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Characterization and Inhibition Studies of Lactate Dehydrogenase Isolated from Chicken Muscles by Aqueous and Ethanolic Leaves Extracts of Allium sativum and Gossypium arboretum

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Abstract: This work was aimed to present isolation and purification studies of lactate dehydrogenase from chicken muscles and the inhibitory effect of extracts of *Allium sativum* and *Gossypium arboreum* as potential anticancer therapies. Lactate dehydrogenase (EC.1. 1. 1. 27) was isolated from chicken muscle and partially purified by Affinity chromatography employing Cibacron Blue, 3GA Agarose and Sephadex G-25. The enzyme was partially purified 11-fold with 52.6% recovery. The enzyme has optimum pH of 7.2 and temperature of 40^0C. It has a Km and Vmax of 12mg/ml and 25µmol/min.mg^{-1} protein and pKa values of 6.8 and 7.4 respectively, which implicated ionisable groups such as imidazole of histidine and hydroxyl of tyrosine in the active site respectively. The Arrhenius plot was non linear with activation energy of 4.0 kcal/mol which could be attributed to more than one step being rate limiting at different temperatures. Double reciprocal plot of the initial velocity data of the inhibition by the extracts of *Allium sativum*, reveal a non competitive type of inhibition for both aqueous and ethanolic extracts of *Allium sativum* and competitive for aqueous extract of *Gossypium arboreum* in a dose dependent manner. The inhibition binding constants (Ki) of the extracts were 2.5, 5.0 and 4.0mg/ml. The low Ki value (2.5mg/ml) indicates high affinity of the extract or the active component (present in the plants) for the enzyme. The results suggest that the extracts of *Allium sativum* and *Gossypium arboreum* have inhibitory effect on LDH activity which reflects their potential use in the treatment of Cancer.

Key words: *Allium sativum, Gossypium arboretum*, Characterization, Inhibition of Lactate Dehydrogenase, Chicken Muscles.

INTRODUCTION

Medicinal Plants may give a chemical blue print for the development of related synthetic drugs and the natural products [1]. This may be used as building blocks for the synthesis of semi-synthetic drugs [2]. Some examples are Quinine and Artemisinin that have been used successfully against resistant strains of malaria parasites[3].

Lactate dehydrogenase has received a great deal of attention since it be may a valid therapeutic target for diseases so different as malaria and cancer[4]. In fact, the isoform expressed by *Plasmodium falciparum*, pfLDH, is a key enzyme for energy generation of malaria parasites [5]. Therefore, inhibitors of pfLDH would potentially cause mortality of *Plasmodium falciparum* and to this purpose; several small organic molecules have been recently designed and developed with the aim of

blocking this new potential antimalarial chemotherapeutic target [3, 1].

Moreover, most invasive tumour phenotypes show a metabolic switch (Warburg effect) from oxidative phosphorylation to an increased anaerobic glycolysis, by promoting an up regulation of the human isoform-5 of lactate dehydrogenase (hLDH-5 or LDH-A), which is normally present in muscles and in the liver[6]. Hence, inhibition of hLDH-5 may constitute an efficient way to interfere with tumour growth and invasiveness [7]. Using a tumour cell mammary gland transplant model, a very significant decrease in tumour growth rate and an increase in animal survival were observed using LDH-A knockdown tumour cells in comparison with tumorigenesis induced by the unmodified control tumour cells [8]. The therapeutic effect presumably is competitive inhibition of available energy molecules in fast growing tumour cells and deprive them of energy and oxygen till the tumour become dysfunctional [9].

The emergence of strains of malaria parasite resistant to conventional drug therapy and search for therapeutic selectivity of cancer cells without significant toxicity to normal cells have stimulated recent researches for antimalarial and anticancer agents with novel modes of action [10,11]. One approach to these problems is to identify an enzyme that plays a vital role both in the life of the parasite and tumour cells and has significantly different properties with enzymes catalyzing similar chemistry in the human host [10]. Such differences can be exploited in the design of inhibitor specific to the parasite and tumour cells protein, with the ultimate aim of developing therapeutic agents to target these diseases. Cancer cells and malaria parasite commonly display altered glucose metabolism (glycolytic phenotype-Warburg-effect) [9]. Several studies have revealed a functional connection between carbohydrate –driven energy production and tumour maintenance, importantly the result suggest that inhibition of a specific aspect of glucose metabolism may represent an attractive and potentially well- tolerated approach to interfering with tumour and malaria parasite growth [9,7]. Studies have shown that, blocking the conversion of glucose to lactate by LDH in tumour cells or/ inhibition of LDH stimulate mitochondrial function and drastically compromise the ability of the tumour cells and malaria parasite to proliferate [10, 7]. Therefore, inhibition of LDH may represent a rational and safe strategy for treatment of cancer and malaria. Hence the search for LDH inhibitors can be used effectively to checkmate these ailments.

A. sativum and *G. arboreum* are two indigenous plants used by herbal traditional practitioners in Tropical West Africa as herbal medicine to treat various ailments-stomach troubles, diarrhoea, dysentery, menstrual cycle disturbances, and as antibiotics, fungistatics etc. Phytochemical studies of Garlic and Onion reveal the presence of amino acid derivative compound- allyl-sulfur called allicin[12,9]. Aqueous extract of fresh Onion inhibits LDH [12]. A disesquiterpene natural product isolated from cotton plant was also effectively shown to inhibit pfLDH [13]. Therefore, this work presents inhibition of lactate dehydrogenase isolated from chicken muscles by aqueous and ethanolic leaves extracts of *Allium sativum* and *Gossypium arboreum.*

MATERIALS AND METHODS

Materials

Cibacron Blue, 3GA Agarose, Sephadex G-25, Phynylmethylsulfonylfluoride (PMSF), β-mercaptoethanol, Oxidized Nicotinamide adenine dinucleotide (NAD^+), Reduced Nicotinamide adenine dinucleotide (NADH), Sodium Chloride (NaCl), Sodium Bicarbonate ($NaHCO_3$) and Ammonium Sulphate (NH_4SO_4) were products of Sigma Chemical company, Germany. Bovine Serum Albumin (BSA) was from Fischer scientific company, Ethylene diamine tetra acetic acid (EDTA) was from Hopkin and Williams, Coomassie Brilliant Blue was supplied by Shandons southern England, and Lactic acid was from BDH Chemicals Ltd, Poole, England. All other chemicals used were of analytical grade.

Equipment

Computer-based spectrophotometer (Pharmacia LKB-BIochrom 4060), High-speed refrigerated centrifuge (Mistle Canada), pH meter (Pierron, Singapore), Weighing balance (Mettle balance; PM 16-K), Deep freezer (Thermacool, China), Pestle and mortar

Animals

A live broiler chicken was purchased from Sokoto central market, Nigeria

Plant materials:

The branches of Cotton Plant (*Gossypium arboreum*) was collected from Runjin Sambo area, Sokoto,Nigeria, while Garlic (*Alliun sativum*) was from Sokoto Central Market, Sokoto North Local Government of Sokoto State, Nigeria and brought to the herbarium, Biological Science Department, Usmanu Danfodiyo University for identification. The Cotton Plant and the bulb of the Garlic were sun-dried after identification.

Treatment of Plant materials

The leaves of *Gossypium arboreum* and the bulb of *Allium sativum* were washed to remove impurities (if any), open-air-dried and cut into small pieces, pulverized into fine powder with wooden pestle and mortar respectively and stored in plastic bags until required for use.

Aqueous Extract Preparation

One hundred grammes (100g) each of powdered *Gossypium arboreum* and *allium sativum* were dissolved in 1.0 Litre of distilled water and kept for 24 hours. Also One hundred grammes (100g) each of powdered *Gossypium arboreum* and *Allium sativum* were dissolved in 1.0 Litre of ethanol and kept for 24 hours. The extracts were then filtered through Muslin cloth and subsequently through Whatman No. 1 filter paper separately. The filtrates were then dried in the drying cabinet [14]. Five percent (5%) and ten percent (10%) concentrations of aqueous and ethanolic extracts of *Gossypium arboreum* and *Allium sativum* were prepared.

General Procedure

A 10-fold dilution of a 1 mg/ml BSA sample was prepared by addition of 100µl BSA to 900µl of distilled water to make 100µg/ml BSA; 25µg/ml, 50µg/ml, 75µg/ml, 150µg/ml and 200µg/ml standards were also made in different test tubes. To each test tube 5ml of Coomassie Blue was added, mixed and allowed to stand for about 5 minutes. Absorbance was taken after adjusting the spectrophotometer to a wavelength of 595nm.

Muscle Homogenization

A total of 50g of chicken breast muscle were cut up into pieces with razor blade. Connective tissue and fat were discarded. Minced tissue was placed in a beaker containing 75ml of cold extraction buffer and transferred to a blender; tissue was disrupted by the use of 4*30 second burst, with at least 10 seconds intervals, just to decrease the temperature of the homogenate.

The homogenized tissue/buffer mixture was transferred into six pre-chilled 15ml centrifuge tubes and balanced well. The homogenate was centrifuged for 20 minutes at 15,000g. Supernatant was collected and kept on ice until required.

