P<0.05$; ND= Not detected

Table 3: Purification and recovery of glycolate oxidase and lactate dehydrogenase

I)	**Glycolate oxidase enzyme from Rat liver:**				
	Enzyme Activity (IU/ml)	Protein conc. (mg/ml)	Specific Activity (IU/mg)	Purification fold	Recovery (%)
Crude Enzyme	0.0766	0.0590	1.298	1	100
Ammonium Sulphate Precipitation	0.0547	0.0172	3.18	2.449	40.83
Dialysis	0.034	0.01	3.4	2.61	38.31
DEAE-Cellulose purified	0.00773	0.0021	3.682	2.83	35.33
II)	**Lactate Dehydrogenase enzyme from Rat liver:**				
	Enzyme Activity (IU/ml)	Protein conc. (mg/ml)	Specific Activity (IU/mg)	Purification fold	Recovery (%)

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Crude Enzyme	1.275	0.098	13.01	1	100
Ammonium Sulphate Precipitation	2.29	0.086	26.50	2.036	49.11
Dialysis	1.543	0.03	51.43	3.95	25.31
DEAE-Cellulose purified	0.64	0.005	128	9.83	10.17

Table 4: Vmax and Km of B.ciliata (ethanolic extracts) for glycolate oxidase inhibition.

Samples	**Concentrations (µg/ml)**	**Vmax (µg/min)**	**Km (µg/ml)**	**Type of Inhibition**	**IC_{50} value (µg/ml)**
B.ciliata	10	0.0281	0.86	Non-competitive	61.77
	25	0.025			
	50	0.022			
	75	0.0213			
	100	0.02			

Table 5: Vmax and m of B.ciliata (ethanolic extracts) for lactate dehydrogenase (LDH) Inhibition.

Samples	**Concentrations (µg/ml)**	**Vmax (µg/min)**	**Km (µg/ml)**	**Type of Inhibition**	**IC_{50} value (µg/ml)**
B.ciliata	10	2	1.75	Non-competitive	61.32
	25	1.66	1.66		
	50	1.28	1.63		
	75	1.25	1.63		
	100	0.89	1.63		

Table 6: Amino acid contents (mole %) of protein glycolate oxidase

Protein: Glycolate oxidase (Source: Rattus norvegicus liver) Length: 370 amino acids Molecular weight: 40966.07 Daltons		
Amino acid	Number	Mol (%)
Ala A	34	9.19
Cys C	5	1.35
Asp D	25	6.76
Glu E	20	5.41
Phe F	11	2.97
Gly G	28	7.57
His H	4	1.08
Ile I	19	5.14

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Lys K	21	5.68
Leu L	37	10.00
Met M	12	3.24
Asn N	12	3.24
Pro P	14	3.78
Gln Q	16	4.32
Arg R	24	6.49
Ser S	21	5.68
Thr T	15	4.05
Val V	36	9.73
Trp W	6	1.62
Tyr Y	10	2.70

Table 7: Amino acid contents (mole %) of protein lactate dehydrogenase

Protein: Lactate dehydrogenase (Source: Rattus norvegicus liver) Length: 332 amino acids Molecular weight: 36448.57 Daltons		
Amino Acid	Number	Mol (%)
Ala A	20	6.02
Cys C	5	1.51
Asp D	19	5.72
Glu E	16	4.82
Phe F	7	2.11
Gly G	25	7.53
His H	6	1.81
Ile I	24	7.23
Lys K	28	8.43
Leu L	37	11.14
Met M	8	2.41
Asn N	14	4.22
Pro P	12	3.61
Gln Q	14	4.22

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

Arg	R	10	3.01
Ser	S	26	7.83
Thr	T	11	3.31
Val	V	37	11.14
Trp	W	6	1.81
Tyr	Y	7	2.11

Table 8: UV-VIS peak values of ethanolic extract of Berginiaciliata.

Wavelength (nm)	**Abs.**
975	0.016
380	3.136
321	3.835
218	3.953
928	0.014
369	2.828
283	3.125

Table 9: FTIR peak values and functional groups of ethanolic extract ofBerginiaciliata.

Peak values	**Functional group**
3445.15	Alcohols (including phenols)
1612.76	Non acid carbonyl, Alkenes, Primary amines
1439.08	Aromatics
1035.81	Aliphatic amines

Table 10:Biologically active chemical compounds of ethanolic extract of Bergenia ciliata (Haw.) Sternb,

***Bergenia ciliata (Haw.) Sternb* (ethanolic extract):**						
CAS	Name of Compound	RT	Purity (%)	Model	Molecular weight	Molecular Formula
17877428	>tromethamine (3,7-Dioxa-2,8-disilanonan-5-one, 2,2,8,8-tetramethyl-	10.5564	38%	73 m/z	234.11	$C_9H_{22}O_3Si_2$
6736965	>Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, à-D-	26.0867	61%	129 m/z	288.75	$C_{14}H_9ClN_2O_6S$
EPA-380134	>D-Pinitol, pentakis(trimethylsilyl) ether	27.1177	63%	260 m/z	555.08	$C_{22}H_{54}O_6Si_5$

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

6736965	>Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, à-D-	27.3895	57%	217 m/z	288.75	$C_{14}H_9ClN_2O_6S$
6736976	>D-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-	28.8936	71%	204 m/z	541.06	$C_{21}H_{52}O_6Si_5$
EPA-380131	>á-D-(+)-Xylopyranose, tetrakis(trimethylsilyl) ether	29.4956	72%	191 m/z	438.85	$C_{17}H_{42}O_5Si_4$
EPA-380131	>á-D-(+)-Xylopyranose, tetrakis(trimethylsilyl) ether	30.5769	65%	204 m/z	438.85	$C_{17}H_{42}O_5Si_4$
2775908	>á-D-Glucopyranose, 1,2,3,4,6-pentakis-O-(trimethylsilyl)-	31.7783	68%	191m/z	541.06	$C_{21}H_{52}O_6Si_5$
2582798	>Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	34.5127	63%	305m/z	613.24	$C_{24}H_{60}O_6Si_6$
30645024	>Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L-	28.0439	53%	295m/z	$C_{21}H_{52}O_6Si_5$	541.06
19159252	>Sucrose (à-D-Glucopyranoside, 1,3,4,6-tetrakis-O-(trimethylsilyl)-á-D-fructofuranosyl 2,3,4,6-tetrakis-O-(trimethylsilyl)-	49.7508	57%	362m/z	910.74	$C_{36}H_{86}O_{11}Si_8$

Table 11: Bioactivity of phytocomponents identified in the ethanolic extract of Bergenia ciliata (Haw.) Sternb.

***Bergenia ciliata*(Haw.) Sternb:**	
Name of the compound	Biological Activity**
>Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-, à-D-	Decrease oxalate excretion, Xanthine oxidase inhibitor, diuretic, Decalcifier, coronary dialator, Decrease Lactate/pyruvate ratio, Anticancer, Antitumor, Inhibit production of uric acid, NADH-oxidase Inhibitor.
>D-Pinotol,pentakis(trimethylsilyl) ether	Smart drug, Anticancer, CNS-depressant, Decalcifier, Decongestant, coronary dialator, Decrease oxalate excretion, Decrease Lactate/pyruvate ratio, Dehydrogenase inhibitor, diuretic, provide vitamin D.
Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L-	Larvicidal, Lactation, Anticancer, Laxative, Anti-leukemia, Litholytic, Lymphatic diseases, oxidase inhibitor.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

>Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	Antidepression, liver problems, panic disorders, and diabetes.

(**Activity source: Dr. Duke's Phytochemical and Ethnobotanical Database)

Table 12: Molecular docking study data

Compounds	**Glycolate oxidase (GOX)**		**Lactate dehydrogenase (LDH)**	
	Docking score (kcal/mol)	Active site (Amino acids)	Docking score (kcal/mol)	Active site (Amino acids)
Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L-	-77	Trp 108 Tyr 124 Trp 127 His 160 Tyr 129	-88	Arg 168 Arg 105 Arg 98 Ile 241
Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-	-91	Trp 1 Tyr 129 His 254 Arg 164 His 264 Tyr 124	-92	Arg 168 Leu 172 Arg 105 Thr 94

