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# Studies on Purification and Characterization of Sweet Potato (*Ipomoea Batatas*) Cultivated in the Northern Part of Bangladesh

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## ABSTRACT

Enzyme activity was also determined from the Red sweet potato and white sweet potato. The activities of Amylase, Cellulase, Invertase, & protease were 0.093±0.01, 0.05±0.02, 0.062± 0.015, 0.043± 0.02, for Red while 0.088± 0.01, 0.073 ±0.02, 0.072 ±0.015 & 0.050± 0.02 for White sweet potato. Amylase was partially purified from the extract of sweet potato using DEAE cellulose column chromatography. On the purity checking and molecular weight determination on SDS-PAGE. The molecular weight was determined by SDS-PAGE to be 64±1 kDa. The partially purified amylase from sweet potato is β-type is confirmed by $HgCl_2$.The optimum pH & temperatures of partially purified β-amylase were 7.5 and 55˚c respectively. Amylolytic activity of this enzyme is 105% and 55% for amylopectin and Amylase. This enzyme does not affect maltose and maltotetraose. Metallic ions like $Fe^{3+,}$ $Mn^{2+}$, $Mg^{2+}$, and EDTA increased amylase activity while other ions like $Cu^{+2}$, $Hg^{+2}$, $Pb^{+2}$, and urea reduced the amylase activity. The study indicates the importance of β-amylase as a starch degrading enzyme. This enzyme showed less toxicity towards brine shrimp nauplii with the Lc50 value of 1970.288µg/ml. This enzyme did not show any antibacterial activity against *Staphylococcus aureus, Escherichia coli, Salmonella enterica, Bacillus subtilis.*

***Keywords:*** *Sweet potato, Ipomoea Batatas, Starch, Amylase, Protease.*

## INTRODUCTION

Sweet potato is an important root crop in tropical and sub-tropical countries like Bangladesh, China, USA, India, Japan, Indonesia, Philippines, Thailand, Vietnam, Nigeria, etc. Among the root and tuber crops grown in the world, sweet potato ranks second after cassava[1]. The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family *Convolvulaceae*. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable [2]. The genus *Ipomoea* that contains the sweet potato also includes several garden flowers called morning glories, though that term is not usually

extended to *Ipomoea batatas*. Some cultivars of *Ipomoea batatas* are grown as ornamental plants; the name tuberous morning glory may be used in a horticultural context[3]. The edible tuberous root is long and tapered, with a smooth skin whose color ranges between yellow, orange, red, brown, purple, and beige. Its flesh ranges from beige to white, red, pink, violet, yellow, orange, and purple. Sweet potato varieties with white or pale yellow flesh are less sweet and moist than those with red, pink, or orange flesh [4, 5]. In Bangladesh, sweet potato is the 4th most important source of carbohydrate after rice, wheat, and potato. It plays a significant role in increasing food security and income for the poor farmers of Bangladesh. It is mainly grown in the marginal land of Bangladesh from October to February. It is consumed in different forms mainly boiled, fried, and roasted. Sometimes it is also eaten as a vegetable in curry. Now a day, in Bangladesh it is commercially cultivated. Sweet potato is extensively grown in all the districts of Bangladesh, particularly by the side of rivers and the char land. In 2009-10, about 0.31 million metric tons of sweet potatoes were produced from 31.1thousand hectors of land in Bangladesh [6]. Bangladesh ranks 23 in the world in terms of sweet potato production in 2011[7.] Enzymes play a significant role in the biochemical system of animals and plants, and day by day enzymes become a powerful tool in biochemical analysis. Sweet potato contains some important enzymes such as amylase, cellulase, invertase, protease, etc. Of these enzymes, amylase plays an important role in the digestion of starch. The industrial importance of this enzyme also makes it a popular subject for study. Many researchers have purified and characterized amylase. Amylase is from different sources but the report from Sweet potato*es* is very rare in Bangladesh. Moreover, the physiological role of amylase in a plant is not clear. To understand this systematic study is required. In this study, we attempted to assay enzyme activities & partial purification & characterization of amylase from sweet potato (*Ipomoea batatas*). This study comprises the determination of the activity of some hydrolytic enzymes such as amylase, cellulase, invertase, protease, and partial purification of amylase enzyme from sweet potato (*Ipomoea batatas)*. In vitro study of cytotoxic properties of the partially purified enzyme and also in vitro study of antimicrobial activity of the partially purified enzyme.

**Plant under investigation:** *Ipomoea batatas* (sweet potato) was derived from the Greek words *ipos*, meaning "bindweed," and *homoios*, meaning "resembling." When this is put together to form "*Ipomoea*" the direct translation is "resembling bindweed." This name makes sense because the sweet potato has a twining habit, much like the bindweed. The species name "*batatas*" was originally the *Taino* name for sweet potato. This name was most likely spread by the Spanish who came in contact with sweet potatoes in Central America and brought them to the West Indies. "*Batata*" is now the name for potato in Spanish. [8]

**Fig.1.1: Cultivation of sweet potato**

**Fig. 1.2: Flower of Sweet potato**

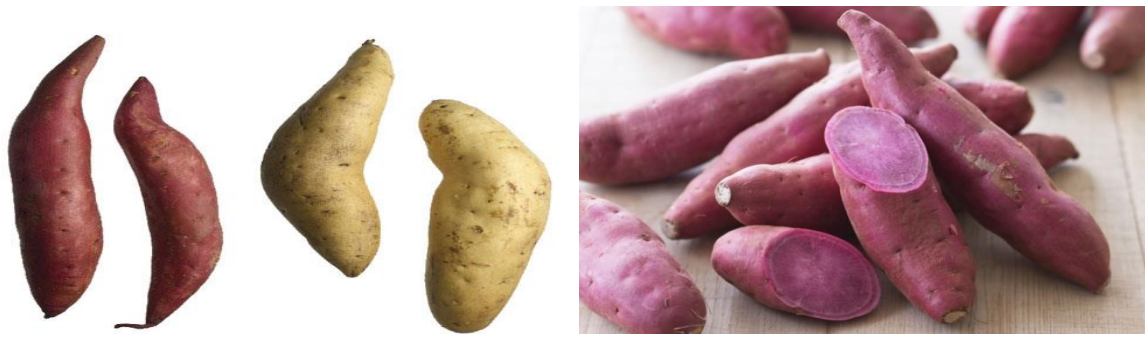


**Fig. 1.3: Sweet potatoes**

## Origin and distribution

The origin and domestication of sweet potatoes are thought to be in either Central America or South America. In Central America, sweet potatoes were domesticated at least 5,000 years ago [9]. In South America, Peruvian sweet potato remnants dating as far back as 8000 BC have been found. One author postulated that the origin of *Ipomoea batatas* was between the Yucatán Peninsula of Mexico and the mouth of the Orinoco River in Venezuela [9]. The 'cultigen' had most likely been spread by local people to the Caribbean and South America by 2500 BC [10]. Sweet potatoes are cultivated throughout tropical and warm regions wherever there is sufficient water to support their growth [11]. Due to a major crop failure, sweet potatoes were introduced to Fujian province of China in about 1594 from Luzon. The growing of sweet potatoes was encouraged by Governor Chin Hsüeh-Tseng [12]. Sweet potatoes were introduced as a food crop in Japan and by 1735 planted in Shogun Tokugawa Yoshimune's private garden. It was also introduced to Korea in 1764[13]. Sweet potatoes became popular very early in the islands of the Pacific Ocean, spreading from Polynesia to Japan and the Philippines [14].

## Nutritional Composition

Sweet potato is a very nutritious vegetable. An analysis of this vegetable shows consists of moisture, protein, fat, carbohydrate-reducing sugar, starch, dietary fiber, vitamins, etc. It also contains trace metals such as Calcium Iron, Manganese, Magnesium, Sodium, Zinc, Phosphorus, Potassium etc[15].

## Uses of enzyme

Enzymes have many uses in addition to their natural function in the body. Enzymes are used in making a wide variety of products. For example, some detergents contain enzymes that break down protein matter such as perspiration which causes stains. Enzymes are also used in the manufacture of antibiotics, beer, bread, cheese, coffee, meat, tenderizers, vitamins, and other products. Physicians use medicines containing enzymes to help clean wounds and dissolve blood clots. Relieve certain forms of leukemia and check allergic reactions to penicillin. Doctors also diagnose several diseases by measuring the value of various enzymes in blood and other body fluids such diseases include anemia, cancer, leukemia, heart and liver ailments.

## Amylases

Amylase is a digestive enzyme classified as a saccharidase (an enzyme that cleaves polysaccharides). It is mainly a constituent of pancreatic juice and saliva, needed for the breakdown of long-chain carbohydrates (such as starch) into smaller units [17].

## Classification of Amylase

There are three different types of amylases. These enzymes are classified according to how the glycosidic bond is attacked. These are:

**α-Amylase**

The α-amylases (1, 4-α-D-glucan glucanohydrolase; glycogenase) are calcium metalloenzymes, completely unable to function in the absence of calcium. By acting at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, ultimately yielding maltotriose and maltose from amylose, or maltose, glucose, and "limit dextrin" from amylopectin. Because it can act anywhere on the substrate, a-amylase tends to be faster acting than (3-amylase. In animals, it is a major digestive enzyme [17]. In human physiology, both salivary and pancreatic amylases are considered as α-amylases.