Ammonium Sulphate Precipitation

Slowly (over a period of 15 minutes) 19.89g ammonium sulphate were added into 51ml supernatant to produce a 60% saturated solution of ammonium sulphate (0.39g of ammonium sulphate per ml of supernatant).The salted supernatant was stirred for additional 15 minutes to dissolve the ammonium sulphate and allow the protein to equilibrate to the presence of the ammonium sulphate. The procedure was carried out on ice. Fifty seven millilitres (57ml) of the sample was centrifuged again, supernatant volume was recorded and used for protein content and enzyme activity determinations. Ammonium sulphate pellet was suspended in 2ml Tris-PMSF (pH 8.6) buffer and mixed gently until the solid material dissolved completely and was kept in the freezer.

Sephadex G-25 Gel Preparation

Seven and half grammes (7.5g) of dry Sephadex G - 25 were suspended in 30ml Tris-PMSF buffer (pH 8.6) solution and allowed to swell for 3 hours at room temperature. The supernatant was decanted and allowed to equilibrate to room temperature.

Column Packing

The entire slurry of (Sephadex G-25) was poured into the column (1.0x20cm) in one portion, using glass rod to prevent bubble formation. Gravity flow was initiated to start packing and the procedure was continued until constant bed height was maintained. Five millilitres (5.0ml) of the sample were loaded on to the column and the remaining buffer was allowed to drain and the eluent was discarded. Protein from the desalting column was eluted by prompt addition of 4.0ml Tris-PMSF buffer (pH 8.6) to the column, eluent was collected and measured. The volume was recorded and the sample kept on ice.

Affinity Chromatography

The column (1.0x30cm) was fixed in a vertical position; small plug of glass wool was pushed into the column tightly against the lower end. Five millilitres (5.0ml) of Tris-PMSF buffer pH 8.6 were added using glass rod against the wall of the column. The fluid was drained until it was just above the top of the glass wool. And no air bubbles were trapped. Five millilitres (5.0ml) of Cibacron blue 3GA agarose suspension were applied on to the column and allowed to settle. The column was equilibrated by passing 10ml Tris –PMSF buffer (pH 8.6) .Flow rate was maintained at 1ml/minute and the flow through was discarded. Five millilitres (5.0ml) of desalted protein solution were loaded on to the column and 5ml fraction was collected, the flow rate was kept constant (1.0ml per minute).

Tris-PMSF Wash 1

The column was filled with Tris- PMSF buffer (pH 8.6) and 5.0ml fractions were collected until when the absorbance was less than 0.1 above the absorbance of Tris- PMSF buffer.

NAD Wash

After the remaining Tris –PMSF buffer (pH 8.6) has drained to the frit ten millilitres (10.0ml) NAD buffer were added on to the column and 5ml fractions were collected at interval of five minutes.

Tris- PMSF Wash 2

When all the liquid had entered the column, Tris-PMSF buffer (pH 8.6) was also applied and 5.0ml fractions were collected at intervals of five minutes until the absorbance was 0.1 above the absorbance of the Tris- PMSF buffer.

NADH Wash

After draining the remaining buffer, 10.0ml NADH buffer were added to the column and 5.0ml fractions were collected and flow rate was maintained at 1ml per minute.

Tris- PMSF Wash 3

Ten millilitres (10.0ml) of Tris –PMSF buffer (pH 8.6) were added to the column and 5.0ml fractions were collected at intervals of five minutes, until absorbance was 0.1 above the absorbance of the Tris –PMSF buffer. All fractions were stored in the freezer at 4^0c.

Characterization of Purified LDH

Lactate dehydrogenase was purified from Chicken muscle by the principle of Affinity Chromatography as described by Michelle [15] using Cibacron blue 3GA Agarose column.

Determination of Activity of LDH from Chicken Muscle

Lactate dehydrogenase activity was determined spectrophotometrically as described by the method of Mcmanus and James [16]. The amount of NAD^+ converted to NADH is measured as soon as the reaction is initiated. This takes the advantage of the fact that NADH has an absorbance peak at 340nm while NAD^+ exhibits little absorbance at this wavelength. Three test tubes were set in test tube rack; assay was run in duplicate and blank for each. The assay mixture contained 0.6ml lactic acid solution in 10mM Tris-Hcl buffer (pH 8.6), 0.4ml NAD stock solution in 10mM Tris-Hcl buffer (pH 8.6) and 0.2ml bicarbonate stock solution in a cuvette. Hence, to the reaction tubes 10µl of the partially purified enzyme was added and covered with aluminium foil and mixed.

At exactly one minute absorbance was read at 340nm wavelength after the spectrophotometer was blanked. The assay was repeated for each fraction.

One unit of activity of the enzyme, defined as level of activity that produces one mole of NADH per minute at room temperature, was calculated using the relation.

$A = e\,cl$

Where l is almost always 1and e, which is a characteristic of the molecule in question, is $6220M^{-1}cm^{-1}$ for NADH.

Effect of Substrate Concentration

Lactic acid concentration was varied over the range of 5-15mM and reacted with an aliquot (10µl) of the partially purified LDH enzyme in the presence of 0.4ml NAD stock solution and 0.2ml Bicarbonate stock solution. Double reciprocal plot (Lineweaver-Burk's plot) of [S] against V_0 was plotted to calculate Vmax and Km of the enzyme.

Effect of pH on the activity of LDH

The buffer (Tris HCl buffer) was prepared at different pH in the range of 4.0-9.0. A solution (10µl) of the partially purified enzyme was dispensed in to two (2) different test tubes containing substrate solutions and plot of the pH versus lactate dehydrogenase activity was constructed to determine the pH optimum.

Effect of pH on Km and Vmax of LDH

The LDH activity was assayed according to the method of Mcmanus and James [16]. The assay procedure was largely the same except for the change in buffer pH for each initial rate study. The initial rate study was conducted using pH, 4, 5, 6, 7, 8 and 9.0. Lineweaver-Burk plots of the values obtained at different pH were used to determine Km and Vmax at each corresponding pH and values of Vmax/Km were also determined by plotting V against V/s.

Effect of Temperature on the Activity of LDH

The effect of temperature on rate of production of NADH from LDH reaction was studied at 20^0C, 30^0C, 40^0C, 50^0C, 60^0C and 70^0C. A solution (10µl) of the partially purified enzyme was dispensed in to twenty (20) different test tubes containing substrate solutions and incubated at each of the temperatures above for five (5) minutes. A plot of temperature versus LDH activity was used to determine optimum temperature.

Effect of the extracts of *A. sativum* and *G. arboreum*. on LDH activity

Both aqueous and ethanolic extracts of *G. arboreum* and *A. sativum* were prepared in duplicates 5% and 10% according to the method of Stephens [14]. One millilitre (1ml) of the extract was taken from each stock and added to the reaction mixture for 5 minutes. Initial velocity studies were carried out as previously described in the determination of activity of LDH.

RESULTS

The summary of the purification results for LDH is presented in Table 1.

Table 1. Purification profile of LDH from Chicken Muscle

Step	Total Protein (mg)	Specific Activity (µmol/min)	Total Activity (unit/min/ml)	Purification Fold	% Yield
Crude	82.7	3.47	285	1.00	100
Desalted	5.4	25	135	7.4	47.4
Fraction 6	5.0	26.4	132	7.8	46.3
Fraction 7	4.5	31	143	9.1	50.2
Fraction 12	4.0	35	143.5	10.6	50.3
Fraction 13	4.0	37.5	150	11.0	52.6

The crude homogenate had the highest amount of LDH as well as the greatest total LDH activity and % yield while producing the lowest specific LDH activity and fold purification. Of the column fractions, 6 had the largest LDH concentration while 13 had the highest specific LDH activity, fold purification and % yield. The desalted protein solution had a larger protein concentration compared to individual column fractions.