FIGURES

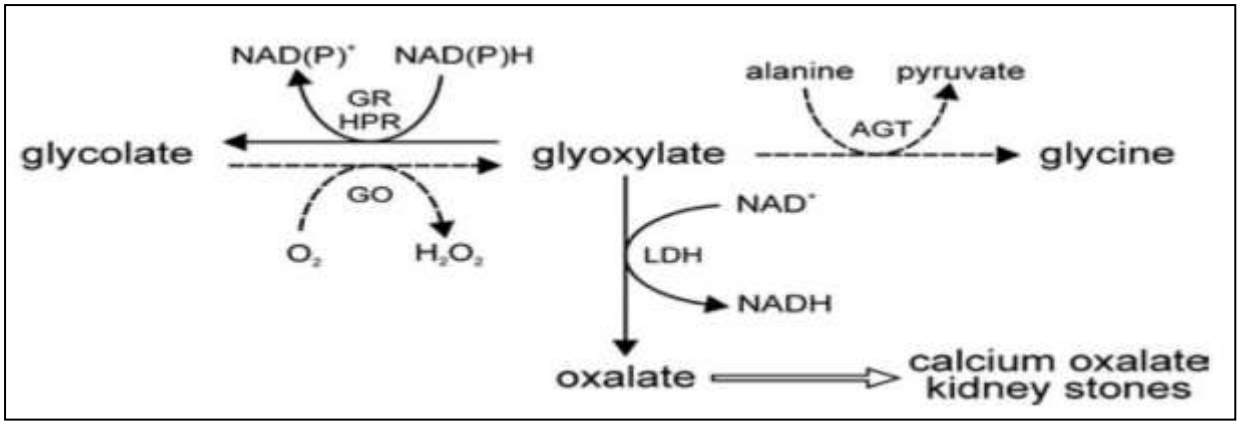


Figure1: Pathway associated with oxalate synthesis

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

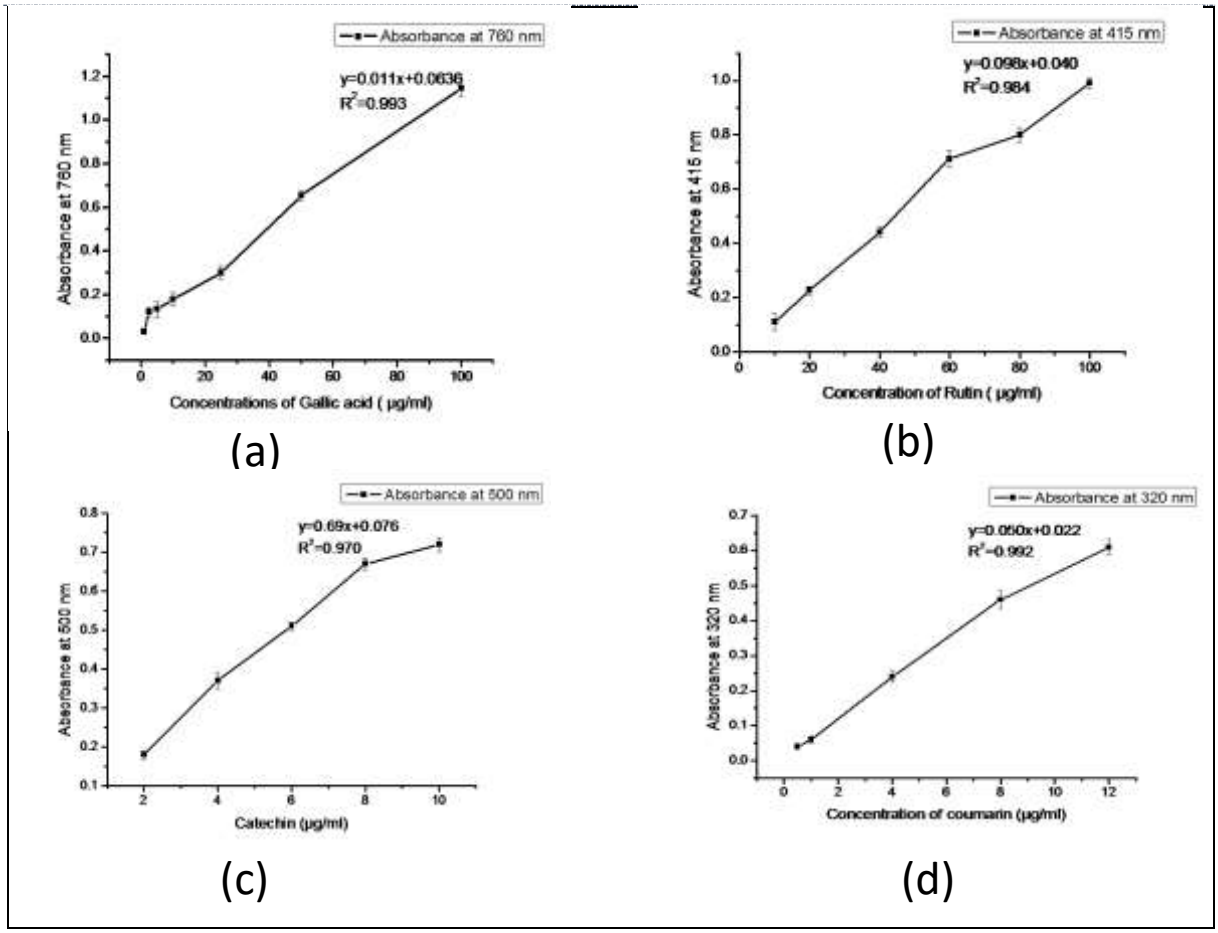


Figure 2: Standard calibration curve for total Standard calibration curve for total phenolic content (a), total flavonoids content for standard Rutin (b), total proanthocyanidinscontent for standard catechin (c) and total coumarin for standard coumarin (d).

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

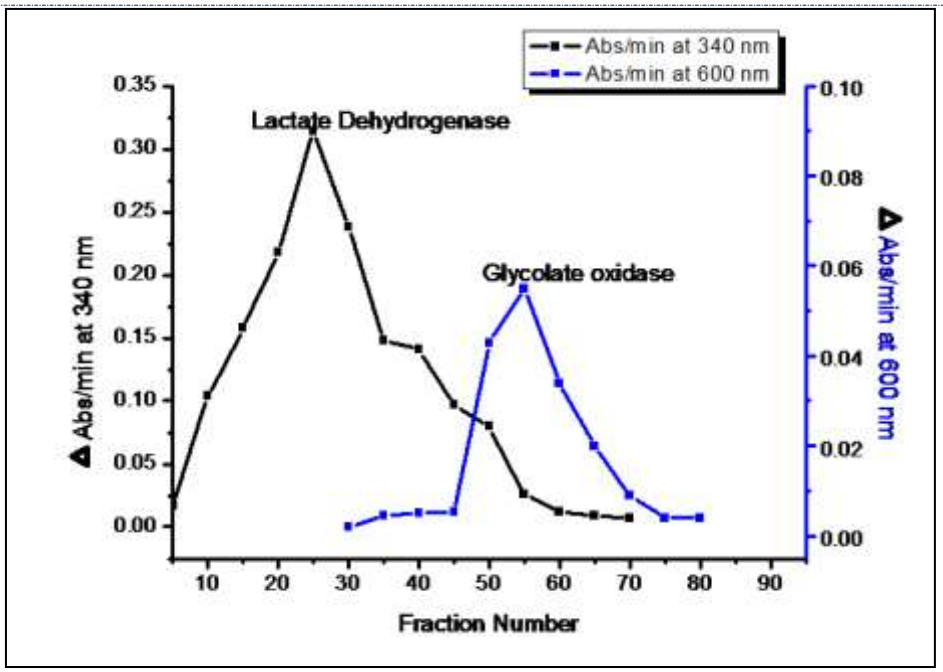
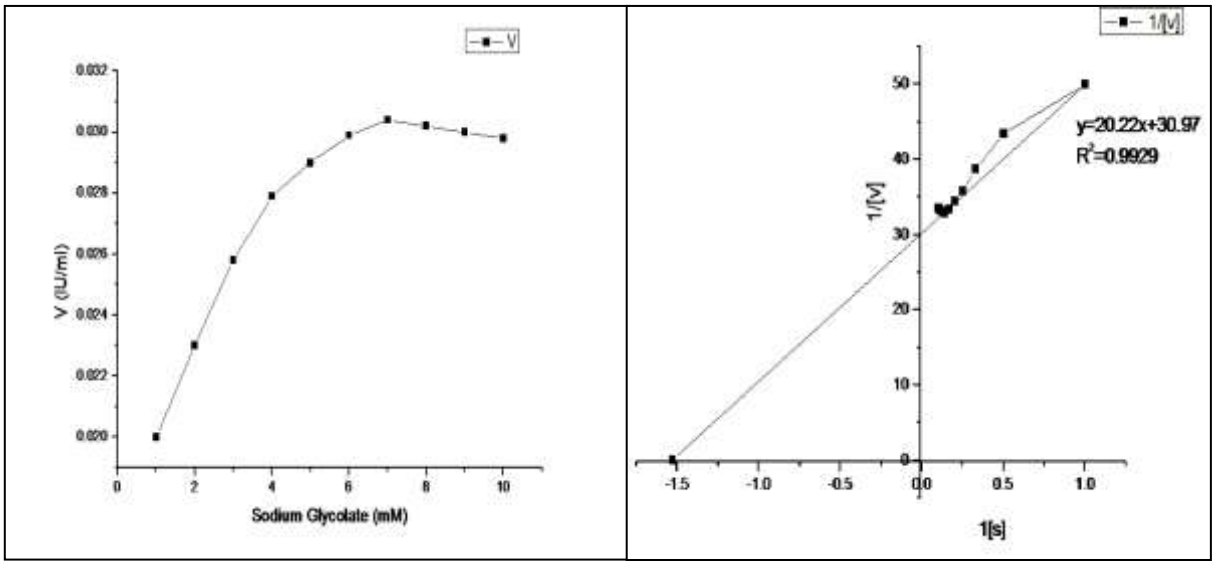