**β-Amylase**

β-Amylase is known as 1,4-α-D-glucan maltohydrolase; glycogenase; saccsharogen amylase. β-amylase [18-19] is an enzyme that hydrolyzes 1, 4-α-glucosidic linkages in starch-type polysaccharide substrates to remove successive maltose units from the non-reducing ends of the chains. β-amylase is present in certain bacteria as well as in plants. The highly conserved sequence regions are found in all known β-amylases. The first of these regions is located in the N-terminal section of the enzymes and contains an aspartate which is known to be involved in the catalytic mechanism. The second, located in a more central location, is centered on glutamate which is also involved in the catalytic mechanism. These proteins belong to family 14 the classification of glycosyl hydrolases [20]. Animal tissues do not contain β-amylase, although it may be present in microorganisms contained within the digestive tract [21].

**γ-Amylase**

γ- Amylase is known as Glucan 1,4-α -glucosidase; amyloglucosidase; Exo-1, 4-α -glucosidase; glucoamylase; lysosomal a-glucosidase; 1,4-α -D-glucan glucohydrolase. Glucoamylase (GA) is an enzyme that catalyzes the release of D-glucose from the non-reducing ends of starch and other oligo-or polysaccharides. Extensive studies of fungal GA have shown [22] those three closely clustered acidic residues play a role in the catalytic mechanism of GA. The region that includes these residues is also conserved in a recently sequenced bacterial GA [23]. These proteins belong to family 14 in the classification of glycol hydrolases [24].

**Importance of Amylase**

Amylase refers to a group of enzymes whose catalytic function is to hydrolyze (breakdown) sugar and starch. Amylase digests carbohydrates (polysaccharides) into smaller disaccharide units, eventually converting them into monosaccharide such as glucose. Amylase digests not only carbohydrates but also dead white blood cells (pus). Amylase is involved in anti-inflammatory reactions such as those caused by the release of histamine and similar substances. The inflammatory response usually occurs in organs that are in contact with the outside world, i.e., the lungs and skin. Salivary α-amylase has been used as a *biomarker* for *stress* that does not require a blood draw [25]. Increased amylase levels may indicate the following abnormalities: a) Acute pancreatitis, b) Cancer of the pancreas, ovaries, or lungs, and c) Cholecystitis. Decreased amylase levels may indicate [26] the following abnormalities: a) Damage to the pancreas, b) Kidney disease, c) Pancreatic cancer, and d) Toxemia during pregnancy. Workers in factories that work with amylase for any of the above uses are at increased risk of occupational asthma. 5-9% of bakers have a positive skin test, and a fourth to a third of bakers with breathing problems are hypersensitive to amylase [27]. An inhibitor of α-amylase called phaseolamin has been tested as a potential diet aid [28].

**Protease**

Protease refers to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins to liberate amino acids

and protease differs in their ability to hydrolyze various peptide bonds. Each type of protease has a specific kind of peptide bond. Example of proteases includes fungal protease, pepsin, trypsin, chymotrypsin papain, bromlain, and subtilisin[29]. Protease medicinal uses include oncology, inflammatory conditions, blood theology control, and immune regulation [30].

**Invertase**

Invertase is an enzyme that catalyzes the hydrolysis (breakdown) of sucrose. The resulting mixture of fructose and glucose is called inverted sugar syrup. Related to invertases are sucrases. invertases and sucrases hydrolyze sucrose to give the same mixture of glucose and fructose. Invertases cleave the O-C (fructose) bond, whereas the sucrases cleave the O-C (glucose) bond [31]. For industrial use, invertase is usually derived from yeast. It is also synthesized by bees, which use it to make honey from nectar. The optimum temperature at which the rate of reaction is at its greatest is 60 °C and an optimum pH of 4.5[32,33].

**Cellulase**

Cellulose refers to a class of enzymes produced chiefly by fungi, and protozoans that catalyze cellulolysis.

## METHODS AND MATERIALS

**Collection of sweet potato tuber**

During the winter season, sweet potato was collected from Rajshahi (Northern part of Bangladesh). After collection, it was cleaned and stored at -8°C temperature in deep refrigeration for experimental purposes.

**Chemicals and Equipments uses (Supplement-1)**

**Enzyme activity assay**

Amylase, Invertase, and cellulase play a major role in carbohydrate metabolism in several plant tissues [34, 35]. Amylase, an important industrial enzyme, is used in the starch industry (Liquefaction of starch for the production of glucose, fructose, and maltose), backed goods, brewing, paper, textiles, and detergent and sugar industries [36]. Starch is the major component of most of the world's crop yield and the degradation of starch is essential in the germination of these plants [37]. Starch degradation in seeds requires the action of α-amylase and β-amylase. Invertase, which hydrolyzes sucrose into glucose and fructose, occurs in many plants and microorganisms. The expression and distribution of plant invertases have been especially well documented because these are considered to play an important role in sugar metabolism [38.] Proteases are proteolytic enzymes that catalyze the hydrolysis of protein. During germination, the rapid mobilization of storage protein in the cotyledons of seedling requires the action of protease. There have been very few reports on the enzyme activity, particularly on sweet potatoes. Therefore, the present work aimed to study the enzyme activity of sweet potatoes.

**Preparation of crude enzyme extract**

At first 5 g rhizome of sweet potatoes were cut into small pieces and pasted in a mortar with a pestle and then homogenized well with Pre-cold Tris-HCl buffer of pH-8.2. The homogenate was filtered through a double layer of muslin cloth. After centrifugation at 9,000 rpm for 15 min, the supernatant was collected in a film tube used as crude enzyme extract.

**Assay of amylase activity**

Amylase activity was assayed following the method described in the Laboratory Manual in Biochemistry (Jayaraman, 1981). One percent starch solution was used as substrate. The amylase activity was measured by estimating the release of maltose. The amount of maltose released was calculated from the standard curve (Fig.-3.1) prepared with maltose. The enzyme activity is measured as the amount of glucose released per minute per ml of crude enzyme extract.

The reagents used for this assay are 0.1 M phosphate buffer (pH 6.7), 1% starch solution in 0.1 M phosphate buffer (pH 6.7), 1% NaCl in distilled water, 2 N NaOH, Dinitrosalicylic acid (DNS), Crystalline phenol, Sodium sulfate, and Standard maltose solution (2 mg/ml).

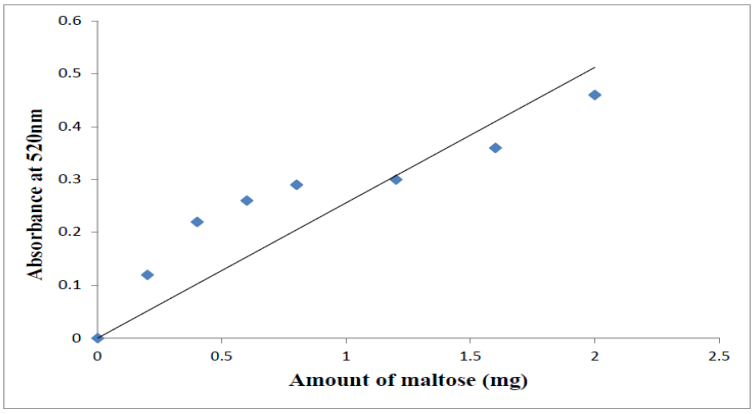



**Fig. 3.1: Standard curve of maltose for determination of activity of Amylase**

**Table 3.1: Activities of amylase, cellulase, invertase and Protease in sweet potato**

| Name of the enzymes | Enzyme activity (mg/min/ml) (Red sweet potato) | Enzyme activity (mg/min/ml) (White sweet potato) |
|---|---|---|
| Amylase | 0.093± 0 .01 | 0.088± 0 .01 |
| Cellulase | 0.052 ± 0.02 | 0.073± 0 .02 |
| Invertase | 0.062 ± 0.015 | 0.072 ± 0 .015 |
| Protease | 0.043 ± 0.02 | 0.050± 0 .02 |

## Preparation of DNS Reagent (Supplement-1)

## Assay of Cellulase Activity

Cellulase activity was assayed following the method described in Biochemical Methods for Agricultural sciences. Hydrolysis of cellulose is a complex process. A minimum of three different types of enzymes are believed to be involved: 1) Endo-b-1, 4 glucanase 2) Exo- b-1,4 glucanase 3) b-Glucosidase. In this process, Carboxy Methyl-Cellulose is used as a substrate that is hydrolyzed to glucose. The enzyme activity is measured as the amount of glucose released (Fig-3.2) per minute per ml of crude enzyme extract.

The reagents used for this assay are Sodium citrate buffer 0.1M (pH 5.0), 1% Carboxymethyl cellulose in citrate buffer (pH-5.0), Dinitratosalicylic Acid (DNS), 40% Rochelle salt solution (Na-K tartrate), and Preparation of the standard: Glucose in conc. range 200mg/100ml.

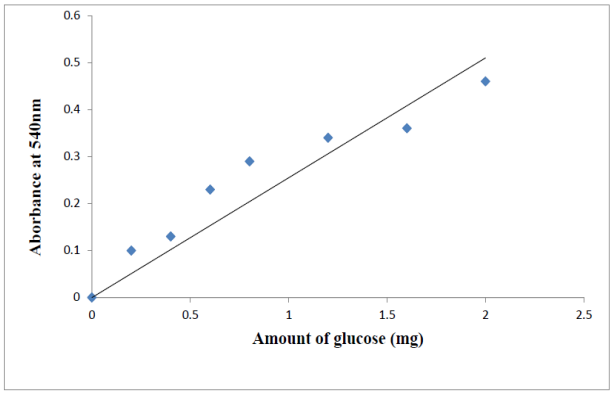



**Fig.3.2: Standard curve of glucose for determination of activity of Cellulase**

**Procedure**

Firstly 0.45 ml of 1% CMC solution was pipetted out and 0.05 ml of enzyme extract was added to it and incubated at 55°C for 15 min. immediately after removing the enzyme-substrate mixture from the bath added 0.5 ml of DNS reagent and then heated for 5 min in boiling water bath. The while tubes were warm, 1.0 ml of Na-K tartrate solution was added. After cooling, distilled water was added to make the volume up to 5 ml and finally, absorbance was measured at 540 nm.