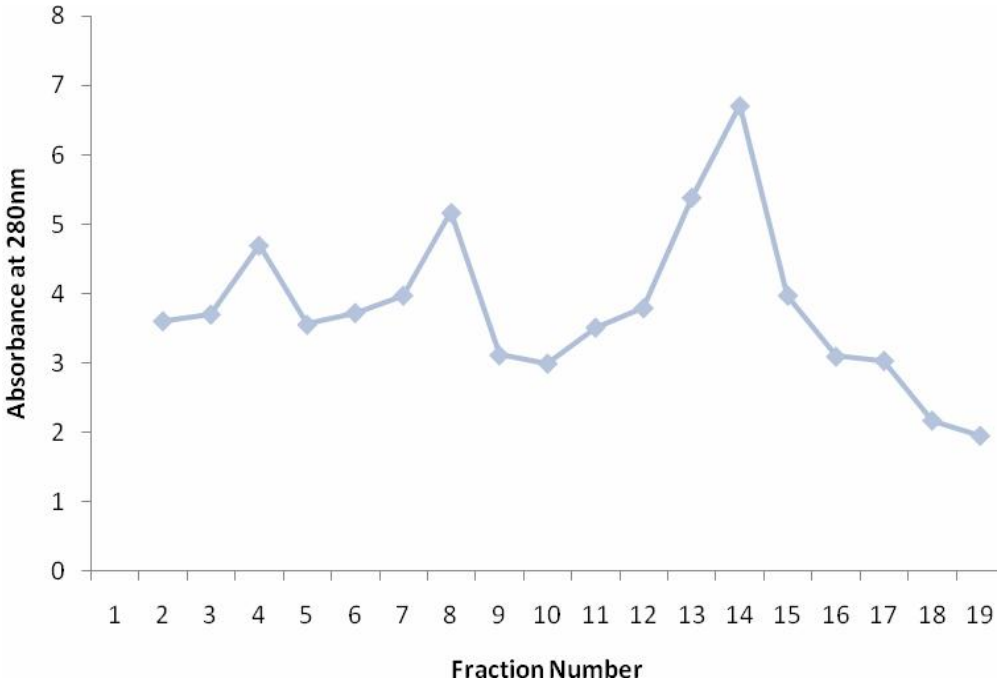

Figure 1. Elution profile

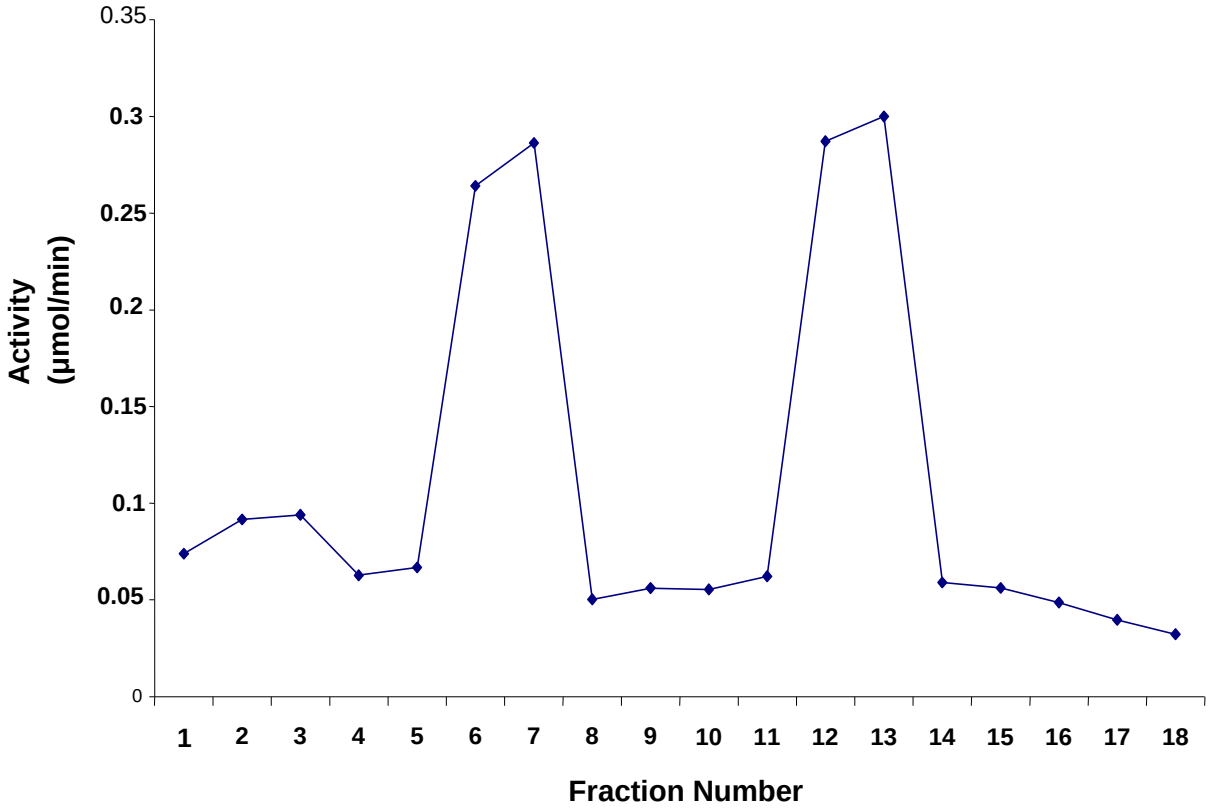

Figure 2. Elution Profile of LDH on Cibacron blue 3GA Affinity Column The active peaks produced a final purification fold of 11.0 for LDH activity and 52.6% recovery.

Table 2. Mean values of Vmax/Km at different pH values

pH	Vmax/Km
4.0	0.0046
5.0	0.0050
6.0	0.0082
7.0	0.0116
8.0	0.0082
9.0	0.0052

pKa values of 6.7 and 7.4 were extrapolated and indicates the imidazole groups of Histidine and hydroxyl groups of Tyrosine respectively.

Table 3. Mean values of double reciprocal plot of LDH at different substrate concentrations.

1/[s]	1/v
0.2	0.15
0.1	0.11
0.07	0.08

Lineweaver-Burk's plot of the data revealed that LDH enzyme has Vmax and Km of 25µmol/min/mg and 12mg/ml respectively as shown in figure 7.

Table 4. Mean values of double reciprocal plot at different aqueous extract of *Allium sativum* concentrations

1/[s]	5%	10%
0.2	0.35	0.69
0.1	0.30	0.51
0.07	0.20	0.34

Table 5. Mean values of double reciprocal plot at different fixed ethanolic extract of *Allium sativun* concentrations.

1/[s]	5%	10%
0.2	0.29	0.38
0.1	0.20	0.25
0.07	0.16	0.20

Table 6. Mean values of double reciprocal plot at different fixed aqueous extract of *Gossypium arboreum* concentrations

1/[s]	5%	10%
0.2	0.20	0.30
0.1	0.17	0.20
0.07	0.13	0.16

Table 7. Mean values of double reciprocal plot at different fixed ethanolic extract of *Gossypium arboreum* concentrations

1/[s]	5%	10%
0.2	0.13	0.14
0.1	0.09	0.12
0.07	0.008	0.10

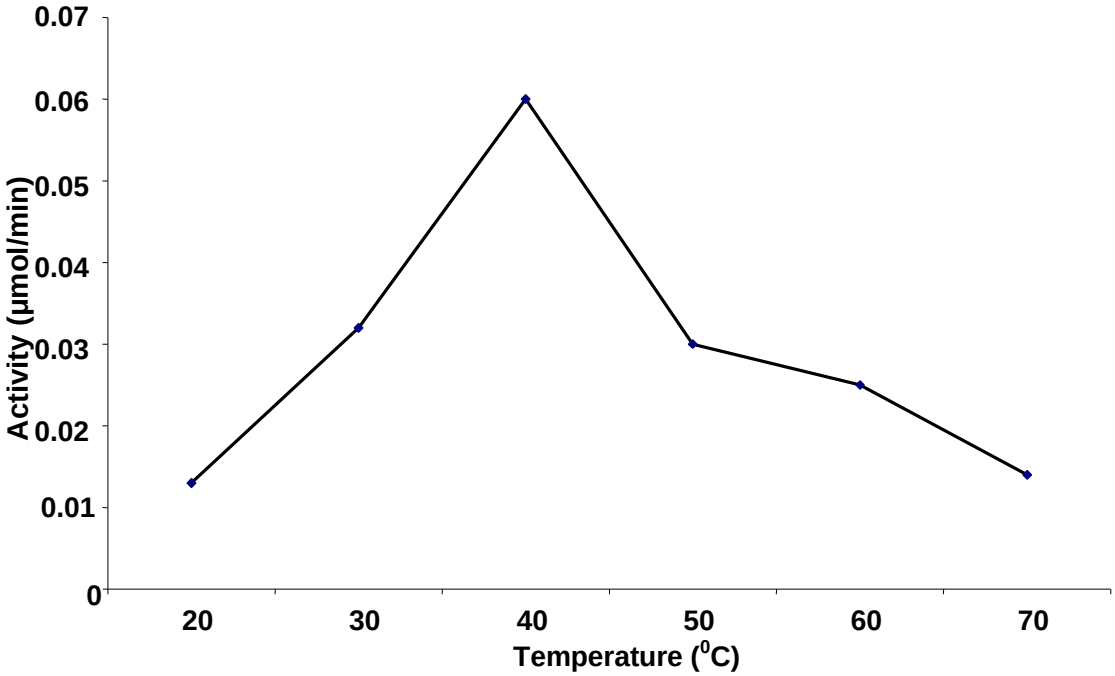

Figure 3. Effect of Temperature on LDH Activity with optimum activity at 40^{o}c.

Thermostability of Chicken Muscle LDH

The values obtained in the above plot, represent a graphic relationship between temperature and LDH activity which depicted a typical dumb-bell shaped curve. The purified enzymes produced a very broad temperature range with optimum activity at 40^{0c}.At incubation temperature of 50^{0c} the enzyme retained 50% activity. However, the activity dropped drastically by more than 75% at incubation temperature of 70^0C.