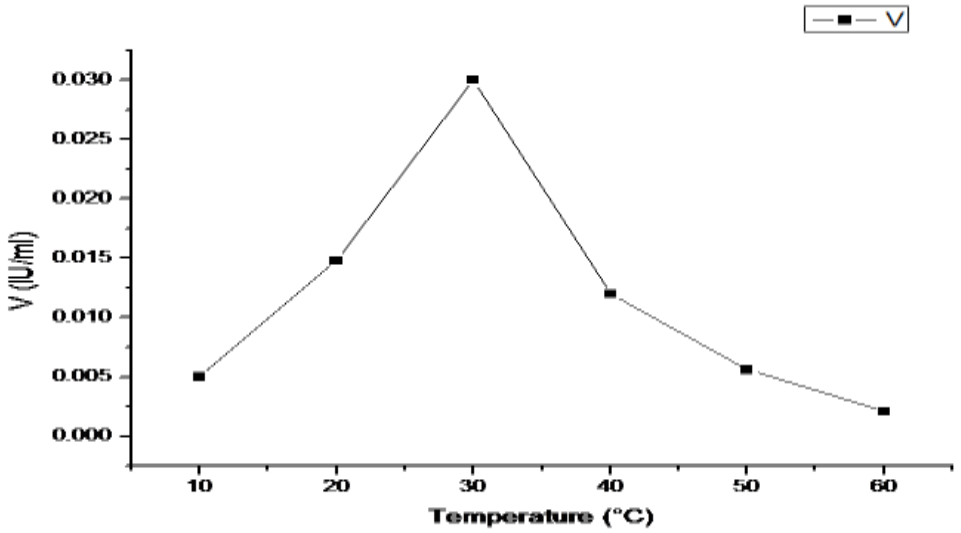
Figure 3: Separation of Lactate dehydrogenase (LDH) and glycolate oxidase (GOX) with ion exchange of chromatography on DEAE-cellulose.



Reaction mixture contained: mM Sodium glycolate as indicated; 0.2 mM potassium phosphate buffer pH-7; EDTA- 3 µmoles; DCPIP- 1 µmoles; BSA-1mg/3ml; extract protein, 16µg; total vol 3ml; at 37°C for 5 min

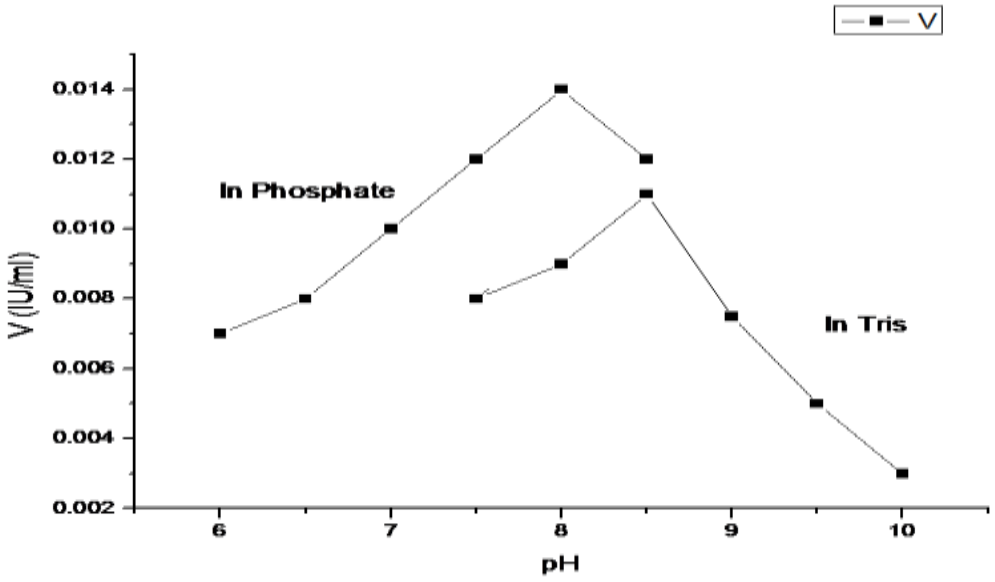
Figure 4:Effect of Substrate concentration on activity of enzyme glycolate oxidase; Lineweaver - Burk plot of glycolate oxidase activity with glycolate as a substrate

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7



Reaction mixture contained Sodium glycolate 2µmoles; 0.2 mM Potassium Phosphate Buffer pH-7.0 ; EDTA- 3 µmoles; DCPIP- 1 µmoles; BSA-1mg/3ml; extract protein 16 µg; total vol 3 ml; temp as indicated; for 5 min.

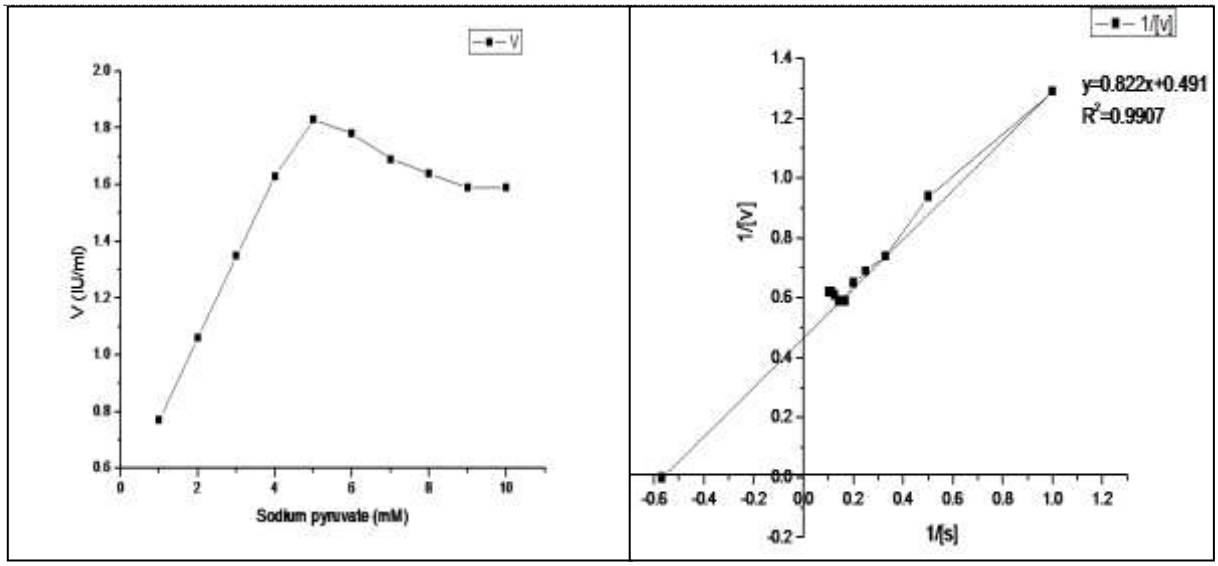
Figure 5:Effect of temperature range on enzyme glycolate oxidase activity



Reaction mixture contained Sodium glycolate 2µmoles; 0.2 mM Potassium Phosphate Buffer & 0.1 M Tris HCl Buffer; EDTA- 3 µmoles; DCPIP- 1 µmoles; BSA-1mg/3ml; extract protein; 16 µg; total vol 3 ml; at 37°C; for 5 min.

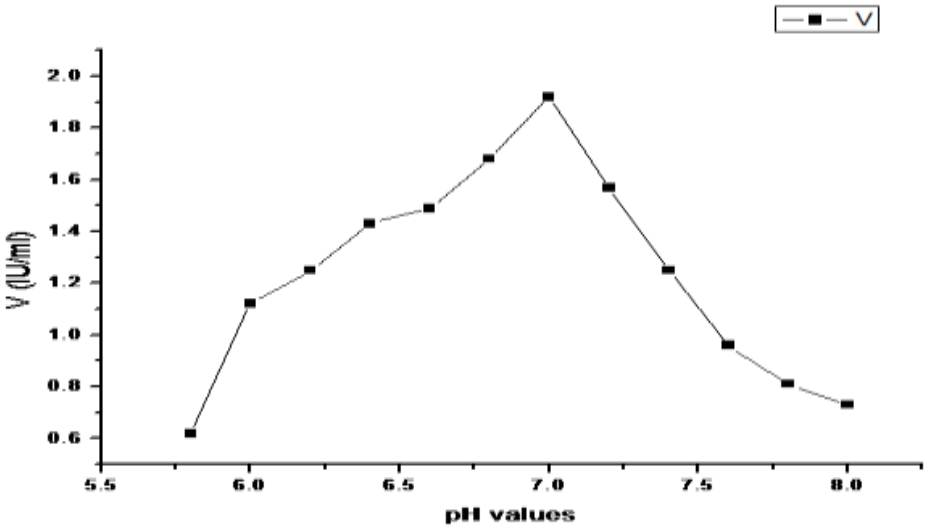
Figure 6:Effect of pH range on enzyme activity of glycolate oxidase

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7



Reaction mixture contained: mM Sodium pyruvate as indicated; 0.1M potassium phosphate buffer pH-7; NADH-4mg/3ml; extract protein, 28µg; total vol 3ml; at 37°C for 5 min.