**Assay of Invertase Activity**

Invertase activity was assayed following the modified method as described in methods in physiological Plant Pathology (Mahadevan and Sridhar, 1982) [97]. Sucrose was used as a substrate. The invertase activity was measured by estimating the release of glucose. The amount of glucose released was calculated from the standard curve (Fig. 3.3) prepared with glucose. The enzyme activity was measured as the amount of glucose released per minute per ml of crude enzyme extract.

The reagents used for this assay are 0.1 M phosphate buffer (pH 7.0), 1% sucrose solution in 0.1 M phosphate buffer (pH 7.0), 1% NaCl in distilled water, 2 N NaOH, Dinitrosalicylic acid (DNS), and Standard solution of Glucose: 200 mg of glucose was dissolved in 100 ml of distilled water.

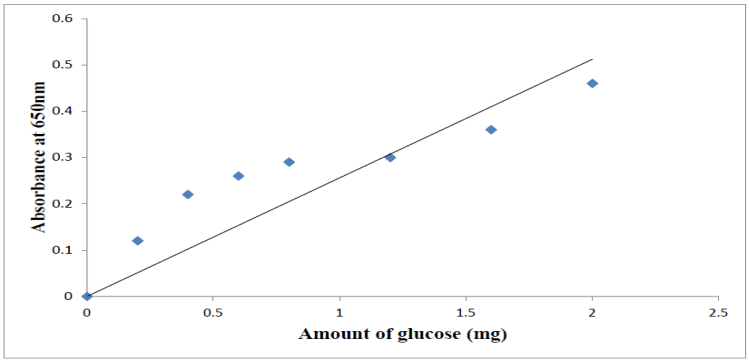



**Fig. 3.3: Standard curve of glucose for determination of activity of invertase**

**Preparation of DNS Reagent (Supplement-1)**

**Measurement of Protease Activity**

The protease activity was measured following the method of Kunitz[98]. The milk protein casein was used as a substrate. The activity was measured by detecting the release of an amino acid (Tyrosine). The amount of tyrosine released was calculated from a standard curve (Fig. 3.4) constructed with tyrosine. The enzyme activity is measured as the amount of tyrosine released per minute per ml of crude enzyme extract.

The reagents used for this assay are 1.2% casein solution: 1.2 g of casein was dissolved in 0.1 M phosphate buffer (pH 7.0), and 0.4 M Trichloro acetic acid (TCA).

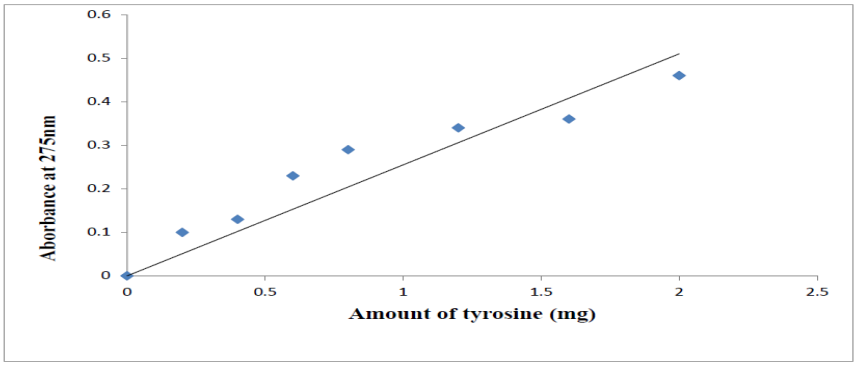



**Fig. 3.4: Standard curve of tyrosine for determination of activity of protease**

**Procedure**

2.5 ml of 1.2% casein was taken in different test tubes. Since casein is sparingly soluble in water, it was dissolved in a minimal amount of 0.1 M NaOH and the volume rose to 100 ml with the buffer. a) For control-1 no. b) For experiment-2 no. and c) for blank-1. no. Then 0.5 ml crude enzyme extract was added to the control and experimental tubes whereas 0.5 ml of buffer was added to the blank test tube. Immediately after the addition of crude enzyme extract, 2.5 ml of TCA was added to the control tube to stop the reaction. The rest of the tubes were incubated at 45°C for an hour and the reaction was stopped by the addition of 2.5 ml of TCA in the test tubes. After cooling, the reaction mixture was centrifuged at 5500 rpm for 7 minutes. The supernatant was collected and absorbance was taken at 275 nm against the reagent blank.

## RESULTS AND DISCUSSION

**The activity of Amylase in Sweet Potato**

Amylase, an enzyme having physiological, commercial, and historical significance, is also called diastase. Two types of amylase are recognized. β- Amylase (in plants and bacteria) which can only remove the terminal two glucose molecules each time it reacts, and α-amylase (animal-derived) which are called endoamylases, because they attack a polyglucan chain randomly, thus rapidly breaking large molecules down into smaller units. [99] Were the first to become aware of enzymatic starch hydrolysis they found that malt extract converted starch to sugar. Starch is the principal storage carbohydrate in plant cells, which is made up of amylose and amylopectin. Traditionally, β-amylase has been associated with starch degradation [100]. Although the entire pathway of starch degradation has not been conclusively established in any plant tissue [101]. It has long been attributed to various combinations of activities of α-amylase, β-amylase, starch debranching enzyme, starch phosphorylase, and α- glucosidase [102]. To make use of the carbon and energy stored in starch, with the help of the enzyme, one must first break down the polymer into smaller assailable sugars, which are eventually converted to individual basic glucose units. The activities of amylase in the red and white color sweet potato were found to be (0.093 ± 0.01 mg /min/ml) and (0.088 ± 0.01 mg /min/ml). Cellulolytic enzymes are a group of hydrolytic enzymes (cellulases) capable of hydrolyzing cellulose to glucose. There are at least three major types of cellulolytic enzymes produced by fungi: endoglucanases, cellobiohydrases, and cellobiase [39]. These are produced by a large number of microorganisms like fungi and bacteria [40]. Many plant pathogens are also known to produce either adaptively proteolytic, cellulolytic, or various polysaccharides [41.] They are used to perform various functions including removing cell walls or crude fiber to release valuable components (flavors, enzymes, polysaccharides, and other proteins) from plant cells to improve the nutritional value of animal feeds or to prepare plant protoplast for genetic research [42]. The activities of cellulase examined in the red and white color sweet potato were found to be (0.052± 0.02mg /min/ml) and (0.073±0.02 mg/min/ml). The enzyme occurs widely in the plant, microbial, and animal sources. Invertase plays an important role in the hydrolysis of sucrose to glucose and fructose in higher plants, especially in the storage organs. Sucrose is an early product of the photosynthetic reaction and is the most abundant transportable free carbohydrate in the plant kingdom. Sucrose serves as an important carbohydrate in plants, especially in such storage organs as tuber, root, and seed, sucrose is a ready degradation source of energy. The activities of invertase examined in the red and white color sweet potato were found to be (0.062±0.015mg /min/ml) and (0.072±0.015 mg/min/ml).

Proteins play an important role in the whole development of sweet potatoes. Protein content rather than plant size may be the

main factor influencing seedling development. Proteases hydrolyze peptide bonds in polypeptides into amino acids. The most well-characterized proteases are associated with seed germination and are employed to mobilize stored reserves to provide amino acids and amides for embryogenesis and/or early seedling development. The activities of protease examined in the red and white color sweet potato were found to be (0.043±0.02 mg/min/ml) and (0.050±0.02 mg/min/ml).

## DISCUSSION

Bangladesh is a developing country and its food industries are developing day by day. Enzymes are playing a significant role in the determination of food quality. From the enzyme activity study, the results are suggested that sweet potato is a good source of amylase, cellulase, invertase, and protease. Here Amylase activity of Red sweet potato is higher than that of White sweet potato. But cellulase invertase and protease activity of White sweet potato are higher than that of Red sweet potato. It is also shown that the activities of Amylase are higher than that of others and if facilities are available, it is possible to purify Amylase enzyme from sweet potatoes and it will be applicable in food industries for improvement of food qualities.

### Preparatory steps for purification

### Preparation of Crude Extract

50g tuber of sweet potato was cut into small pieces and then 100ml of pure water was added and the sample was homogenized by a homogenizer. The homogenate was occasionally stirred and then filtered through a muslin cloth. The filtrate was collected and clarified further by centrifugation at 8000 rpm, at 4°C for 15 minutes. The clear supernatant was collected and dialyzed against cold distilled water for 2 hours. After centrifugation, the clear supernatant was used as crude protein extract and also preserved in the deep freeze for experimental purposes.

### Purification of Amylase

### DEAE-Cellulose Column Chromatography

### Procedure

**a) Activation of DEAE-cellulose powder:**

The DEAE-cellulose powder was suspended in 0.2M NaOH in a beaker and left to swell for 3 hours. During swelling, it was stirred gently at short intervals to prevent the formation of lumps. Then it was transferred to a filtering funnel and washed with distilled water several times until its pH reached 4.0. The gel suspension was then transferred to another beaker containing 0.2 M NaOH and left for another 3 hours. It was again washed with distilled water several times until its pH reached 7.0.

**b) Packing in the column:**

The activated DEAE-cellulose suspension was taken in a filtering flask and deaerated by a vacuum pump. A column of (1.5 ×12 cm) in length was packed with the activated column material. The precaution was taken to avoid the trapping of air bubbles during packing.

**c) Equilibration of the column:**

After packing, the column was equilibrated with 10 mM Tris-HCl buffer, pH-8.2.