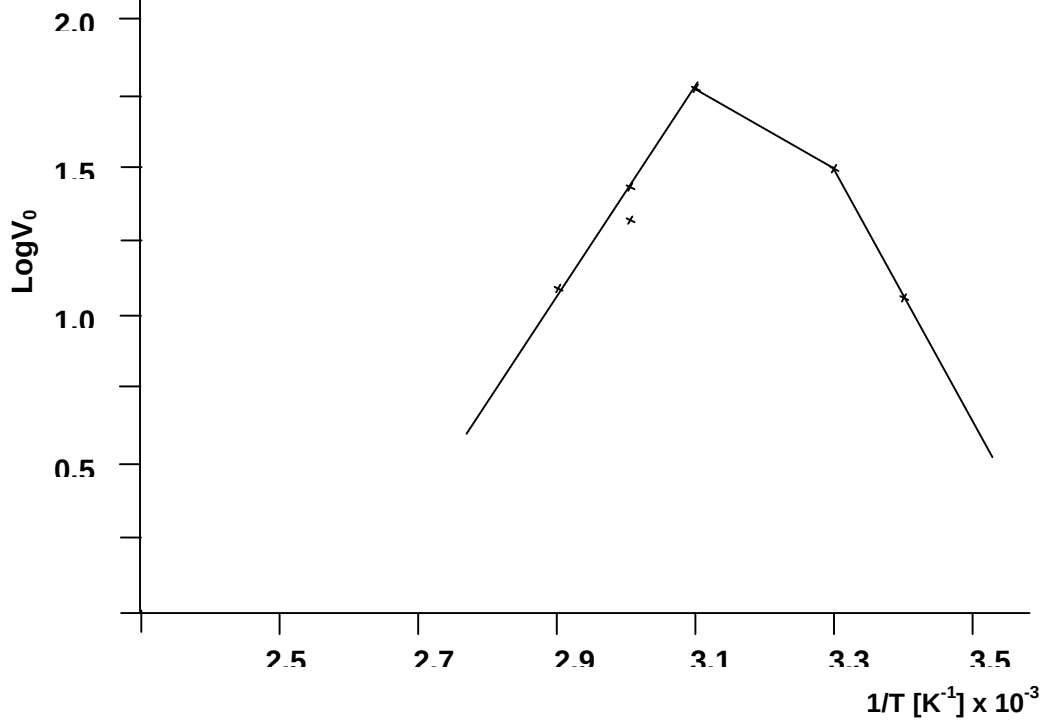

Figure 4. Arrhenius plot for LDH Activity: Depicts a plot of log of initial velocity versus the reciprocal of the temperature in Kelvin with activation energy (Ea) of 4.0 Kcal/mol.

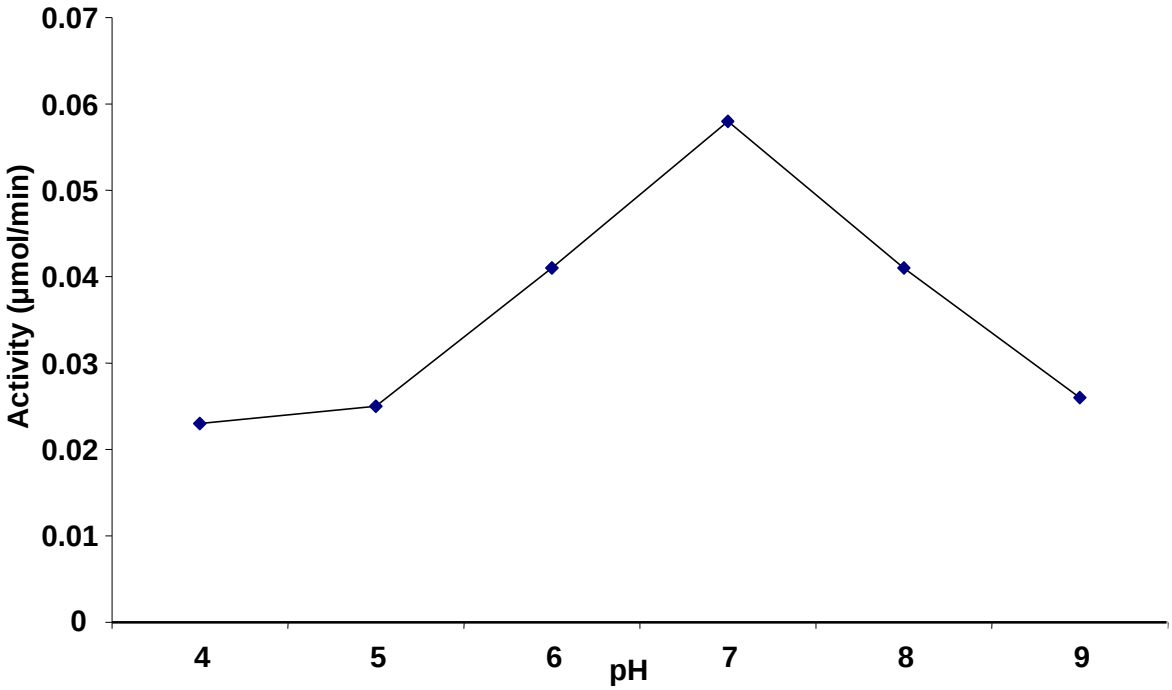

Figure 5. Effect of pH on Activity of LDH from Chicken Muscle

Effect of pH on the Activity of LDH from Chicken Muscle

The LDH activity was determined at pH 4.0-9.0 using lactic acid as substrate and maximum activity was recorded at pH 7.2. The enzyme was highly active over a narrow range (from 6.8-7.4). The enzyme activity declined in alkaline pH of 9.0 as well as acidic pH (below 6.0). Figure 3.5.

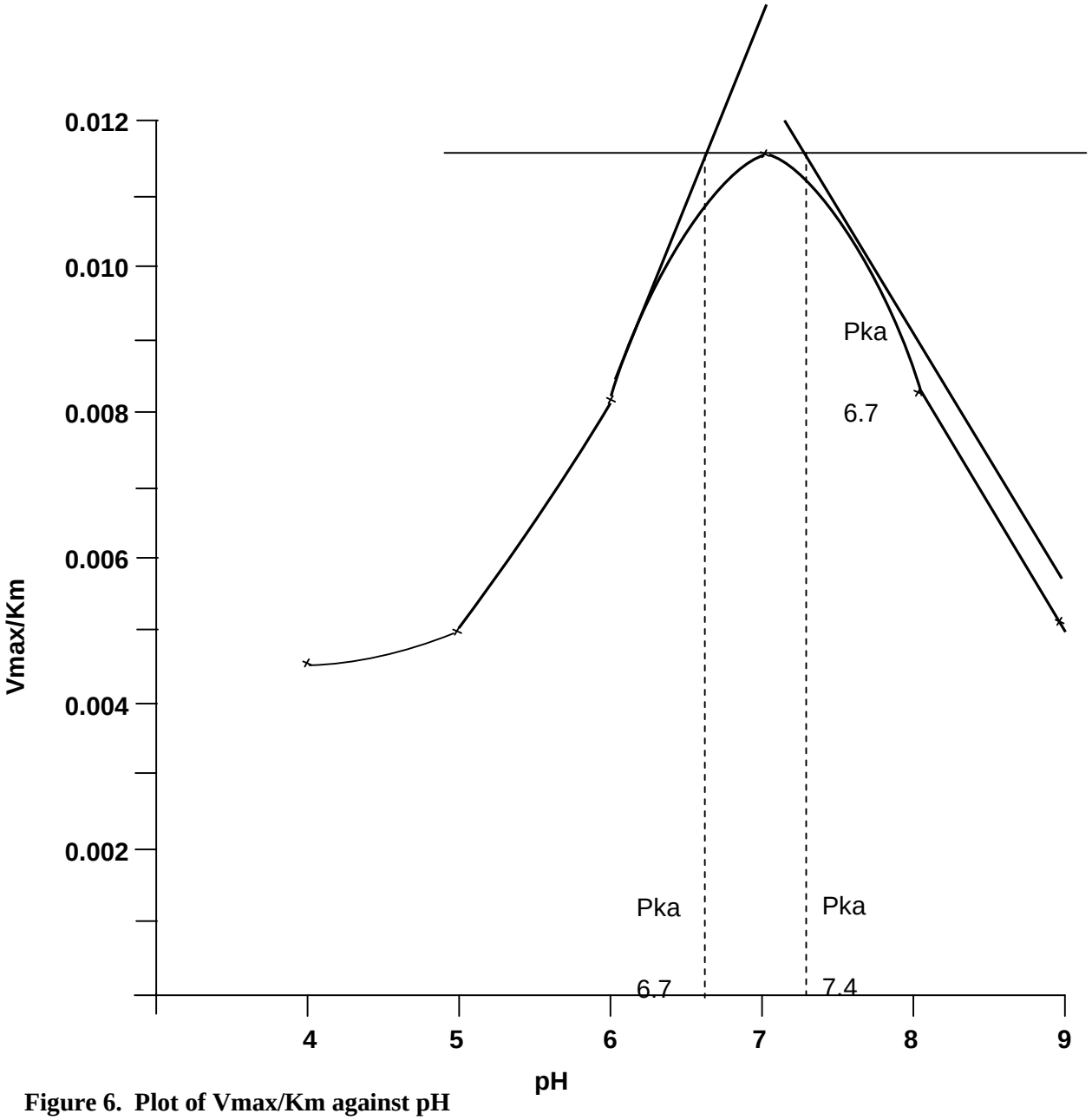

Figure 6. Plot of Vmax/Km against pH

Identification of amino acid residue in active site of LDH from Chicken Muscle

Fig. 6 shows the pKa values of 6.7 and 7.4 which implicate the imidazole groups of Histidine and hydroxyl groups of Tyrosine respectively. Consequently, these amino acids are predictably responsible for the catalytic activities or binding of the substrate to the enzyme.