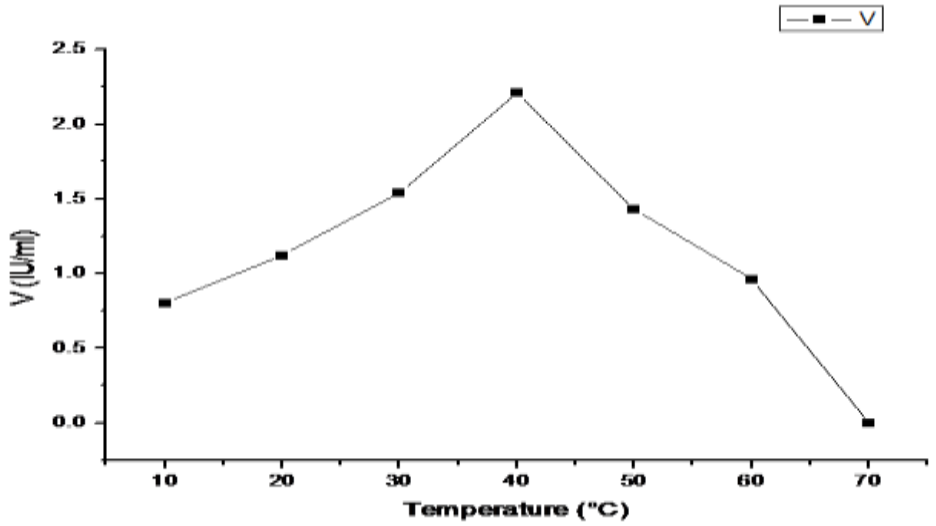
Figure 7: Effect of Substrate concentration on activity of enzyme lactate dehydrogenase; Lineweaver - Burk plot of lactate dehydrogenase activity with pyruvate as a substrate



Reaction mixture contained Sodium pyruvate 3.3mmoles; 0.1 M Potassium Phosphate Buffer ; NADH-4mg/3ml; extract protein; 28 µg; total vol 3 ml; at 37°C; for 5 min.

Figure 8:Effect of pH range on enzyme lactate dehydrogenase activity

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7



Reaction mixture contained Sodium pyruvate 3.3mmoles; 0.1 M Potassium Phosphate Buffer pH-7.0 ; NADH-4mg/3ml; extract protein 28 µg; total vol 3 ml; temp as indicated; for 5 min.

Figure 9:Effect of temperature range on enzyme lactate dehydrogenase activity

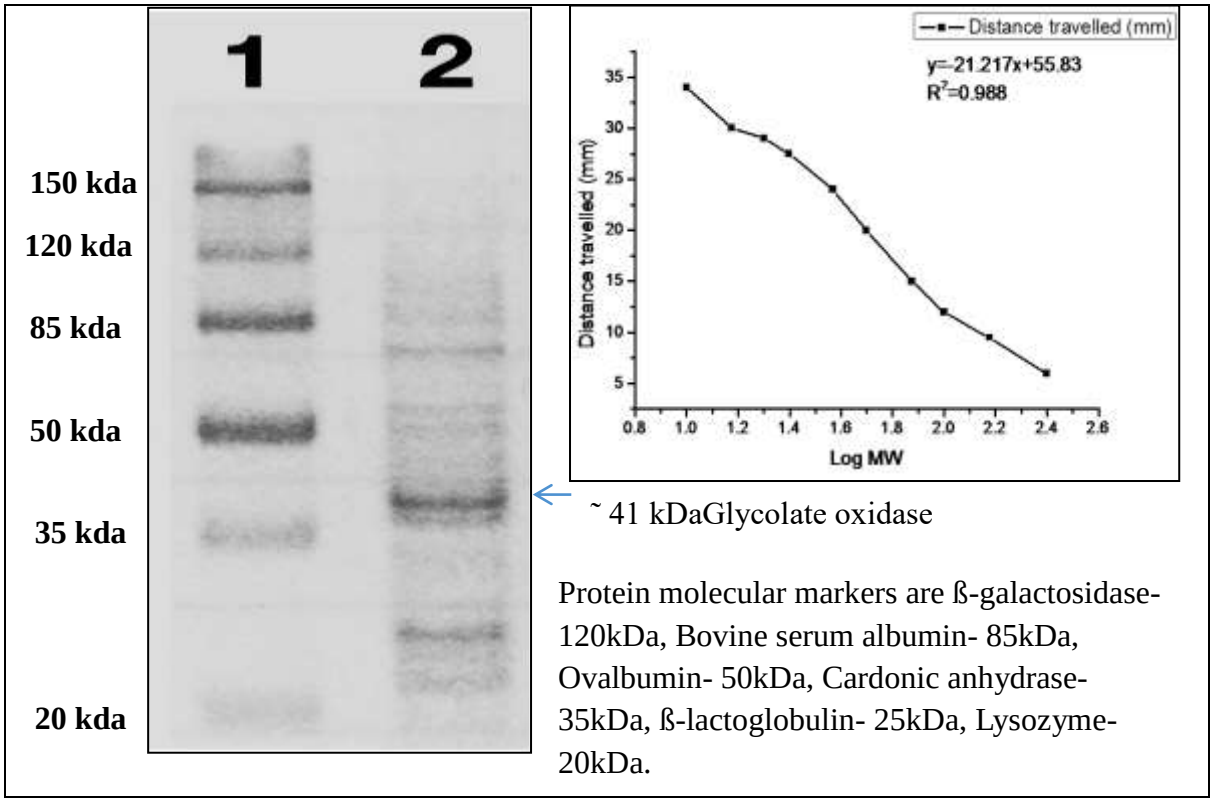


Figure 10:SDS-PAGE: Coomassie brilliant blue staining of Glycolate oxidase from rat liver (1= Marker protein, 2= DEAE-cellulose purified protein)

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

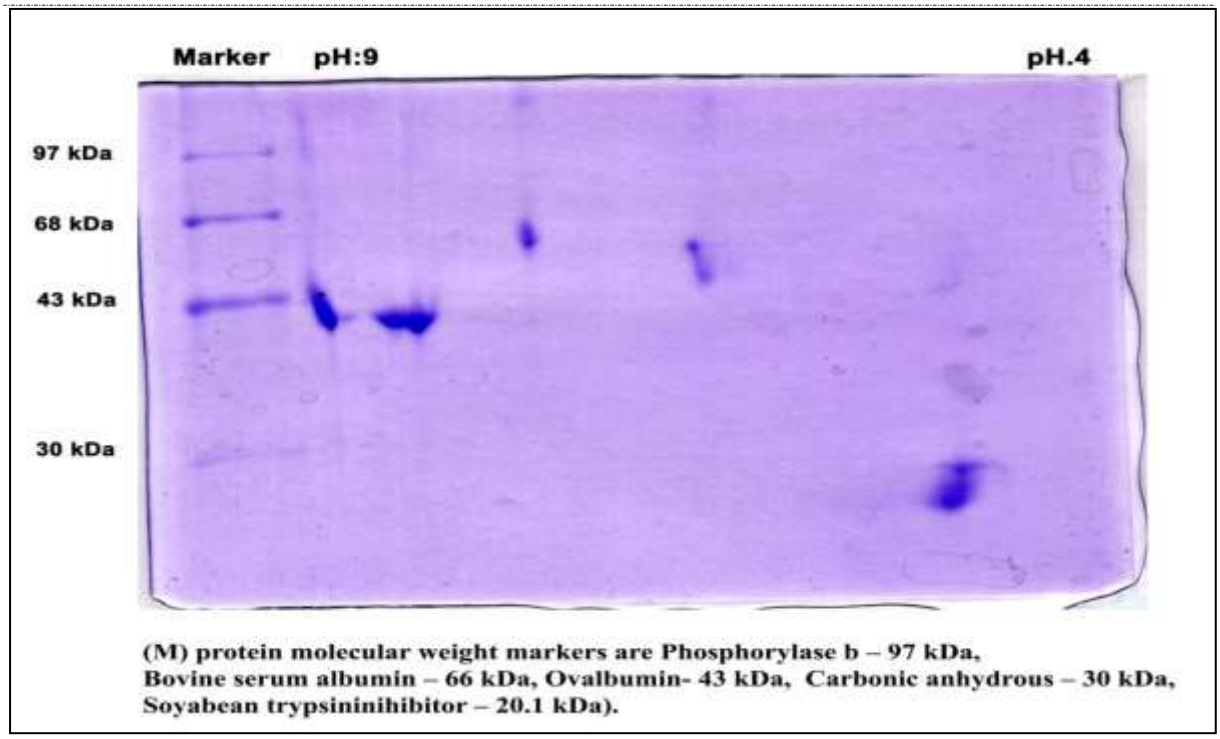


Figure 11: Determination of Isoelectric point (PI) of glycolate oxidase from rat liver by Isoelectric Focusing