**d) Preparation and application of sample:**

The dialyzed sample was loaded on to DEAE column at 4°C. The elution was performed by an increase of sodium chloride salt concentration from 0 to 0.5 M in the same buffer. An automatic fraction (SF-160) collector was used to collect the eluent. The absorbance of each fraction was measured at 280 nm and the protein concentration was determined by the Lowry method (Lowry *et al.* 1951).

### Determination of Protein Concentration (by Lowry Et Al., 1951) [Supplement-3]

### Characterization of Amylase

### Effect of pH on Amylase activity

The activity of amylase was measured at different pH values (pH 4.0-11 using suitable buffers) at 37°C following the procedure as described in the Materials and Methods section (3.2.2.1) of Chapter 3.

Starch solution (1 %) was prepared in the above-mentioned buffer, of different pH values and was used as substrates for the enzyme.

**Effect of temperature on Amylase activity**

The activity of the amylase was measured at different temperatures (30-80°C) using 0.1 M phosphate buffer, pH 6.7 as described in the Materials and Methods section (3 2.2.1) of Chapter 3.

**Substrate specificity**

To determine the substrate specificity of the enzyme amylase, amylopectin, maltotetraose, and maltose, Starch (pea) and starch (potato) were used as substrates instead of starch during the assay.

**Treatment with various metal salts**

The effect of different metal ions on enzyme activity was tested by pre-incubating the enzyme (0 .25-0 .30 mg /ml) at specified concentrations of 0.5 ml reagent for 15 minutes at 37°C and the enzyme activity was assayed.

**Determination of cytotoxicity of the purified enzyme by Brine-Shrimp bioassay**

Brine shrimp lethality bioassay is a recently developed method for bioactive compound assessment. This bioassay indicates toxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds. Extracts and isolated compounds from plant origin can be tested for their bioactivity by this method. Here *in vivo* lethality bioassay is conducted by using the simple zoo logic organism; brine shrimp naupli (*Artemia salina*, Leach). The method has the advantages of being very simple, rapid (24 hours), and inexpensive. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of samples. In the present study, TCSL was used for its cytotoxicity study using the brine shrimp lethality test.

**Materials**

a) *Artemia salina* leach (brine shrimp eggs)
b) Sea salt (non-ionized NaCl)
c) Small tank with a perforated dividing dam to grow shrimp, cover, and lamp to attract shrimp.
d) Pipettes
e) Micropipette (10µl- 100µl)
f) Vials, (4 ml)
g) Magnifying glass. (3X magnifying glass)

**Procedure**

**a) Preparation of simulated seawater**

38 g of sea salt (non-ionized NaCl) was weighed accurately, dissolved in one liter of sterilized distilled water, and then filtered off to get a clear solution. The pH of the seawater was maintained between 8 and 9 by using a $NaHCO_3$ solution.

**b) Hatching of brine shrimp**

*Artemia salina* leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated sea water was taken in the small tank and the shrimp eggs (1.5 g/l) were added to one side of the tank and this side was covered. The shrimps were allowed for one day to hatch and immature as nauplii (larvae). The constant oxygen supply was carried out and constant temperature (around 37˚C) was maintained during the hatching time. The hatched shrimps were attracted to the lamp on the other side of the divided tank through the dam. These nauplii were taken for this bioassay.

**Preparation of sample**

The test sample contained an enzyme. This solution was used as a stock solution.

**c) Application of the test sample and brine shrimp nauplii to the vials**

Twelve clean vials were taken for the sample in four concentrations (Three vials for each concentration) and another three vials were also taken for control. Then the concentration of every three vials was 30, 60, 80, 100, 120, 140, 160, and 180µg/ml respectively enzyme solution containing the

sample was added to every three vials gradually and finally marked up to 5 ml by seawater. With the help of a Pasteur pipette, 10 living shrimps were taken to each sample vial and control vial respectively.

**d) Counting of nauplii**

After 24 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial was counted and noted. From this, the nauplii were counted averagely of each three vials, which contained the same conc. of the sample, the percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Probit analysis [122].

**Antibacterial activity study**

Constituents from natural resources as well as synthetic organic and inorganic compounds have been receiving much attention in biological systems. Many of these compounds are being used as chemotherapeutic agents. The pathogenic organisms are developing resistance heriditically towards some antibacterial agents. It is, therefore, necessary to find out consistently new, safer, effective, and inexpensive agents for the purpose. In this connection, it is very important to determine whether crude protein solutions are active against test organisms or not. It is well known that a large number of human and animal diseases are caused by pathogenic microorganisms. Infections due to these microorganisms have been a major cause of morbidity and mortality in both developed and developing countries. Synthesized compounds are necessary to determine their spectrum against various types of gram-positive and gram-negative bacteria. The prime objective of performing the antibacterial screening is to determine the susceptibility of the microorganism to any agent and which can be measured *in vitro* by several techniques, such as Bio autographic method, Serial dilution method, Disc diffusion method, and Streak test. Among them, the disc diffusion method using different concentrations of the agents absorbed on sterile filter paper discs is widely acceptable in an investigation for preliminary screening of compounds. The antibacterial activity of the compound was studied by using the "Disc diffusion technique".

**Test organisms used for the study**

The choice of test organisms will depend greatly on the purpose of the investigation. The pure cultures were collected from the Institute of Biological Science, University of Rajshahi, Bangladesh. The following pathogenic bacteria were used for the study of antibacterial activity.

**Conditions necessary for the growth of the pathogen**

For optimum growth of bacteria, we must have the required nutrients, the permissive temperature, enough moisture in the medium, the proper gaseous atmosphere, proper salt concentration, and appropriate pH in the absence of growth-inhibiting factor.

**Apparatus and materials**

| | |
|---|---|
| Filter paper discs (sterilized) | Autoclave (ALP Co. Ltd. KT-30L, Tokyo) |
| Petri dishes | Refrigerator (Aritstion, Italy) |
| Sterile cotton | Nutrient Agar Media (Difco) |
| Sterile forceps | Ethanol & chloroform |
| Spirit burner | Standard disc |
| Micropipette (10-100 µl) | Vial |
| Incubator (OSK-9639A, Japan) | Punch machine |
| Nose mask and Hand gloves | |
| Laminar airflow (Bio-Craft & Scientific Industries, India) | |

**Preparation of media**

The following media were used for the study of the antibacterial activity of the above test bacteria.

1. Nutrient agar medium
2. Nutrient broth medium

**Principle of the disc diffusion method**

The solution of known concentration (mg/ml) of the test samples was made by dissolving the

measured amount of the sample in a definite volume of solvents. Dried and sterilized filter paper discs (5 mm diameter) soaked with a known amount of test agents were placed on the nutrient agar media solidified in Petri-dishes (120 mm diameter) and inoculated with test organisms. These plates were then kept at a low temperature (4°C) for 24 h to allow maximum diffusion. During this period the following events took place simultaneously.

a) The dried discs absorbed water from the surrounding agar medium and the test samples/material are dissolved.
b) The test material diffuses from the discs to the surrounding medium. The diffusion took place according to the physical law that controlled the diffusion of molecules through agar gel. As a result, there was a gradual change in test materials concentration in the agar media surrounding each disc.
c) The plates were then kept in an incubator at 37°C for 24 h to allow maximum growth of the organisms. If the test materials would have any antibacterial activity, they would inhibit the growth of microorganisms and moderate zones of inhibition would be observed surrounding the discs.

**Subculture preparation as standard protocol.**

**Principle of agar discs diffusion method**

In the disc's diffusion assay, the surface of a nutrient agar medium contained in a petri dish was uniformly inoculated with the test bacterial culture. The test sample solution was applied on a filter paper disc with the help of a micropipette and dried at room temperature. The filter paper discs were then placed on each of the Petri dishes previously inoculated.

**Procedure to determine antibacterial activity**

Antibacterial activity was determined by keeping the Petri-dishes at room temperature for 6-12 hours. This method was developed by Bondi and standardized by Bauer et al in 1966 for susceptibility tests.

## RESULTS

**Purification of sweet potato amylase by using DEAE-Cellulose Column Chromatography**

The supernatant of protein solution after centrifugation was dialyzed against distilled water for 12 hours. After removal of the insoluble material, the clear supernatant was applied to the DEAE-cellulose column previously equilibrated with 10mM Tris-HCl buffer, pH 8.2 of the same pH at 4°C. The unbound protein fraction was eluted with the same buffer and the bound fraction was eluted with the linear gradient of NaCl (0-0.5M) as shown in Fig-4.2 unbound (not shown) and bound fractions-2, 3, and 4 did not show any amylase activity. The fraction shown in the figure gave amylase activity and each tube was subjected to SDS-PAGE for purity check. Only the tubes indicated by the solid bar gave three bands as shown in Fig-4.5. The proteins of crude enzyme extract from sweet potato were eluted as one major peak (F-1) and three minor peaks (F-0, F-2, F-3). The major fraction F-1, eluted by 0.25M NaCl gradient contained the amylase activity while the other three minor fractions eluted had no amylase activity. The active fraction F-1 was pooled separately and the purity was checked by the slab gel electrophoresis (Fig. 4.5). As shown in the figure, the F-1 fraction gave more than 1 band indicating that F-1 fraction contained more than 1 protein.

**Gel-Filtration Chromatography of F-1 Fraction**

The active fraction F-1 after DEAE-cellulose column chromatography was dialyzed against 10 mM tris buffer, pH 8.2 overnight, and charged onto gel-filtration column previously equilibrated with the same buffer at 4°C. The contents of the F-1 fraction were eluted with 10 mM tris buffer; pH 8.2. The elution profile of the F-1 fraction is shown in Fig. 4.3. The F-1 fraction is separated into three protein peaks F-la, F-lb, and F-lc. Of these, only F-lb contained the amylase activity. The active fraction (F-lb) was obtained from gel-

filtration chromatography. The data on the purification of sweet potato amylase are presented in Table 4 .4. The specific activity of the enzyme was about 132.05-fold purification achieved. The decrease in yield may be due to the denaturation of the enzyme during purification or to some other reasons.