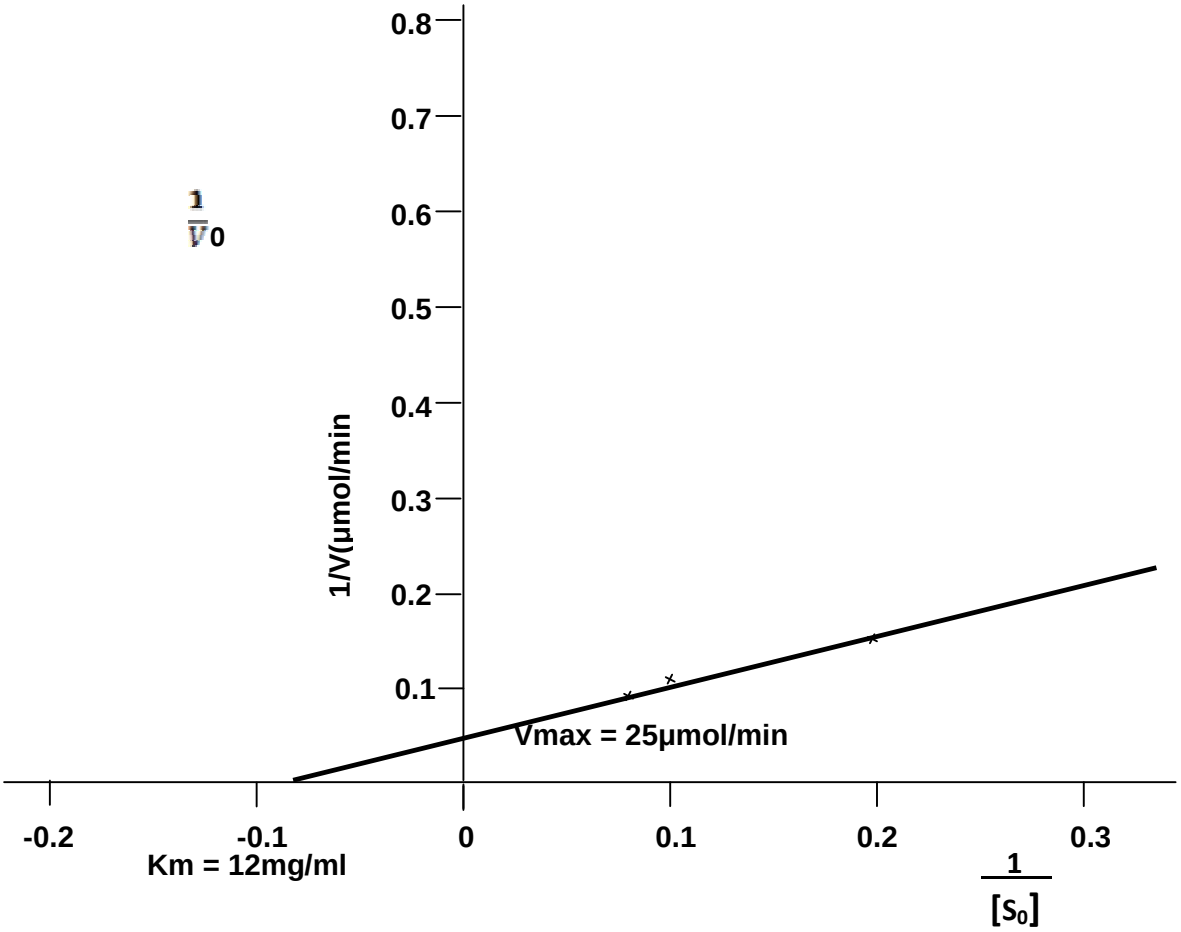

Figure 7. Double Reciprocal Plot ($^{1}/_{V}$ against $^{1}/_{[S]}$) of Activity of LDH against Substrate Concentrations. It depict Lineweaver-Burk's plot and shows Km and Vmax values of 12mg/ml and 25µmol/min respectively.

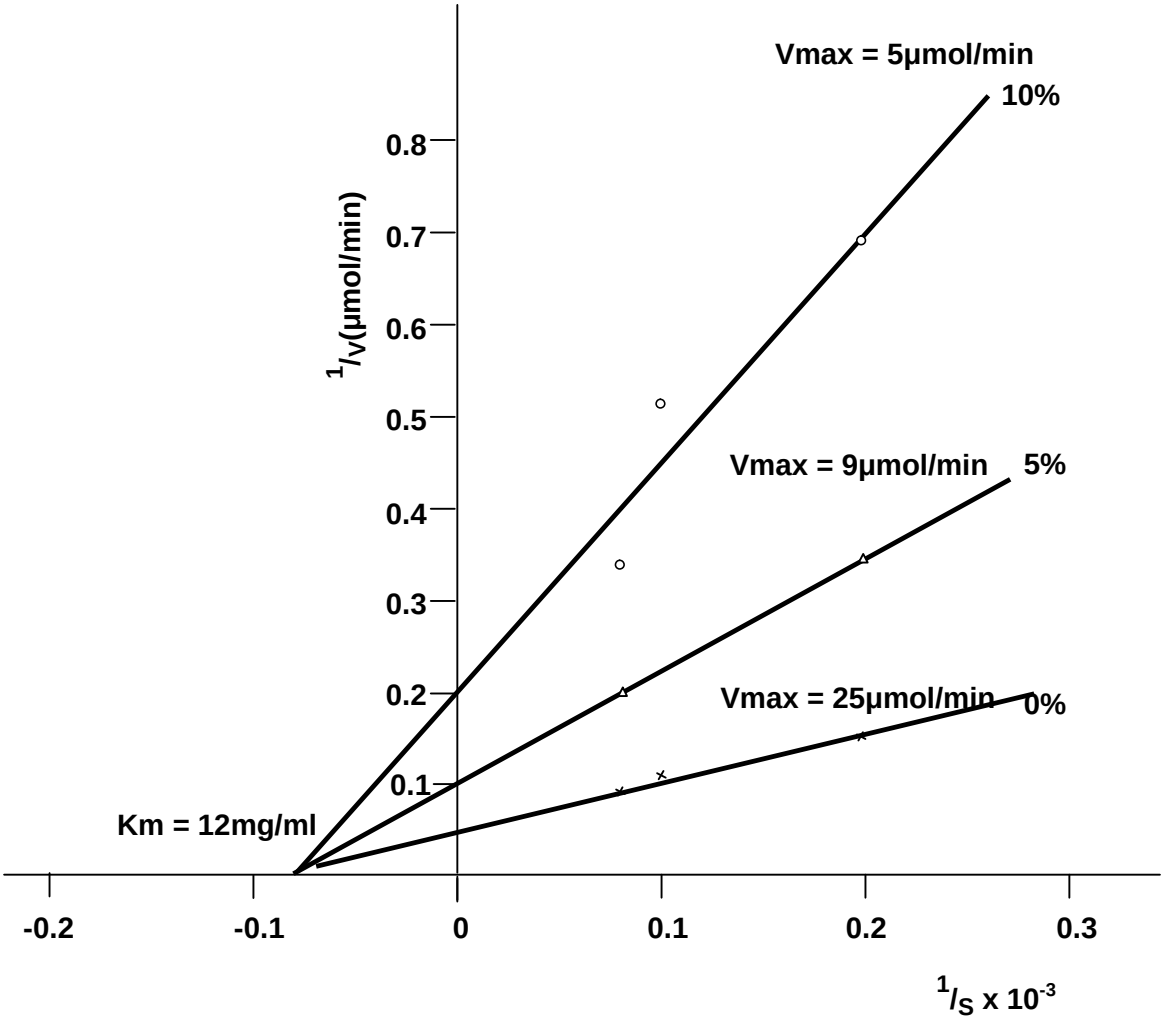

Figure 8. Lineweaver-Burk's Plot of the Effect of Aqueous Extract of *Allium Sativum* on LDH Activity