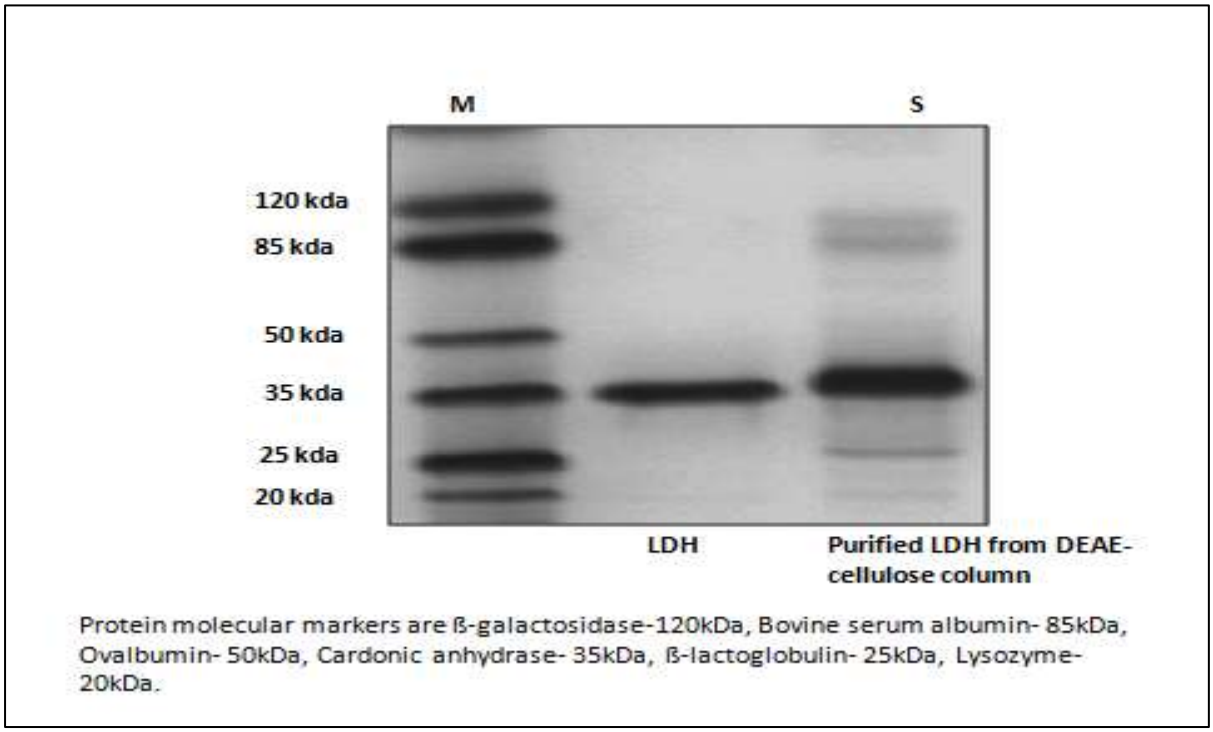


Figure 12:SDS-PAGE: Coomassie brilliant blue staining of lactate dehydrogenase from rat liver (M= Marker protein, S= DEAE-cellulose purified protein)

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

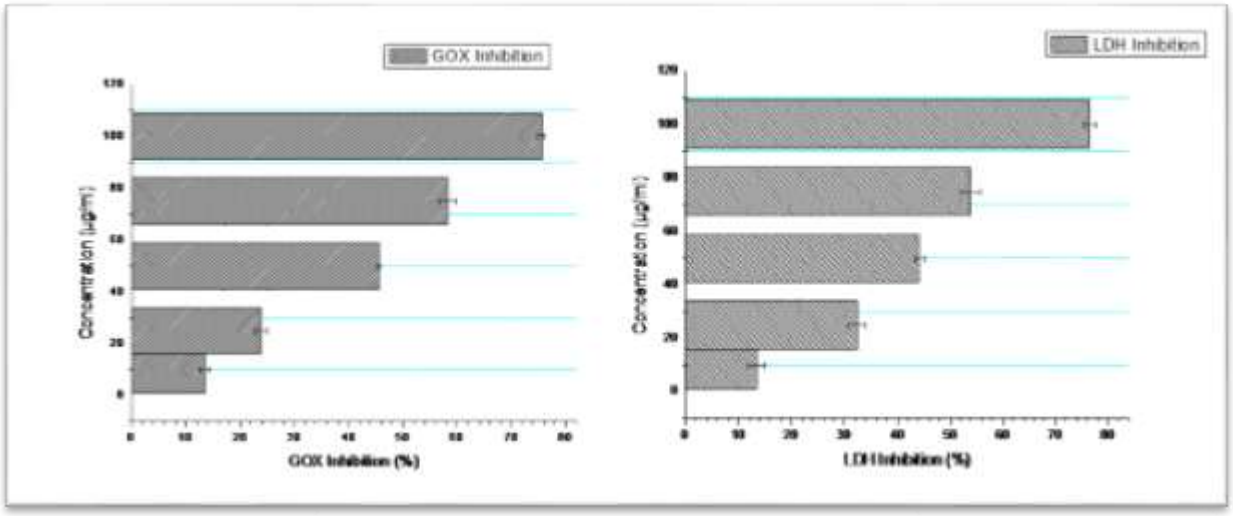


Figure 13: Glycolate oxidase and Lactate dehydrogenase enzyme inhibitor activity of ethanolic extract of Bergenia ciliata.