**Determination of Molecular weight by SDS-PAGE Method**

The molecular weights of the partially purified amylase were determined by SDS-polyacrylamide gel electrophoresis using Phosphorylase B (Mr. 97.4 kDa), Bovin Serum Albumin (Mr. 66.2 kDa), Carbonic anhydrase (Mr. 31 kDa), Lysozyme (Mr. 14.4kDa). Aprotinin (Mr.6.5 kDa) as reference proteins. The molecular weight was calculated from the standard curve of reference proteins which was constructed by plotting molecular weight against the relative mobility of the reference proteins on the gel after electrophoresis and the molecular weight of the partially purified amylase as determined by SDS-PAGE. It assumed that there was amylase presented and estimated to be 64±1kDa of molecular weights. To obtain a better result, the enzyme should purify completely. But for the shortage of time, we assume that the enzyme is partially purified. There need more study and investigation regarding this field.

**Characterization of Amylase**

**Determination of the Type of Purified Amylase**

The purified amylase enzyme gave 100% hydrolytic activity when treated with the substrate in absence of $HgCl_2$, but no hydrolytic activity was found if the substrate solution was pre-mixed with $2x10^{-3}$M $HgCl_2$, an inhibitor of β-amylase. Further, the hydrolytic activity of the purified enzyme was found to be unchanged in the presence of $2x10^{-3}$M EDTA (α-amylase inhibitor) [26]. These findings indicated that the purified amylase was of β-type. Generally, it is found that the amylases having a molecular weight of less than 50kDa are α-amylase, and amylases having a molecular weight of more than 50kDa are β-amylase. The purified amylase enzyme was found to have a molecular weight of 64 kDa, so the purified amylase might be β-amylase.

**Effect of pH on the Enzyme Activity**

The enzyme activity of β-amylase against starch as substrate was assayed using buffers of different pH values varying from pH 4.0 to 11.0 (pH 4.0-6.0 citrate buffer, pH 6.5.0-7.5 phosphate buffer, pH 8.0-8.5 Tris-HCl buffer pH9.0-11.0 glycine buffer). As shown in Fig. 4.10, the pH - activity profile of sweet potato β-amylase gave a characteristic bell-shaped curve. It was observed from the curve that the optimum pH of amylase was 7.5

**Effect of Temperature on the Amylase Activity**

The effect of temperature on the activity of β-amylase of sweet potato was examined in the range of (30-90) °C. As shown in Fig. 4.7 the optimum activity of amylase was observed at 55°C. There was a sharp increase in activity with a gradual increase in temperature up to 60°C while the activity gradually decreased with a further rise in temperature indicating the loss in the active conformation of the enzyme. The enzyme was stable below 65ºC and its activity significantly decreased at and above 90º C.

**Substrate Specificities**

As given in Table4, the β–amylase gave about 100% activity when soluble starch and amylopectin were used but gave more than 200% activity when starch grains from pea were used. It was also shown that when amylose and starch grains from potatoes were used, low activity was found. On the other hand, no hydrolytic activity was observed when the enzyme was incubated with maltose and maltotriose.

In brine-shrimp lethality bioassay, the amylase showed results indicating that the amylase is less cytotoxic. The mortality rate

of brine-shrimp nauplii was found to be constant with the increase of concentration of the amylase and a plot of concentration vs. percentage mortality gave a linear correlation (Fig 4.8). By using Probit analysis, $LC_{50}$ was determined by exploration and the $LC_{50}$ values are 1970.288 µg/ml. The antibacterial activity of partial pure enzyme solution was determined at doses 123.2µg/disc and 184.8 µg/disc that did not give a zone against *Staphylococcus aureus, Escherichia coli, Salmonella enterica, Bacillus subtilis.*

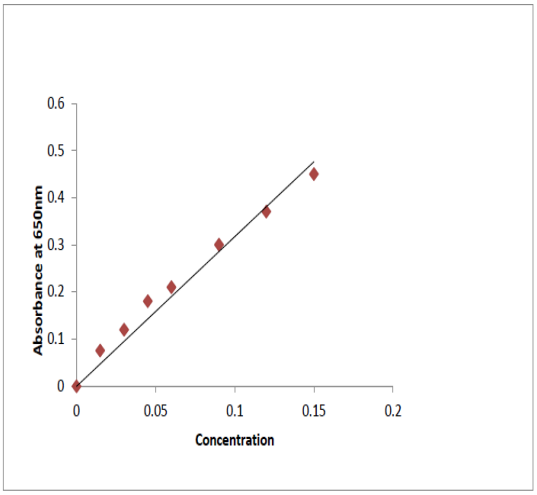
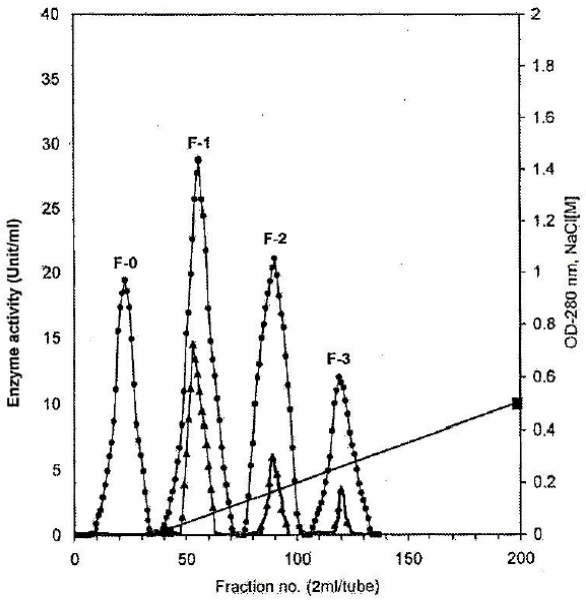



**Fig.4.1: Standard curve of BSA for the determination of protein concentration.**

**Fig. 4.2: Elution profile of crude enzyme from DEAE-cellulose column. The crude enzyme was applied to a DEAE-cellulose column (2. lx 15 cm) pre-equilibrated with 10 mM tris buffer, pH 8.2. The column was eluted with the same buffer until the absorbance of the eluate at 280 nm returned to base line. The column was then eluted with a linear gradient of NaCl (0 to 0.5 M) in the same buffer. The flow rate of the column was 30 ml/h.**




***Symbols:*** **(●) absorbance at 280 nm, (◆) enzyme activity and (■) NaCl gradient**

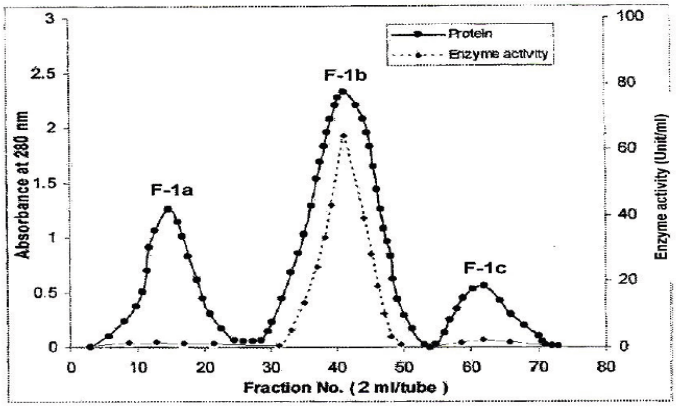



**Figure-4.3: Elution Profile of F-1 fraction from DEAE-cellulose column. Fraction F-1 obtained from DEAE-cellulose column chromatography was applied to a Gel-filtration column (0.5x35 cm) pre-equilibrated with 10 mM sodium tris buffer, pH 8.2. The column was eluted with same buffer until the absorbance of the eluate at 280 nm returned to base line. The flow rate of the column was 20 ml/h.**