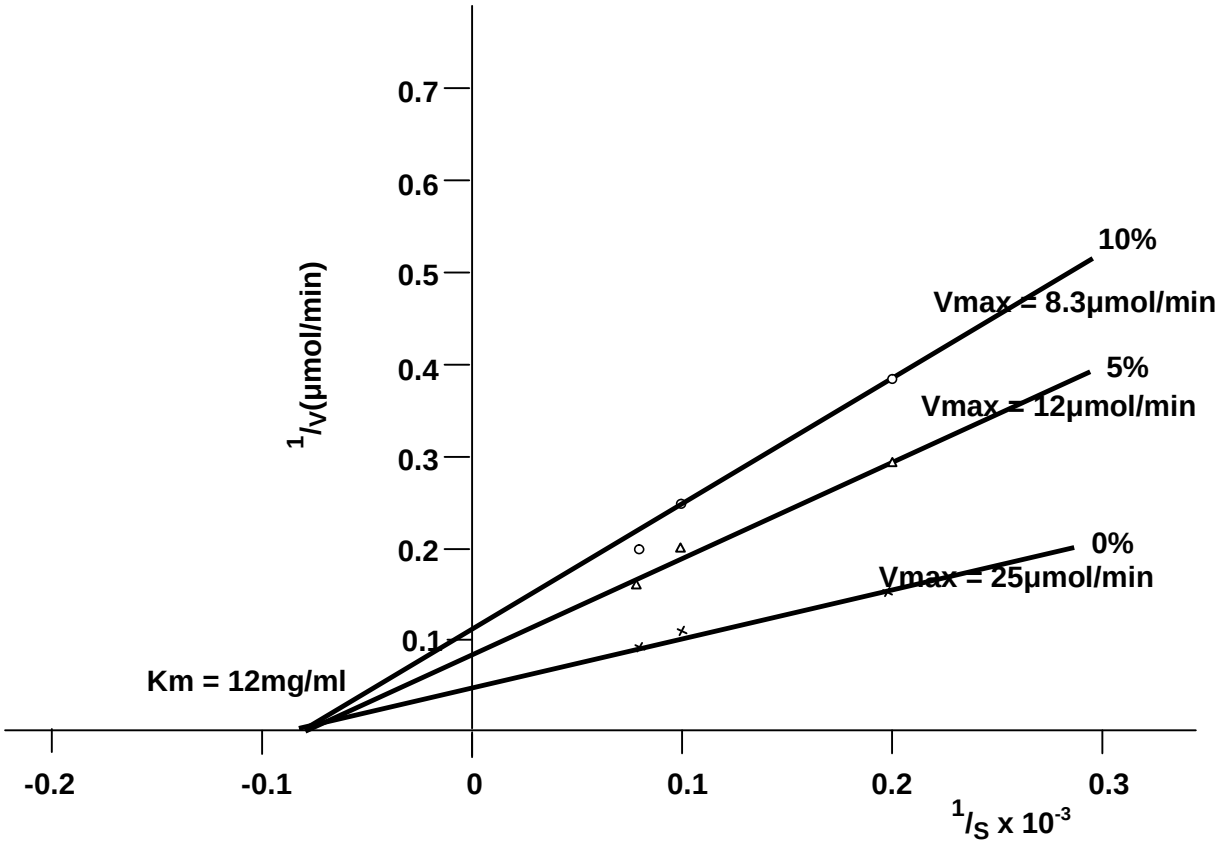

Figure 9. Lineweaver-Burk's Plot of the Effect of Ethanolic Extract of *Allium sativum* on LDH Activity showing a characteristic non competitive

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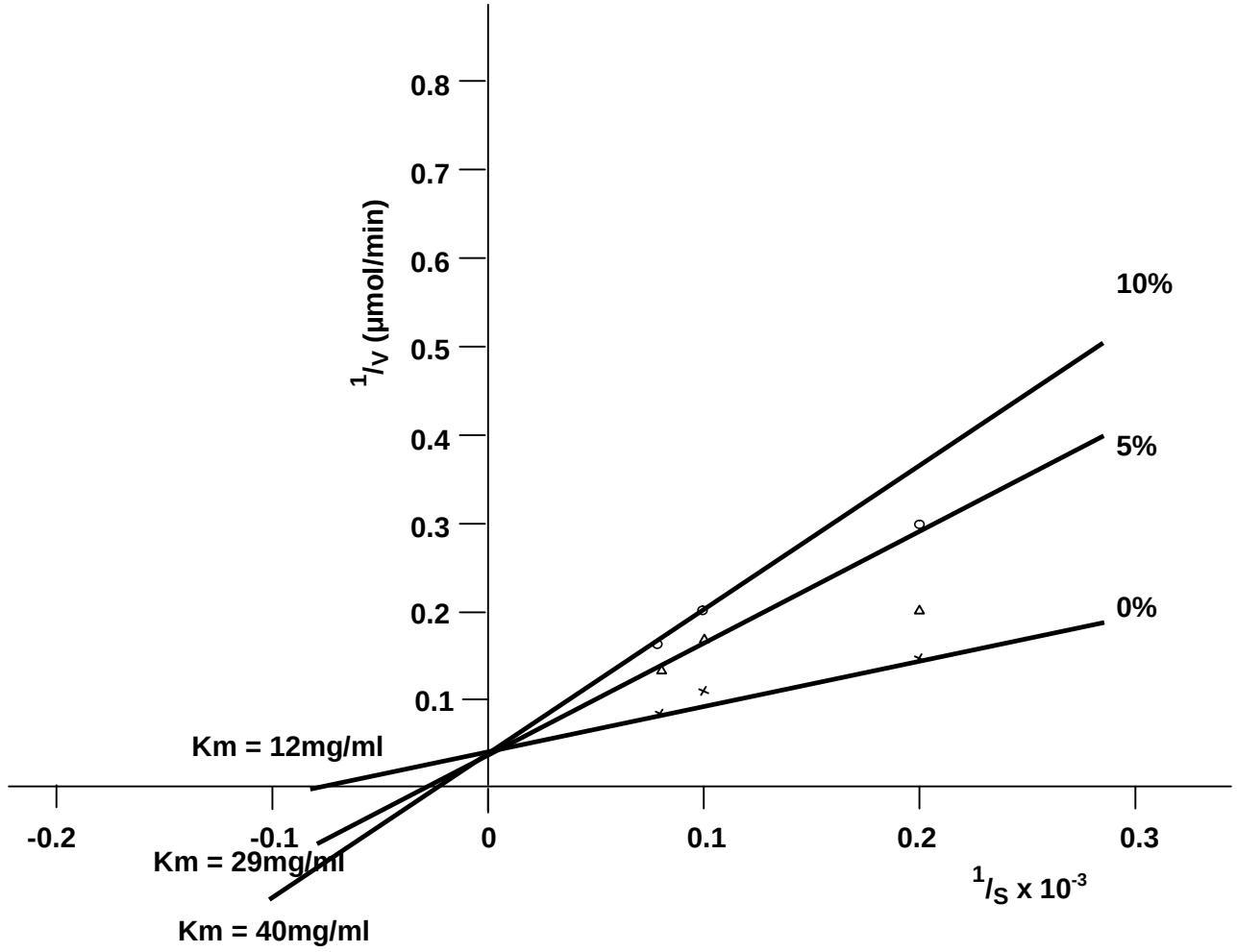

Fig.10 Lineweaver-Burk's Plot of the Effect of Aqueous Extract of *Gossypium arboreum* on LDH Activity showing a characteristic competitive type of

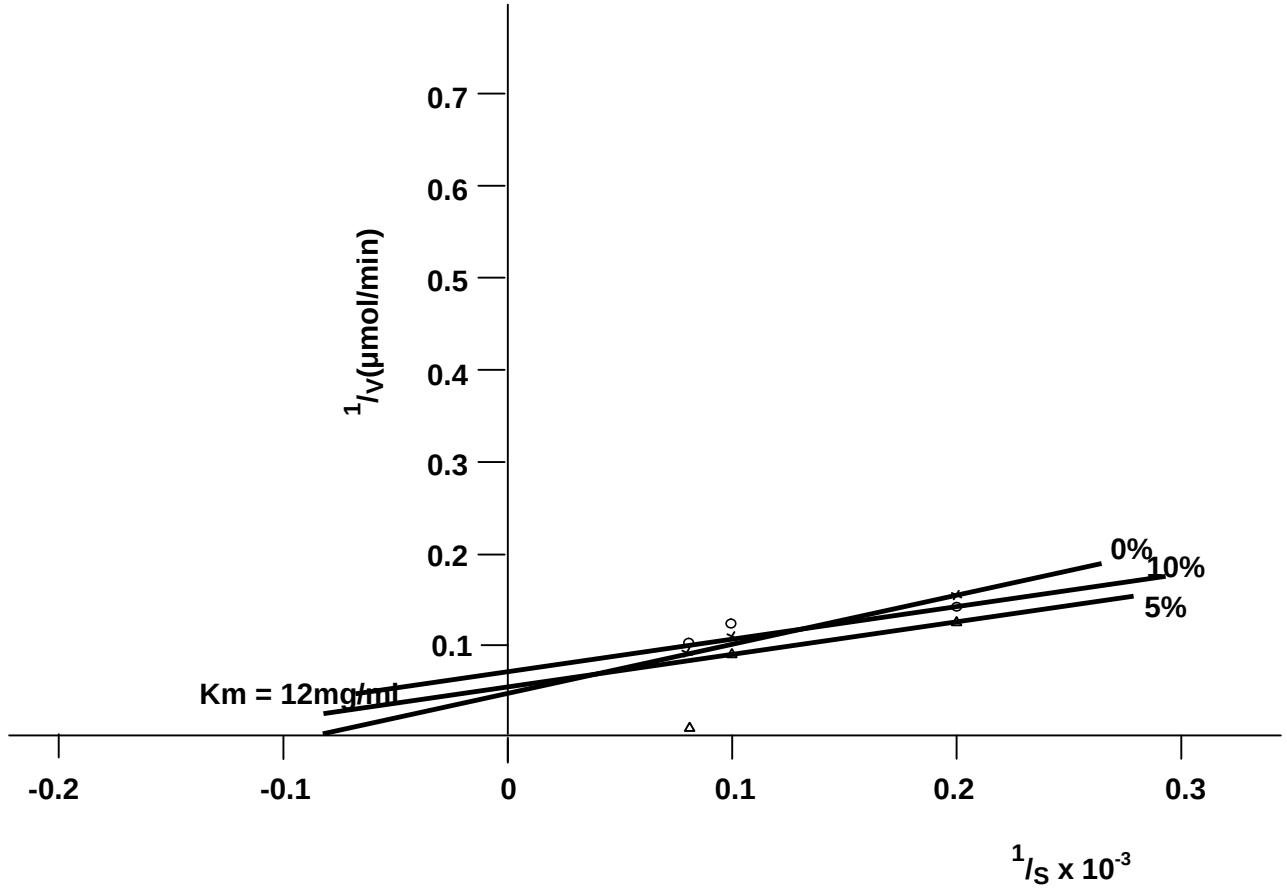

Figure 11. Lineweaver-Burk's Plot of Effect of Ethanolic Extract of *Gossypium arboreum* on LDH Activity showing no effect on the LDH activity