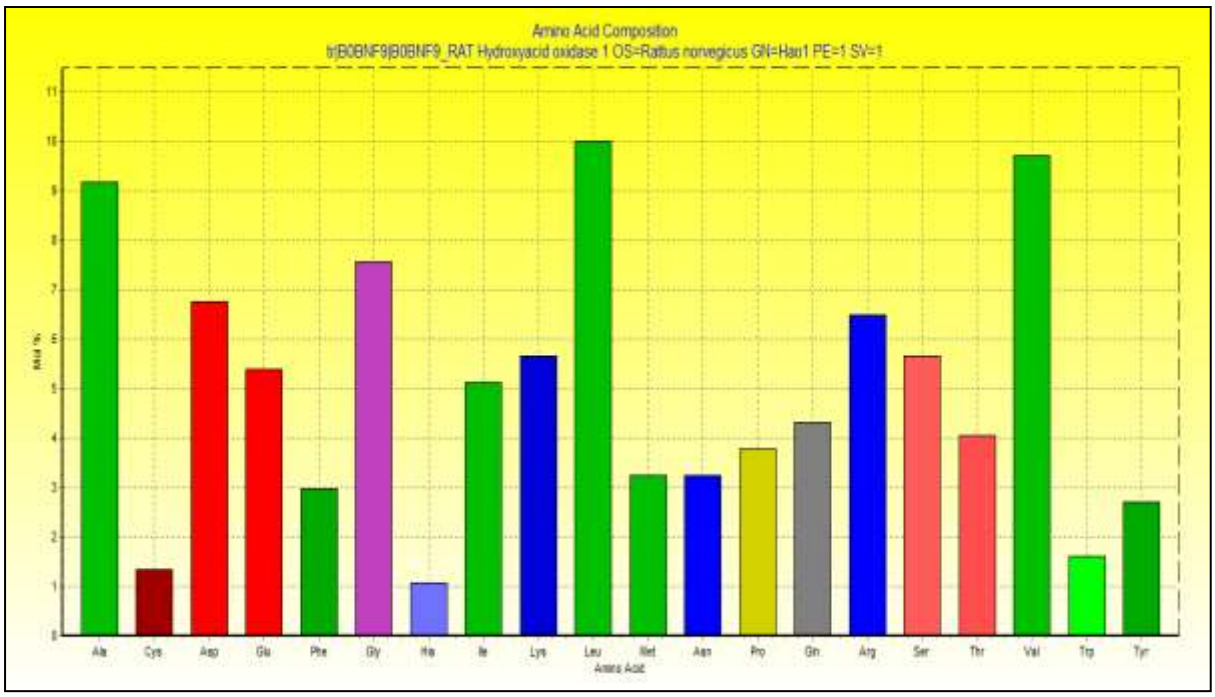


Figure 14: Determination of amino acid composition Enzyme: Glycolate oxidase

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

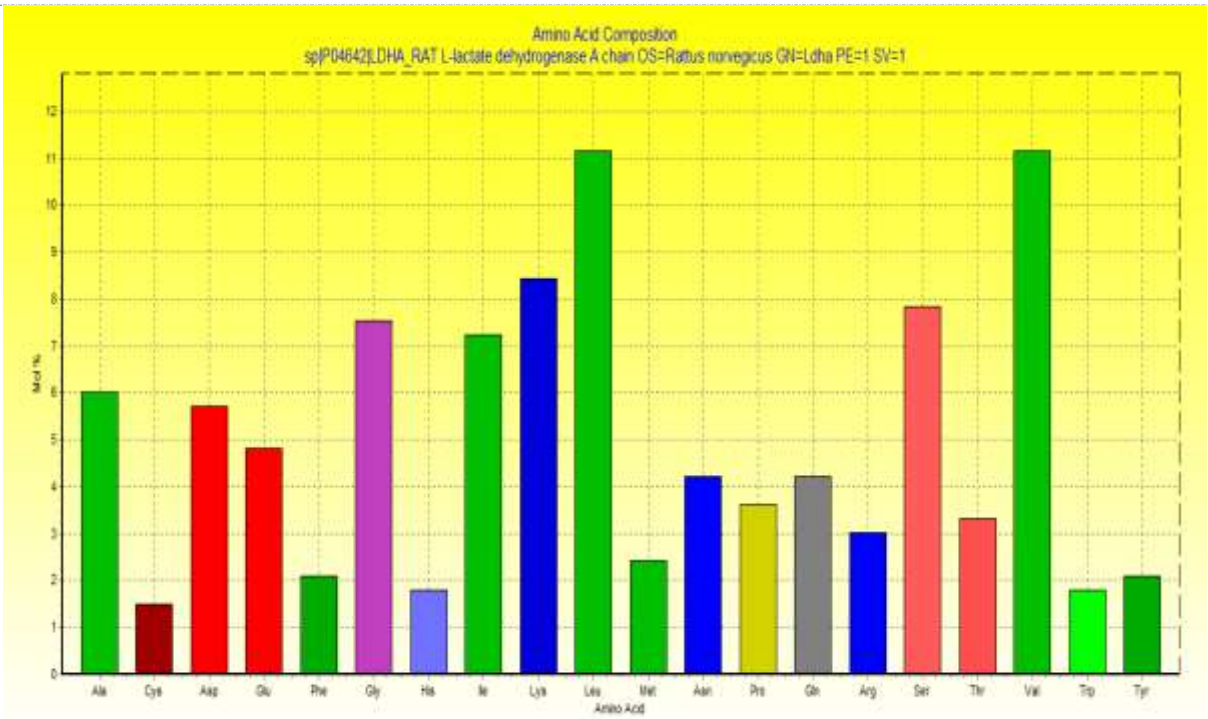


Figure 15: Determination of amino acid composition Enzyme: Lactate dehydrogenase

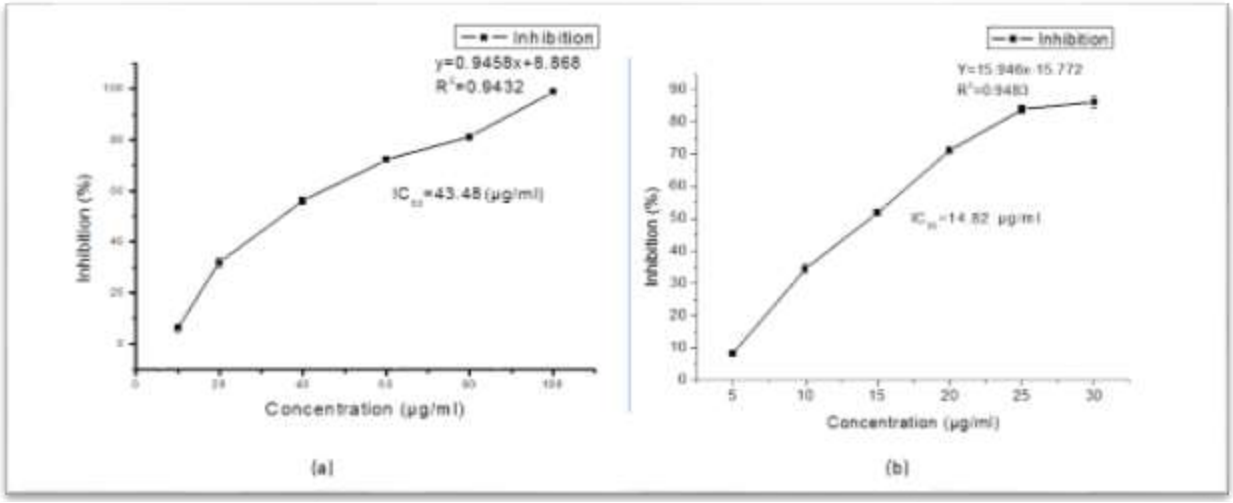


Figure 16:DPPH radical scavenging activity of ethanol extract of B.ciliata (a) and Ascorbic acid as standard (b).

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

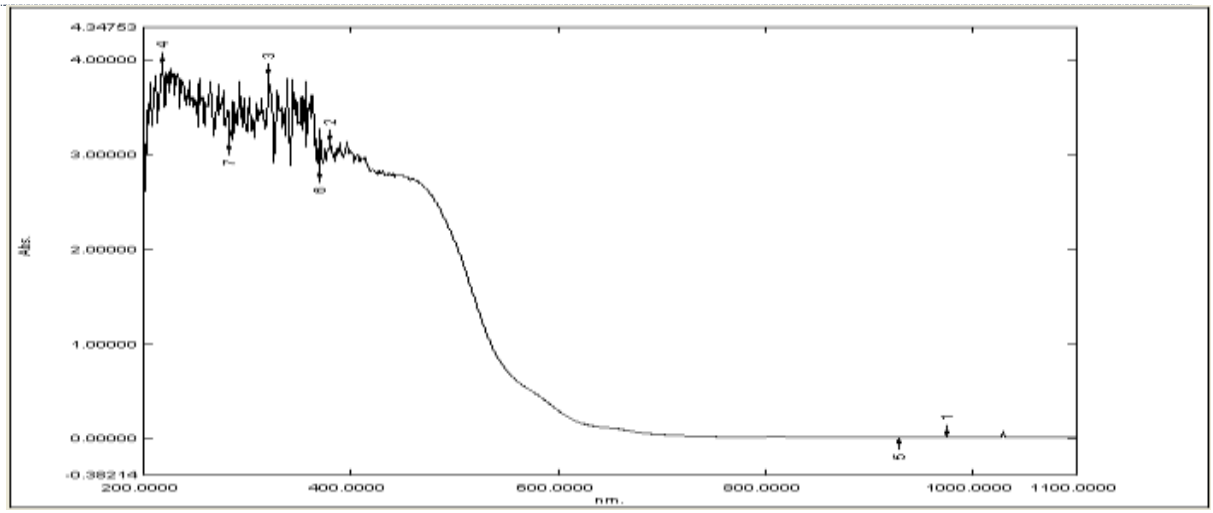


Figure 17: UV-Visible spectrum of ethanolic extract of B.ciliata.