***Symbols:*** **(●) absorbance at 280 run, (◆) enzyme activity**

## A brief scheme of the overall purification steps of amylase from sweet potato

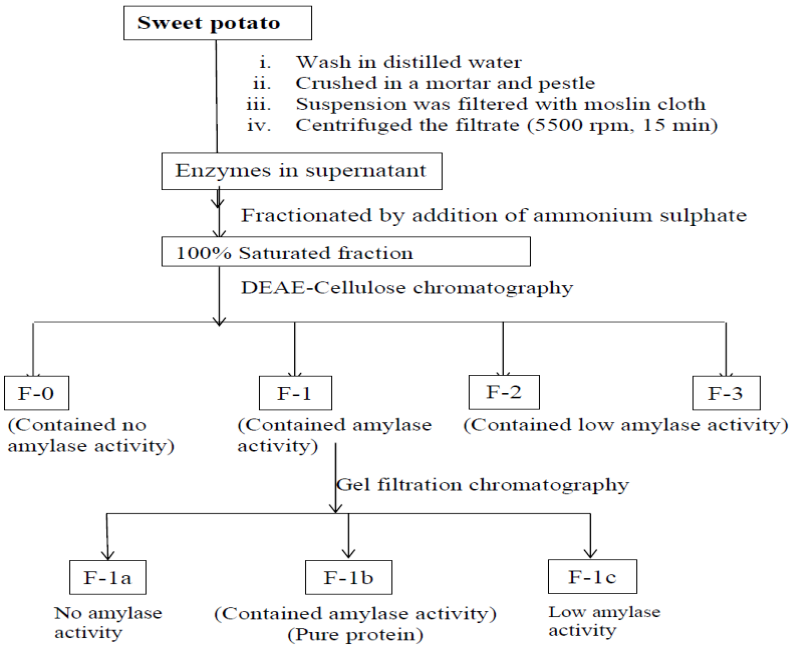



**Fig. 4.4: Schematic representation of the purification steps of amylase enzyme.**

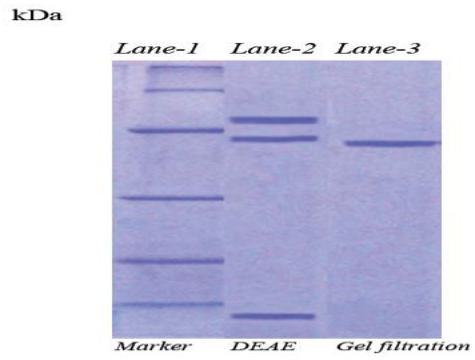



**Fig. 4.5: Purity check and Molecular weight determination of amylase by SDS-PAGE.**
**Lane-1: marker protein;**
**Lane-2: after DEAE cellulose column;**
**Lane-3: partial pure amylase by gel filtration.**
**Standard = Phosphorylase B (Mr. 97.4 kDa), Bovin Serum Albumin (Mr. 66.2 kDa), Carbonic anhydrase (Mr. 31 kDa), Lysozyme (Mr. 14.4kDa). Aprotinin(Mr.6.5 kDa)**

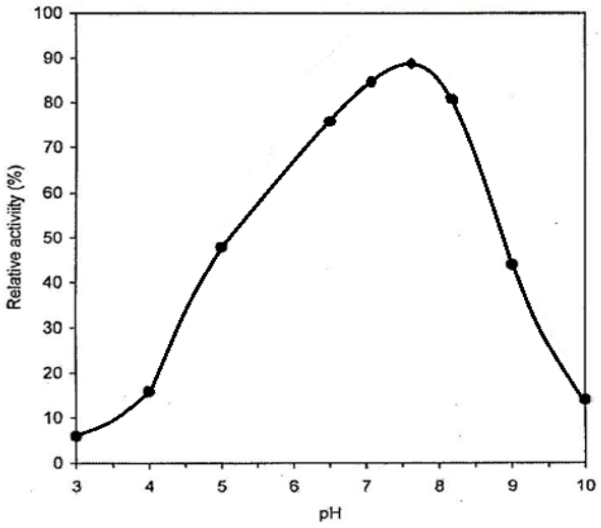



**Fig.4.6: Effect of pH on the activity of sweet potato β-amylase.**

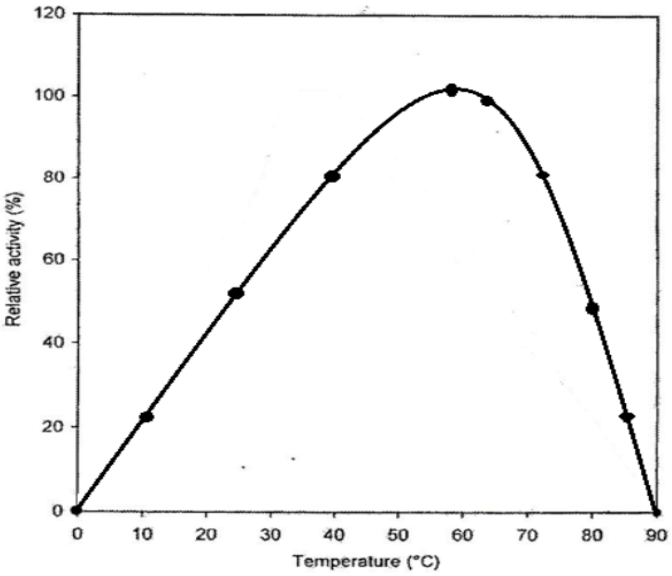



**Fig.4.7: Effect of temperature on the activity of sweet potato β-amylase**

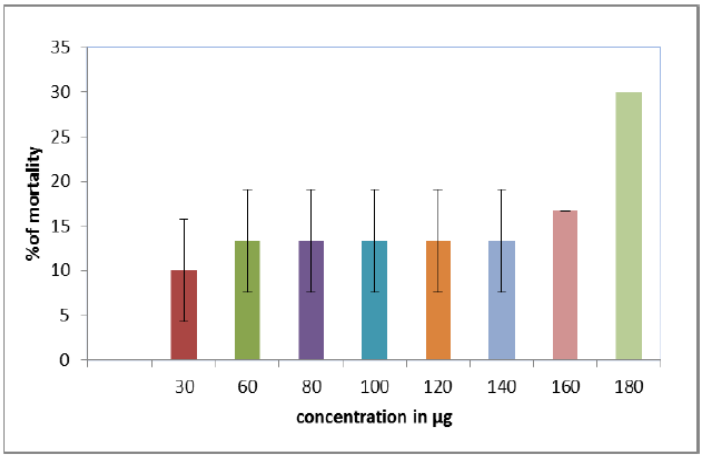



**Fig. 4.8: Percent mortality of brine shrimp treated with enzyme solution after 24 hrs. exposure**
**Lc 50=1970.288 µg/ml**
**Log LD50=3.29**

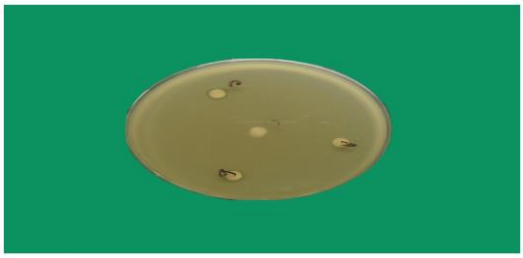


**Fig.4.9: Effect of partially purified amylase**

**Table 4.1: List of the test pathogenic bacteria**

| **Gram positive** | |
|---|---|
| **Serial No.** | **Name of test organism** |
| 1 | *Staphylococcus aureus* |
| 2 | *Bacillus subtilis* |
| **Gram negative** | |
| 3 | *Salmonella enterica* |
| 4 | *Escherichia coli* |

**Table 4.2(a): Composition of nutrient agar culture medium**

| **Ingredient** | **Amount** |
|---|---|
| Bacto peptone | 0.5 gm |
| Sodium chloride | 0.5 g |
| Bacto yeast extract | 1.0g |
| Bacto agar | 2.0 g |
| Distilled water | 100 ml |
| pH | 7.2±0.1 at 25 °C |

**Table 4.2(b): Composition of nutrient broth culture medium**

| **Ingredient** | **Amount** |
|---|---|
| Bacto yeast extract | 1.0 gm |
| Sodium chloride | 0.5 gm |
| Bacto peptone | 0.5 gm |
| Distilled water | 100 ml |
| pH | 7.2±0.1 at 25 $^0$C |

**Table 4.3: Characteristics of the ingredients used in media**

| **Raw materials** | **Characteristic** | **Nutritional value** |
|---|---|---|
| 1. Peptone | The product resulting from the digestion of materials, e.g.; meat, casein and gelatin, digestion of the proteins materials is accomplished with acids or enzymes, many different peptones (depending upon the protein used and the method of digestion) are available for the use in bacteriological media, and peptones differ in their ability to support growth of bacteria. | Principal source of organic nitrogen may also contain some vitamins and sometimes carbohydrates depending upon the kind of proteinaceous material digested. |
| 2. Agar | A complex carbohydrate obtained from certain marine algae: Processed to remove extraneous substances. | Used as a solidification agent for media, agar dissolved in aqueous solutions, gels when the temperature is reduced below 45°C: Agar is not considered as a source of nutrient to the bacteria. |
| 3. Yeast extract | An aqueous extract of yeast cells, commercially available as powder | A very rich source of the vitamins B: also contains organic nitrogen and carbon compounds. |

**Table-4.4. Summary of purification of amylase from sweet potato:**

| **Steps** | **Total protein (mg)** | **Total activity (Unit/mg)** | **Specific activity** | **Yield (%)** | **Purification fold** |
|---|---|---|---|---|---|
| Crude extract | 565 | 1070 | 1.84 | 100 | 1 |
| DEAE-cellulose | 14.32 | 502 | 41.61 | 46.15 | 20.38 |
| Gel-filtration chromatography | 4.00 | 232 | 245.73 | 23.41 | 132.05 |

**Table-4.5. Substrate specificity of the purified β–amylase from sweet potato.**

| **Substrate** | **Relative activity (%)** |
|---|---|
| Soluble starch | 100 |
| Amylopectin | 103 |
| Amylose | 55 |
| Maltose | 0 |
| Maltotriose | 0 |
| Starch (pea) | 205 |
| Starch (potato) | 50 |

**Table-4.6. Effects of Metal ions and Chemical Reagents on β–amylase activity from sweet potato**

| Metal or Chemical Reagent | % Relative activity |
|---|---|
| Control (soluble starch) | 100 |
| $CuCl_2$ | 4.0 |
| $HgCl_2$ | 2.6 |
| $FeCl_3$ | 106 |
| NaCl | 90 |
| $CaCl_2$ | 89 |
| $MgC_2$ | 92 |
| $MnCl_2$ | 107 |
| $PbCl_2$ | 5.0 |
| EDTA | 110 |
| Urea | 2.5 |