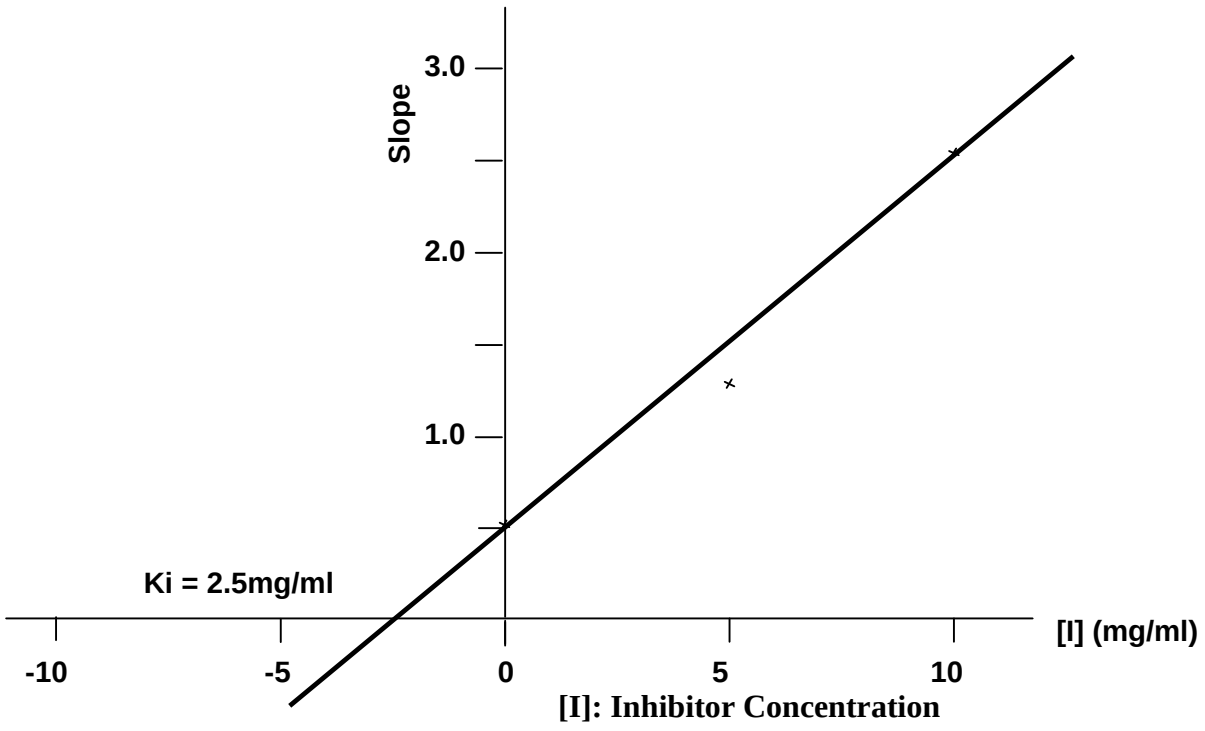

Figure 12. Plot for Determination of Inhibition Constant (Ki) of Aqueous Extract of *Allium sativum* showing inhibition constant (Ki) of 2.5mg/ml.

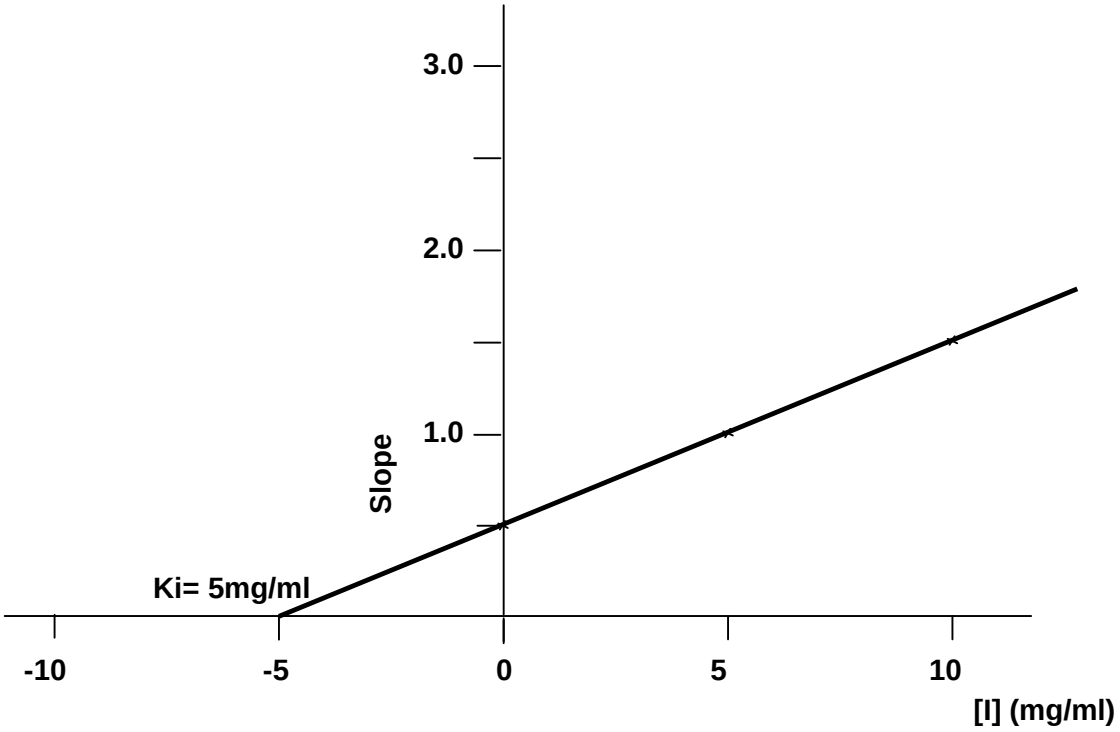

Figure13. Plot for Determination of Inhibition Constant (Ki) of Ethanolic Extract of *Allium Sativum* with inhibition constant (Ki) of 5.0mg/ml.

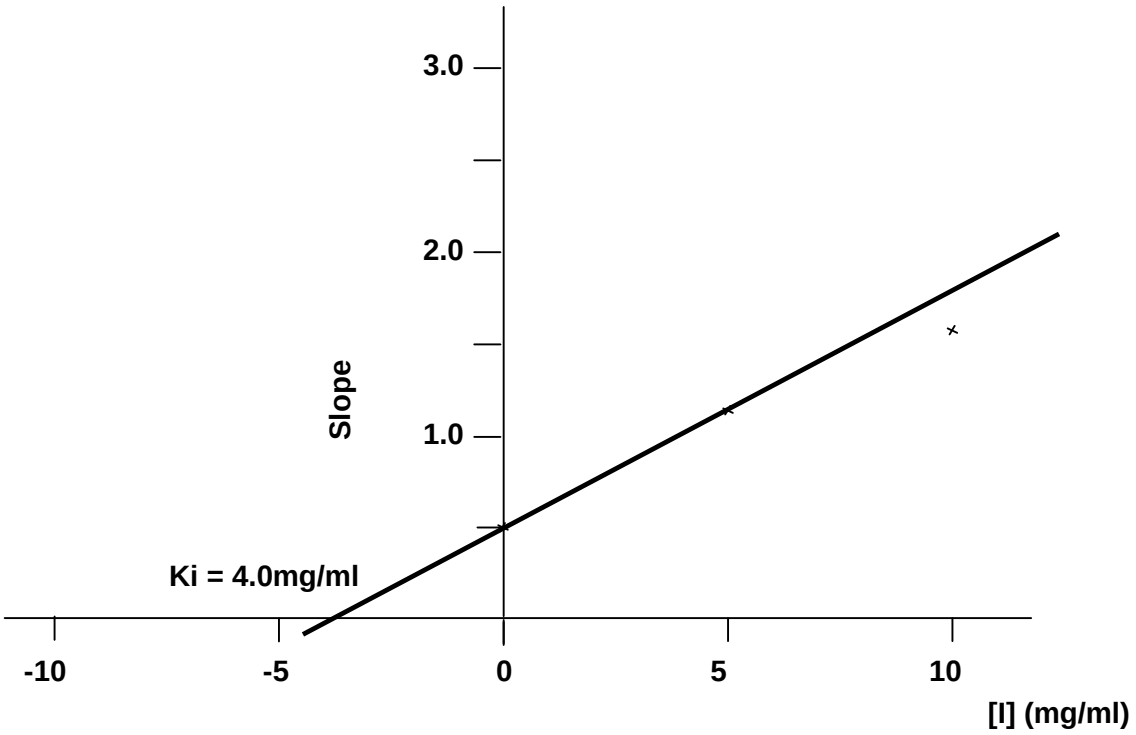

Figure 14. Plot for Determination of Inhibition Constant (Ki) of Aqueous Extract of *Gossypium arboreum* with inhibition constant of 4.0mg/ml.