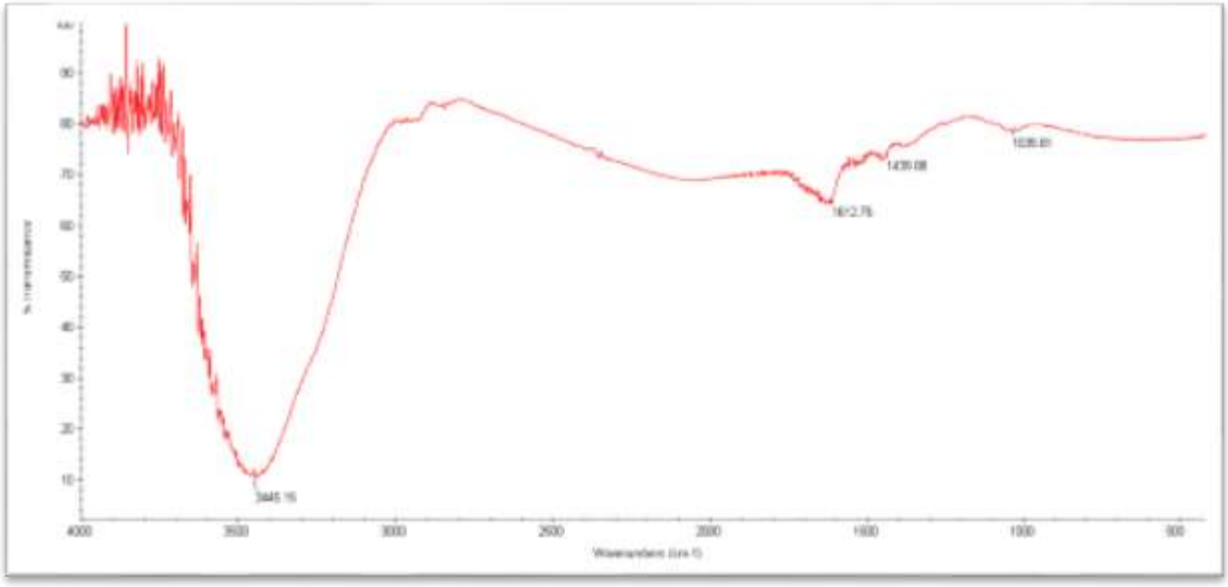


Figure 18: FTIR spectrum of the ethanolic extract of Bergenia ciliata

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

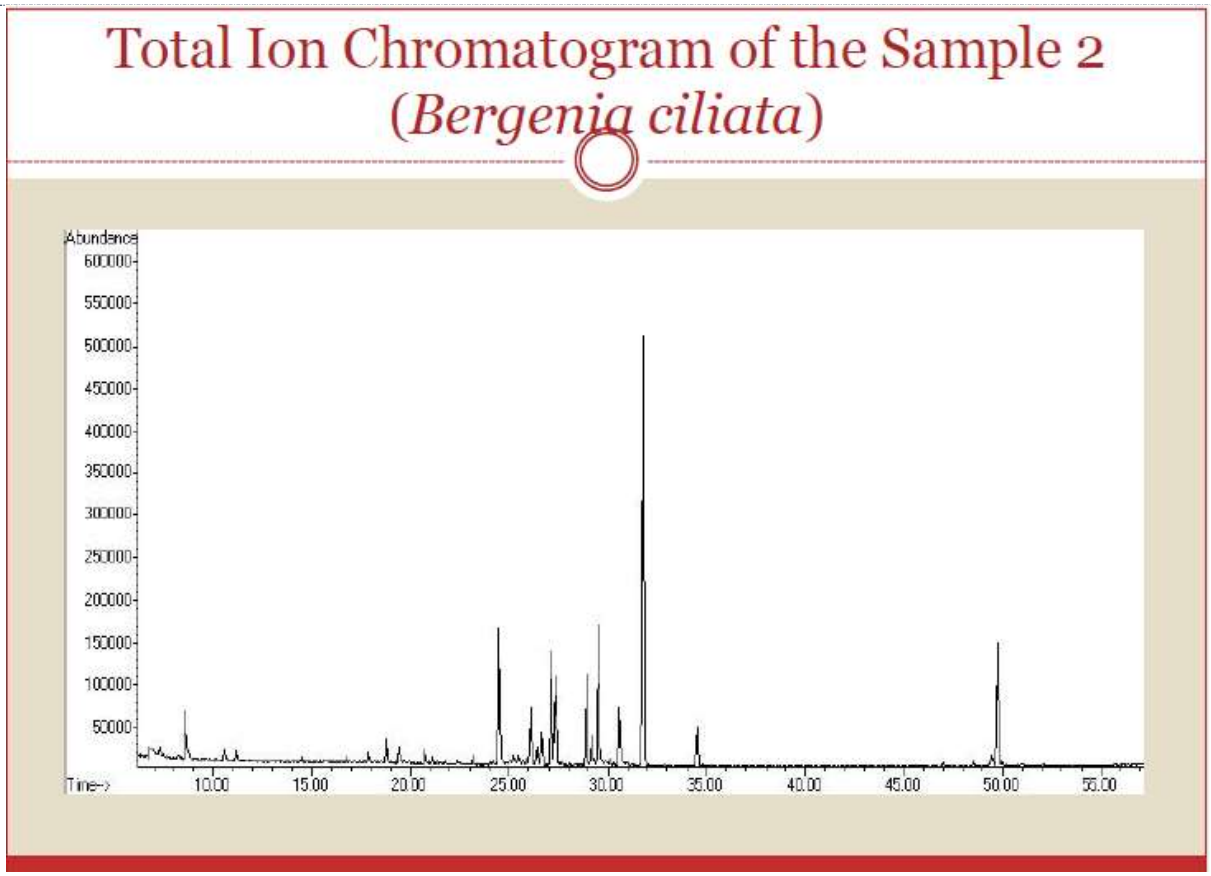


Figure 19:GC-MS of the ethanolic extract of Bergenia ciliata.

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

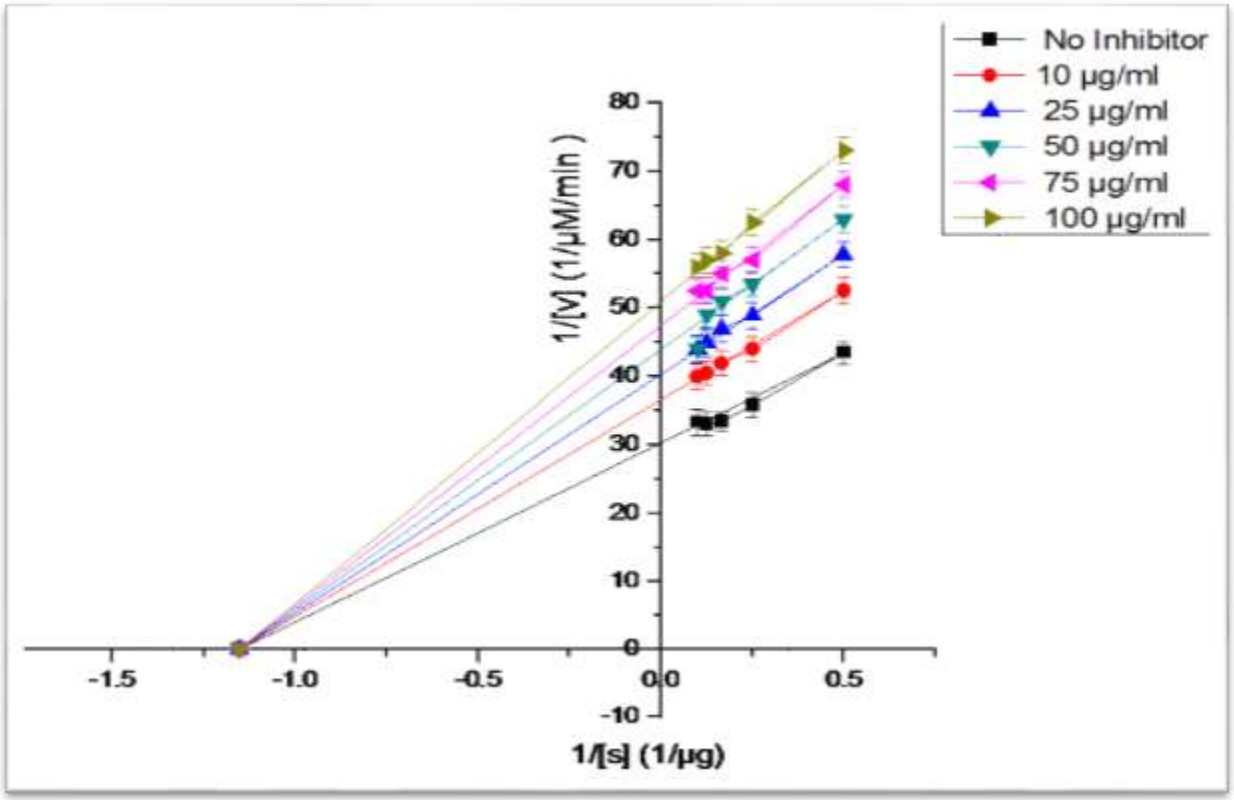


Figure 20: Lineweaver-Burk Plot of B. ciliata ethanolic extract exhibiting inhibition on liver glycolate oxidase enzyme activity. The data is indicated as the mean ± SEM; (n = 3); (P <.05).

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

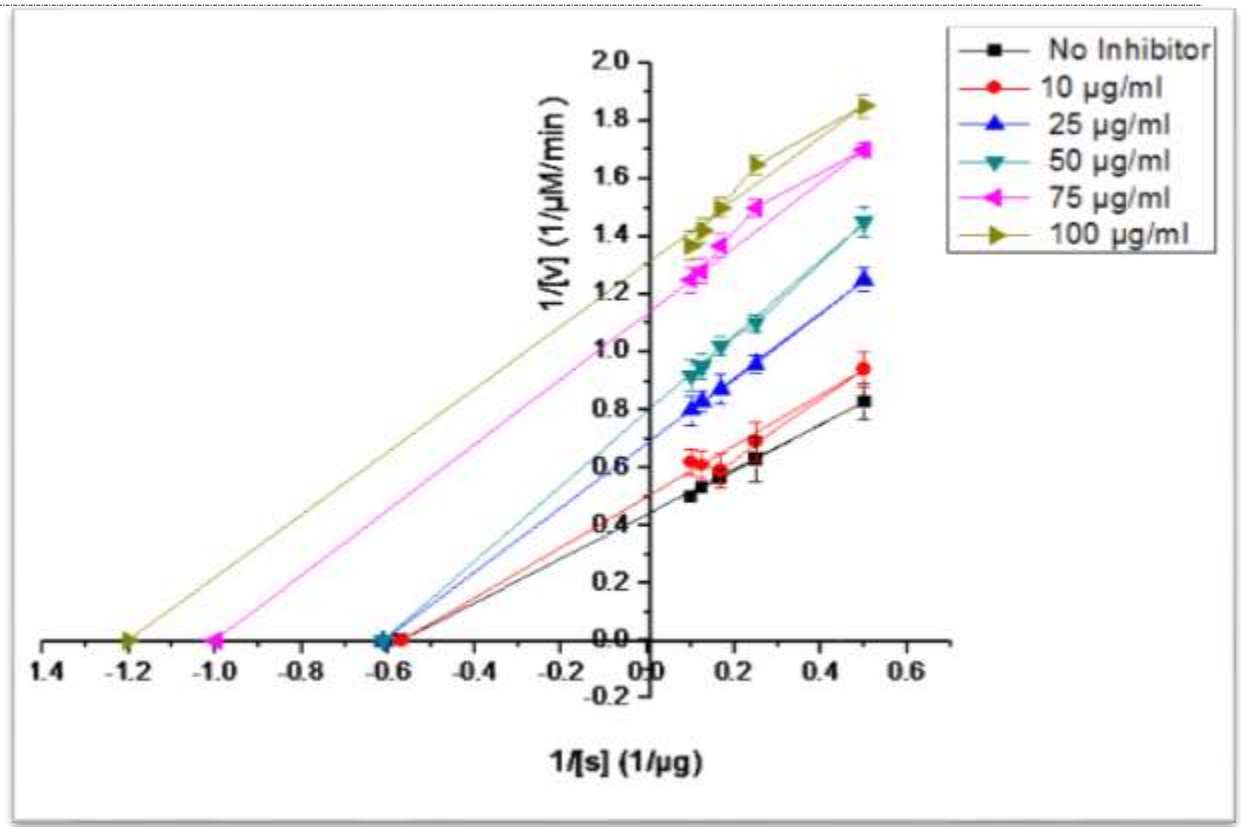


Figure 21: Lineweaver-Burk Plot of B.ciliata ethanolic extract exhibiting inhibition on liver lactate dehydrogenase enzyme activity. The data is indicated as the mean ± SEM; (n = 3); (P <.05).