## DISCUSSION

The enzyme amylase has high amylolytic activity. It rapidly hydrolyzes poly- and oligoglucans from the nonreducing ends of the chains releasing successive maltose units. The molecular weight of β–amylase from sweet potato determined by Shephadex G-75 gel filtration column was 64±1 kDa. This is in good agreement with the molecular weight (66.2 kDa) determined by SDS-PAGE (Figure 4.5). β-amylase from sweet potato has a pH optimum for starch hydrolysis at 7.5 (Fig. 4.6). However, the optimum pH of amylase activity in extracts of sprouted potato tubers was reported by Ross & Davies (1987) as 6.0. Hydrolysis of soluble starch by β–amylase isolated from pea epicotyl and leaves of Arabidopsis had optima at pH 6.0 and 6.0-6.5 respectively, Cereal β–amylases have pH optima at 5.0-6.0 reported by Yamamoto (1988), with which our result is in good agreement. The optimum temperature for β–amylase activity from sweet potato was (Figure 4 7) reported that the temperature optimum was 75º C for Clostridium thermos sulphurogenes β–amylase,] reported 55º C for bulbs of G. Klattianus which are similar our result. β–amylase and reported as 50º C for Bacillus polymyxa N3 β–amylase, which is low than our result. Some disaccharides and polysaccharides were tested as substrates for β–amylase activity shown in Table 4.5. From the results it is clear that the rate of hydrolysis is highest in the case of starch (pea) followed by amylopectin and soluble starch (potato). This result is very similar in substrate specificity for pea epicotyl β-amylase as reported earlier. The effects of metal ions and chemical reagents on the sweet potatoβ–amylase enzyme activity was studied (Table 4.6). The purified enzyme was inactivated in presence of $Cu^{2+}$, $Hg^{2+}$, $Pb^{2+}$, and Urea, and was strongly activated in presence of $Fe^{3+}$, $Mn^{2+}$, and EDTA. These results are in agreement with those reported by Okamato & Akazawa (1978) from rice seed β–amylase, radish root, and small abalone. This Amylase also showed less toxicity towards brine shrimp nauplii, with the $LC_{50}$ value of 1970.288µg/ml. The antibacterial activity of partially purified Amylase solution was determined at doses 123 µg/disc and 184.8µg/disc that did not give any zone against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella enterica.* So, from the results discussed above, we can say that partially pure amylase solution has no antibacterial activity against these bacteria.

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**Conflict of Interest:** None

**Source of Funding:** None

## REFERENCES

1. Ray RC and Ravi V. Post-harvest spoilage of sweet potato and its control measures. *Critical Review of Food Science and Nutrition* 2005; 35: 623-644.
2. Purseglove and John Williams. Longman Scientific and Technical. New York: John Wiley and Sons. *Tropical crops: Dicotyledons* 1968.
3. Woolfe and Jennifer A. Sweet Potato: An Untapped Food Resource. *Cambridge University Press and the International Potato Center (CIP), Cambridge, UK* 1992.

4. Loebenstein G and Thottappilly G. *The sweet potato* 2009; 391–425.
5. Scott, Best, Rosegrant, Bokanga. Roots and tubers in the global food system: A vision statement to the year 2020. *International Potato Center, and others* 2000.
6. Bangladesh Bureau of Statistics (BBS) 2010.
7. "Crop Production, Worldwide, 2010 data". FAOSTAT, *Food and Agriculture Organization of the United Nations*; 2011.
8. Austin and Daniel F. The taxonomy, evolution, and genetic diversity of sweet potatoes and related wild species. In: Gregory P. Exploration, Maintenance, and Utilization of Sweet Potato Genetic Resources. First Sweet Potato Planning Conference, 1987. *International Potato Center (CIP), Lima, Peru* 1988; 27–60.
9. Steingold A C. The Uber Tuber. *Hana Hou!* 2008; 11(4): 2.
10. Zhang D P, Ghislain M, Huaman Z, Cervantes J C, Carey E E. *AFLP Assessment of Sweetpotato Genetic Diversity in Four Tropical American Regions* International Potato Center (CIP) Program report 1997-1998. Lima, Peru: *International Potato Center (CIP)* 1999.
11. Stephen K. O'Hair. "Tropical Root and Tuber Crops". In: J. Janick and J.E. Simon (eds.), Advances in new crops. Timber Press, Portland, OR. 1990; p 424–8. Retrieved 26 September 2014.
12. Spence, Jonathan D. (1993). *Chinese Roundabout: Essays in History and Culture* (illustrated, reprint, revised ed.). W. W. Norton & Company. p 167.
13. Takekoshi and Yosaburō. (1930). *Economic Aspects of the History of the Civilization of Japan*, p 352
14. Kim, Jinwung. (2012). *A History of Korea: From 'Land of the Morning Calm' to States in Conflict*, p 255
15. Bazzano L A, He J, Ogden LG, Loria C M, Vupputuri S, Myers L, Whelton P K. Fruit, and vegetable intake and risk of cardiovascular disease in US adults: the first National Health and Nutrition Examination Survey Epidemiologic follow-up study. *American Journal of Clinical Nutrition* 2002; 76: 93–99.
16. Deb A C. Fundamental of Biochemistry. p 118.
17. Burtis C A, Ashwood E R. *Tietz Textbook of Clinical Chemistry, 3rd ed* Philadelphia: W. B. Saunders Company, 1999; 1318: 689-698. ISBN 0-7216-5610-2.
18. Mikami B, Morita Y, Fukazawa C. Primary structure and function of beta-amylase. *Seikagaku* 1988; 60: 211-216.
19. Friedberg F, Rhodes C. Segments of amino acid sequence similarity in beta-amylases. *Protein Sequence & Data Analysis. 1988;* 1: 499-501.
20. Nitta Y, Isoda Y, Toda H, Sakiyama F. Identification of glutamic acid 186 affinity-labeled by 2, 3-epoxypropyl alpha-Dglucopyranoside in soybean beta-amylase. *Biochemistry Journal 1989;* 105: 573-576.
21. Totsuka A, Nong V H, Kadokawa H, Kim C-S, Itoh Y, Fukazawa C, (1994). Residues are essential for catalytic activity of soybean beta-amylase. *European Journal of Biochemistry* 1994; p 649-654.
22. Sierks M R, Ford C, Reilly P J, Sevensson B, (1990). Catalytic mechanism of fungal glucoamylase as defined by mutagenesis of Asp 176, Glu 179, and Glu 180 in the enzyme from *Aspergillus awan1ori. Protein Engineering* 1990; 3: 193-198.
23. Ohnishi H, Kitamura H, Minowa T, Sakai H, Ohta T. Molecular cloning of a glucoamylase gene from a thermophilic clostriditun and kinetics of the cloned enzyme. *European Journal of Biochemistry* 1992; 207: 413-418.
24. Henrissat B. A classification of glycosyl hydrolases based on amino acid sequence similarities. *Biochemistry Journal* 1991; 280: 309-316.
25. Noto Y, Sato T, Kudo M, Kurata K, Hirota K. The Relationship Between Salivary Biomarkers and State-Trait Anxiety Inventory Score Under Mental Arithmellc Stress: A Pilot Study. *Anesthesia & Analgesia* December 2005; 101 (6): 1873-1876. United States: Lippincott Williams & Wilkins.
26. Lehrer J K and MD. *Medical Encyclopedia*, Department of Gastronterology, Frankford-Torresdale Hospital, Jefferson Health Syste1n, Philadelphia, PA 2007.
27. Mapp C E. Agents, old and new, Causing occupational astluna. *Occup linviron Med.* 2001 58(5): 354-60, 290.
28. Udani J, I-lardy M, Madsen DC, (2004). *Blocking carbohydrate absorption and weight loss: a clinical trial using Phase 2 brand proprietary .fractionated white bean extract. A/tern Med Rev.,* 9(1 ): p. 3-9.

29. Sulzenbacher G, Shareck F, Morosoli R, Dupont C, Davies G J. "The Streptomyces lividans family endoglucanase: construction of the catalytic cre, expression, and X-ray structure at 1.75 Å resolution". *Biochemistry* 1997; 36: 16032–16039. doi:10.2210/pdb1nlr/pdb. PMID 9440876.; rendered with PyMOL
30. Watanabe H, Noda H, Tokuda G, Lo N. A cellulase gene of termite origin. *Nature* 1998; 394: 330-331; Brune A and Okhume M. Role of the termite gut macrobiota in symbiotic digestion, in Bignell D E, ed., Biology of Termites: A Modern Synthesis 2010: ch. 16.
31. Hill R and Needham J. The Chemistry of Life: Eight Lectures on the History of Biochemistry, Effects of pH (Introduction to Enzymes). *London, England: Cambridge University Press* 1970.
32. Maton, Anthea, Hopkins J, McLaughlin C W, Johnson S, Warner M Q, LaHart D, Wright J D. *Human Biology and Health. Englewood Cliffs, New Jersey, USA: Prentice Hall* 1993; ISBN 0-13-981176-1.
33. Mapp CE (May 2001). "Agents, old and new, causing occupational asthma". Occup Environ Med 58 (5): 354–60, 290. doi:10.1136/oem.58.5.354. PMC 1740131. PMID 11303086.
34. WHO(1985). Energy and protein requirements. Report of a joint FAO/WHO/UNU Expert consolation. WHO Tech. Rep. Ser. 724. Geneva, WHO 206p and
35. NHMRC(1881). Recommended Dietary Intakes for use in Australia, Rosemary Stanton (1994) Eating for peak performance.
36. Crueger W and Gruger A. Biotechnology:a textbook of industrial Microbiology (ed. T.D. Brock). *Sinauer Associate Inc. Sunderland, Illinois, USA* 1990; p 191-193.
37. Yoshiki and Yamasaki. B-amylase in germinating millet seeds. *Phytochemistry* 2003; 64: 935-939.
38. Krishan H B, Blanchette J T, Okita T W. Wheat invertase. *Plant Physiology* 1985; 78: 241-245.
39. Klysov, A.A. (1990). Trendsi n Biochemistry and enzymology. Biochemistry. 29. 10577-10585.
40. Enari, T. M. (1983).Microbial cellulases. In Microbial enzymes and Biotechnology. W.F. Rofgaty(Ed), Applied Sciences publishers, London. P 83-223
41. Wood. R.K.S(1960). Pectin and cellulolytic enzymes in plant diseases Ann. Rec.PL. Physiol. 2. 229-232.
42. Mandels, M (1985) Application of cellulose. Biochemical society Translocation. 13, 414-416.