DISCUSSION

A partially purified lactate dehydrogenase (4.0mg) was obtained from 82.7mg crude extract with specific activity of 37.5µmolmg^{-1}. This was about 11.0 fold of the specific activity in the crude extract (Table 1). These values however, differ from chicken muscles LDH values obtained by Michelle [15] with specific activity of 30.8 and 14.0 fold purification. The discrepancy could be as result of different buffers used or type of chromatography employed in the purification.

The partially purified lactate dehydrogenase from chicken muscle shows a characteristic pH optimum of 7.2. Yoshikuni [17] reported optimum pH for crystalline LDH enzyme to be 7.5 (rat LDH) and 7.4 for human. Optimum pH for the LDH enzyme purified from different sources reported so far, mostly fall within a range of pH 7.2-8.0 in the direction of lactate oxidation [18,19,20,21, 17].

However, similarity or discrepancy of optimum pH across different species could be related to pKa of different catalytic groups in the enzyme's active site [22]. pH can affect activity consequent to changing the structure or changing the charge on a residue functional in substrate binding or catalysis [23]. The LDH was highly active over a narrow pH range which indicates possible denaturation, in acidic and in high alkaline pH values.

Ionization of amino acid residue in the catalytic site of an enzyme is pH dependent since catalytic activity relies on a specific state of ionization of these residues [22]. Eenzyme activity is also pH dependent[24].

Plot of Vmax/Km (Fig. 6.0) against pH yields a bell-shaped curve indicating the presence of two important ionisable groups with pKa values (Table 2) of 6.8 and 7.4 .This is consistent with other reports[25,26,27]. Predictably, they are responsible for the catalytic activities or binding of the substrate to the enzyme. Therefore, the pKa values are for imidazole groups of histidine and hydroxyl group of tyrosine respectively [28]. These amino acid residues are in the catalytic site of LDH from chicken muscle [28]. These findings are in conformity with the reports of Gerstein and Chothia, [29] and Peter [27]. It has been shown that LDH cannot form the enzyme cofactor- substrate ternary complex unless the imidazole group of Histidine 195 is protonated [28].

During the study of the effect of temperature on the activity of lactate dehydrogenase (Fig. 3), the purified enzyme exhibited a very broad temperature range with optimum activity at 40^0C .This finding is similarly reported by Roig [30]. The reaction is dependent on temperature and hence is consistent with transition theory. The energy barrier is, of course, lowered equally for both the forward and reverse reactions, so that position of equilibrium is unchanged [24].

The tertiary structure of an enzyme is maintained primarily by a large number of weak noncovalent bonds [22]. In practical terms, an enzyme molecule is a very delicate and fragile structure. If the molecule absorbs too much energy, the tertiary structure will be disrupted and the enzyme will be denatured, and lose catalytic activity [22]. Therefore, the decline in activity observed at high temperature values could be due to denaturation of the enzyme at that temperature. It has been established that LDH-1 is more stable than LDH-5 at high as well as low temperatures [27].

Data obtained from temperature studies was subjected to Arrhenius plot (Fig. 4), with activation energy of 18kcal/mol. And for such low activation energy (Ea) to be thermodynamically favourable, implies that less frequency of collision is required to surmount the activated complex to form product.

In a chemical reaction, reactants achieve their Ea by collision [22]. But in the presence of catalyst the Ea is reduced and less energy is required for the reaction to proceed [22]. It would be deduced therefore that, for LDH from chicken muscle to effectively function, activation energy of 18kcal/mol is required otherwise the reaction will not proceed [24].

The type of inhibition of LDH by both aqueous and ethanolic extracts of *Allium sativum* suggest non competitive inhibition (Fig. 9) with inhibition constants (Ki) of 2.5 and 5.0mg/ml respectively. The extract could have bound at a different site from the substrate, in this case, it is considered that, the extract (inhibitor) destroyed the catalytic activity of the enzyme, by distorting the conformational arrangement of the catalytic site, but not affecting substrate- binding. However, the total enzyme concentration is effectively reduced by the inhibitor, decreasing the value of Vmax but not altering Km, since neither inhibitor nor substrate affects the binding of the other. Moreover, values of Km and Vmax obtained from the double reciprocal plots suggest non competitive inhibition. In non competitive inhibition Vmax decreases, with increasing extract (inhibitor) concentration but the Km remains unaltered [31].

The double reciprocal plot (Fig. 7)for the evaluation of inhibition by aqueous extract of *G. arboreum* reveal competitive inhibition (Table 7) with Ki value of 4.0mg/ml. Thus, LDH inhibition by the extract of *Gossypium arboreum* may have occurred due to binding at the active site of the enzyme causing inhibitory action and that molecule(s) in the extract is similar in structure to the LDH substrate, enough to compete for the enzyme's active site. Since the substrate (lactate) and molecule(s) in the extract is competing for the same site on the enzyme, as extract (inhibitor) concentration increases, Km also increases, as is typical of competitive inhibition[31].

In many different species, the reaction catalyzed by LDH constitutes a major checkpoint of anaerobic glycolysis, by reduction of pyruvate into lactate[32]. This enzyme has recently received a great deal of attention since it may constitute a valid therapeutic target for different diseases as malaria, cancer and other parasitic infections [7]. Lactate dehydrogenase plays an important role in predicting response to therapy and prospects of remission in leukaemia and colon cancer [33]. Lacate dehydrogenase is also an important clue to the diagnosis of reactive haemophagocytic syndrome (RHPS) in febrile cytopaenic patients with immunodeficiency [33].

Increased serum lactate dehydrogenase activity was also observed during acute *P. falciparum* malaria infection. Therefore, this can be accounted for by a synergy between the two pathophysiological processes usually associated with acute *P. falciparum* malaria infection, i.e. the hepatic activity of the invading aporozoites leading to centrilobular liver damage and the destruction of the host RBCs consequent to erythrocytic merogony [34]. Being rich sources of lactate dehydrogenase, the acute liver injury and RBCs destruction will be followed by the release of lactate dehydrogenase into the circulation, hence, serum lactate dehydrogenase activity can serve as an index in the monitoring of acute *P. falciparum* malaria infection, particularly when all other possible causes of increased serum levels have been eliminated [35].

In fact, the isoform expressed by *Plasmodium falciparum* (pfLDH) is a key enzyme for energy generation by the parasite [36]. These species mostly depend on anaerobic glycolysis for energy production, since they lack a citric acid cycle for ATP formation[36,7].

However, cancer cells unlike their normal counterparts, exhibit a metabolic switch (Warburg effect) from oxidative phosphorylation to an increased anaerobic glycolysis, by promoting an upregulation of the human isoform-5 of LDH [7,9].This is the so called Warburg's effect both in cancer cells and parasites cells [36].

It has been known for nearly a century that both cancer cells and *P. falciparum* commonly display altered glucose metabolism[37]. Several studies reveal a functional connection with carbohydrate-driven energy production [9]. Importantly, inhibition of this vital metabolic enzyme (LDH) stimulates mitochondrial function and drastically compromise the ability of the tumour cells to proliferate and potentially cause mortality of *P. falciparum* [36,7,9]. The low Ki values obtained from the leaves extract of *G. arboreum* and cloves of *A. sativum* towards this enzyme is an indication of high affinity which the enzyme has for the extracts.

The inhibition of LDH has been a topic of interest in modern medicine and new information about LDH inhibition, in terms of molecular and clinical significance is predicted to be a promising opportunity in designing a novel drug with high efficacy to treat *P. falciparum* trophozoite and Cancer cells [36,9].

Lactate dehydrogenase constitutes a major check point of anaerobic glycolysis by catalyzing the reduction of pyruvate to lactate. Therefore, inhibition of this enzyme may represent a rational and safe strategy for treatment of cancer and malaria parasite. From this research, aqueous and methanolic extracts of *Allium sativum* and aqueous extract of *Gossypium arboreum* were shown to inhibit the enzyme in a dose- dependent fashion. This inhibition is predicted to be a promising opportunity in designing a novel drug with high efficacy to slow down the rate at which tumour cells proliferate and consequently become energy starved. However, nature and inhibition constants were determined. This will no doubt provide a clue for drug design and formulation in pharmaceutical industry.

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