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7

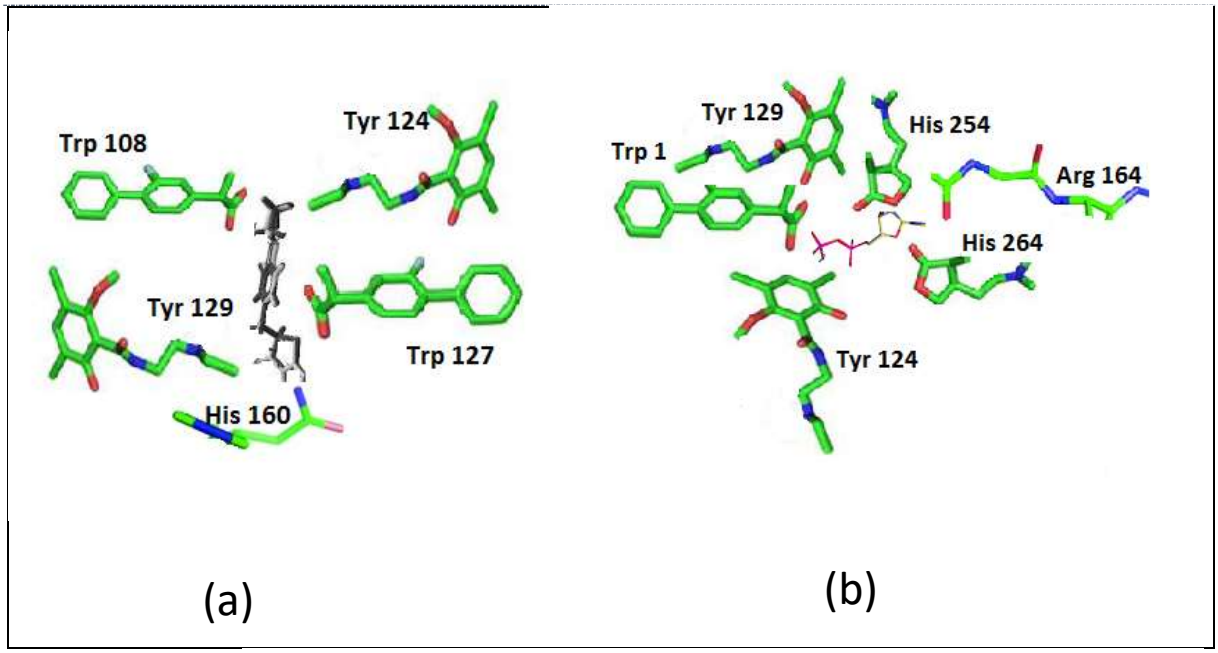


Figure 22: Docking of Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L- (a), Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- (b) with glycolate oxidase (GOX) active site

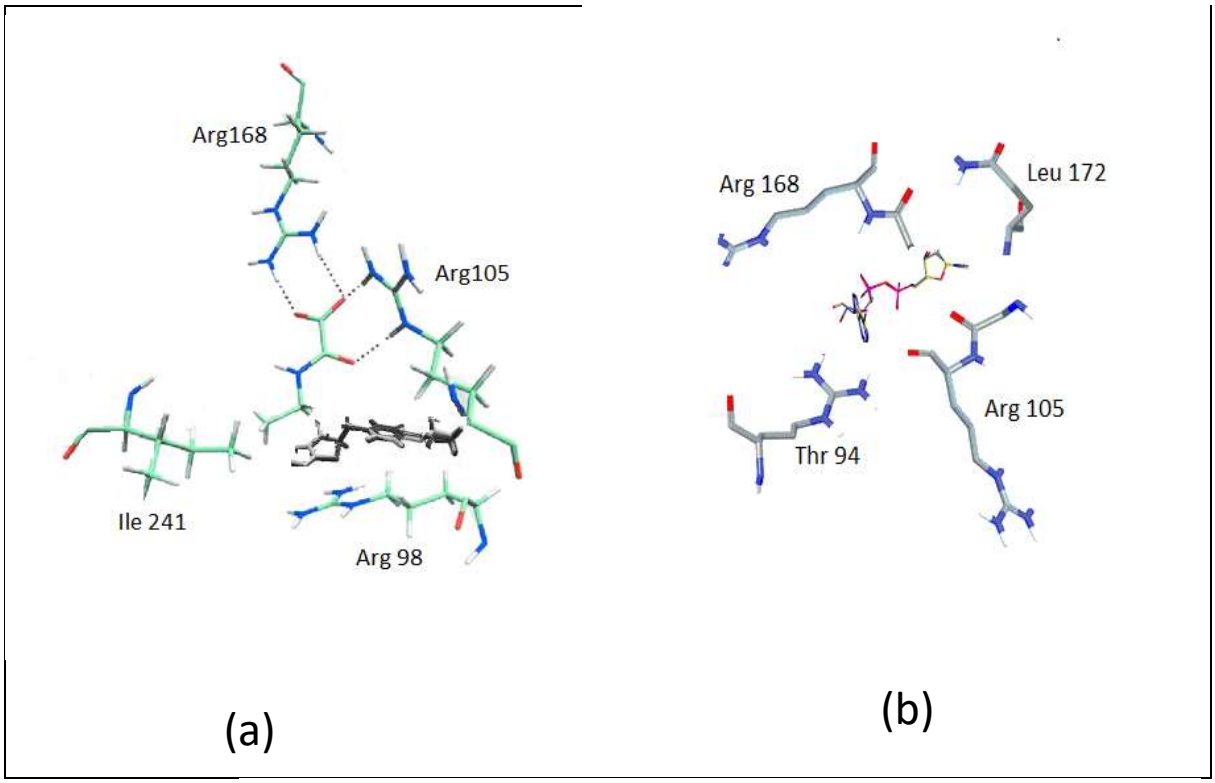
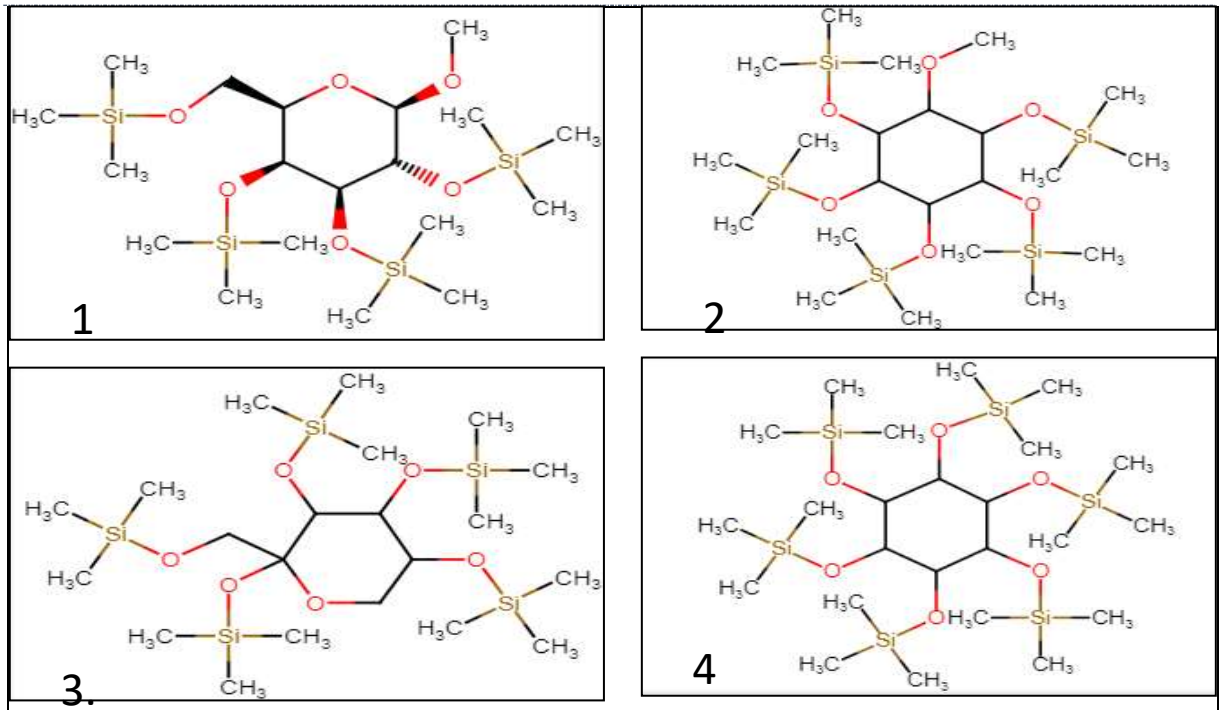


Figure 23: Docking of Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L- (a), Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)- (b) with lactate dehydrogenase (LDH) active site

ISSN: 2277-9655
Impact Factor: 5.164
CODEN: IJESS7



1. Glucofuranoside, methyl 2,3,5,6-tetrakis-O-(trimethylsilyl)-; à-D-
2. D-Pinotol,pentakis(trimethylsilyl) ether
3. Sorbopyranose, 1,2,3,4,5-pentakis-O-(trimethylsilyl)-, L-
4. Myo-Inositol, 1,2,3,4,5,6-hexakis-O-(trimethylsilyl)-

Figure 24: GC-MS analysed compounds with structure