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## Supplement

**Chemicals and Equipments uses (Supplement-1)**

**The chemicals used in this study are mentioned below with their manufacturers:**

Acetone: BDH Chemical Ltd., Poole England.
Acrylamide: Sigma Chemical Company. U.S.A.
Albumin Bovine (BSA): Sigma Chemical company. U.S.A.
Ammonium persulfate: Bio-Rad Laboratories, Richmond, U.S.A.
Ammonium sulfate: Merck, Germany
Aniline: BDH Chemicals Ltd. Poole England.
Arabinose: Sigma Chemical Company. U.S.A.
Borate (Natrium tetraborate): Sigma Chemical Company. U.S.A.
Bromophenol Blue: Bio-Rad Laboratories, Richmond, U.S.A.
Butanol: BDH Chemical Ltd., Poole England
Coomassie Brilliant Blue-250: Bio-Rad Laboratories, Richmond, U.S.A.
Copper sulfate: BDH Chemical Ltd., Poole England.

DEAE- cellulose: Pharmacia fine Chemicals Co. Ltd. Sweden
Di-sodium hydrogen orthophosphate dehydrates BDH Chemical Ltd., Poole England.
Galactose: Sigma Chemical Company. U.S.A.
Glucose: Sigma Chemical Company. U.S.A.
Glycerol: Bio-Rad Laboratories, Richmond, U.S.A.
Hydrochloric acid: BDH Chemical Ltd., Poole England.
Isopropanol: Merck, Germany
Lauryl Sulfate (SDS): Sigma Chemical Company. U.S.A.
Lysozyme: Sigma Chemical Company. U.S.A.
Mannose: Sigma Chemical Company. U.S.A.
N, N-methylene-bis-acrylamide: Sigma Chemical Company. U.S.A.
Orthophosphoric acid: BDH Chemical Ltd., Poole England.
Phenol: Sigma Chemical Company. U.S.A.
Potassium Sodium Tartrate: BDH Chemical Ltd., Poole England
Raffinose: Sigma Chemical Company. U.S.A.
Riboflavin: BDH Chemical Ltd., Poole England
Ribose: Sigma Chemical Company. U.S.A.
Rhamnose: Sigma Chemical Company. U.S.A.
Silica gel-G: Merck, Germany
Sodium dihydrogen orthophosphate: BDH Chemical Ltd., Poole England
Sodium chloride: Merck, Germany
Sodium hydroxide: Merck, Germany
Sodium azide: Sigma Chemical Company. U.S.A.
Sodium carbonate: BDH Chemical Ltd., Poole England
Sulfuric acid: BDH Chemical Ltd., Poole England
TEMED (N, N, N, N-tetramethylene diamine): Sigma Chemical Company. U.S.A.

## EQUIPMENT

**The important equipment used throughout this study is listed below:**

1) Centrifuge (Refrigerated): Eppendorf-5430R
2) Electric balance- Mettler H18
3) Fraction collector-SF-160 (Advantec, Japan)
4) Homogenizer: NISSIN (NX-220)
5) Incubator: GALLENKAMP-Size 2 Incubator
6) Micropipette: Microlit (10-100 µl)
7) pH meter: HANNA-pH 211
8) Shimadzu Spectrophotometer: Model-1200RS
9) Stirrer
10) Slab gel electrophoresis apparatus
11) Volac pipette controller
12) Water bath
13) Cold chamber
14) Laminar air flow unit: Horizontal (Model: Equ/04-EHC)
15) Refrigerator
16) Autoclave: HIRAYAMA (Model-HA-30D)
17) Freeze dryer: TAITEC (VD-800F)

### Preparation of DNS Reagent (Supplement-2)

Simultaneously 1 g of DNS is mixed with 50ml distilled water then add 30 g of Na-K tartrate and mixed. Add 20 ml 2M NaOH solution & makeup to 100 ml by adding distilled water.

### Procedure

Three sets of experiments (Blank, Control, and Sample) were performed for the measurement of invertase activity. The following different solutions were taken in different test tubes.

| Substances | Blank | Control | Sample |
|---|---|---|---|
| 0.1 M phosphate buffer, pH 6.7 | 2.5 | 2.5 | 2.5 |
| 1% Sucrose solution | 2.5 | 2.5 | 2.5 |
| 1% NaCl | 1.0 | 1.0 | 1.0 |
| Enzyme extract | ¾ | 0.5 | 0.5 |

The contents in the test tubes were mixed uniformly and the test tubes were incubated in a water bath at 37°C for 10 min. Then 0.5 ml of crude enzyme extract and 0.5 ml of distilled water were added to the sample and control tubes respectively, whereas 1 ml of distilled water was added to the blank test tube. Immediately after the addition of crude enzyme extract and distilled water, 0.5 ml of 2 N NaOH was added to the control tube. The rest of the test tubes were incubated at 37°C for 15 min and the reaction was then stopped by the addition of 0.5 ml of 2 N NaOH. Then 0.5 ml of DNS reagent was mixed into all the tubes. The tubes were heated in a boiling water bath for 5 min. After cooling at room temperature the absorbance was measured at 650 nm.

**Supplement-3: Determination of Protein Concentration (Lowry Et Al., 1951)**

**Reagents:**

a) Alkaline sodium carbonate solution (20 gm/liter $Na_2CO_3$ in 0.1 M NaOH solution).
b) Freshly prepared copper sulfate and sodium potassium tartrate solution (5 gm/l $CuSO_4$ 5H2O in 10 gm/liter Na-K tartrate).
c) Alkaline solution: Mixture of solution (a) and (b) in the proportion of 50:1 respectively.
d) Folin-Cicolteau's reagent (Diluted with an equal volume of $H_2O$ just before use).
e) Standard protein (Bovine serum albumin 10 mg/100 ml in dist. $H_2O$) solution.

**Determination of cytotoxicity of the purified enzyme by Brine-Shrimp bioassay [Supplement-4]**

Brine shrimp lethality bioassay is a recently developed method for bioactive compound assessment. This bioassay indicates toxicity as well as a wide range of pharmacological activities (e.g. anticancer, antiviral, insecticidal, pesticidal, AIDS, etc.) of the compounds. Extracts and isolated compounds from plant origin can be tested for their bioactivity by this method. Here *in vivo* lethality bioassay is conducted by using the simple zoo logic organism; brine shrimp naupli (*Artemia salina*, Leach). The method has the advantages of being very simple, rapid (24 hours), and inexpensive. It easily utilizes a large number of organisms for statistical validation and requires no special equipment and a relatively small amount of sample. In the present study, TCSL was used for its cytotoxicity study using the brine shrimp lethality test.

**Materials**

a) *Artemia salina* leach (brine shrimp eggs)
b) Sea salt (non-ionized NaCl)
c) Small tank with a perforated dividing dam to grow shrimp, cover, and lamp to attract shrimp.
d) Pipettes
e) Micropipette (10µl- 100µl)
f) Vials, (4 ml)
g) Magnifying glass. (3X magnifying glass)

**Procedure**

**a) Preparation of simulated seawater**

38 g of sea salt (non-ionized NaCl) was weighed accurately, dissolved in one liter of sterilized distilled water, and then filtered off to get a clear solution. The pH of the seawater was maintained between 8 and 9 by using a $NaHCO_3$ solution.

**b) Hatching of brine shrimp**

*Artemia salina* leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated sea water was taken in the small tank and the shrimp eggs (1.5 g/l) were added to one side of the tank and this side was covered. The shrimps were allowed for one day to hatch and immature as nauplii (larvae.). The constant oxygen supply was carried out and constant temperature (around 37˚C) was maintained during the hatching time. The hatched shrimps were attracted to the lamp on the other side of the divided tank through the

dam. These nauplii were taken for this bioassay.

**c) Preparation of sample**

The test sample contained an enzyme. This solution was used as a stock solution.

**d) Application of the test sample and brine shrimp nauplii to the vials**

Twelve clean vials were taken for the sample in four concentrations (Three vials for each concentration) and another three vials were also taken for control. Then the concentration of every three vials was 30, 60, 80, 100, 120, 140, 160, and 180mg/ml respectively enzyme solution containing the sample was added to every three vials gradually and finally marked up to 5 ml by seawater. With the help of a Pasteur pipette, 10 living shrimps were taken to each sample vial and control vial respectively.

**e) Counting of nauplii**

After 24 hours of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial was counted and noted. From this, the nauplii were counted averagely of each three vials, which contained the same conc. of the sample, the percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Probit analysis.

**Method**

For the construction of standard curves 0.0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard protein solution (1 mg/ml) were taken in different test tubes and made up to the volume of 1 ml of distilled water. The protein solution (1 ml) was also taken in duplicate in different test tubes and 5 ml of the alkaline solution (solution-c) was added to the standard protein solution in different test tubes and mixed thoroughly. It was allowed to stand at room temperature for 10 minutes. Then 0.5 ml of diluted Folin-Cicolteau's reagent was added rapidly with immediate mixing and left for 30 minutes. The dark blue color formed was measured at 650 nm against the appropriate blank. By the same procedure described above, the absorbance of the collected fractions was measured and a graph was constructed by plotting concentration versus OD. The protein concentration was determined in the fraction from the graph.

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