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RESEARCH ARTICLE

EXTRACTION AND CHARACTERIZATION OF B-GALACTOSIDASE ENZYME FROM ZIZIPHUS SPINA-CHRISTI FOR FOOD APPLICATIONS

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Abstract

Galactosidaseis an industrially important enzymewitha number of applications in dairy, fermentation, and food industries. The study was carried out to extract and characterize the galactosidase enzymefromZiziphusspina-christi,a therapeutic herb. The enzyme was studied for pH, temperature sensitivity, and heavy metal effects on enzyme activity. The enzyme activity of galactosidase was analysed for its capacity to hydrolyze 2-nitrophenyl -D-galactopyranoside (ONPG). The results show that at a temperature of 50 °Cand pH 5.5, the enzyme was most active.The Km and Vmax of the enzymewas 3.65 mmand 0.18 mol/min. HgCl2 and KCN was fully inhibited by the activity ofβ-galactosidase from Ziziphusspina-christi.After 4h, Ziziphusspina-christiβ-galactosidase reduced milk lactose by 38.5% and 70%. Hg+2was decreased most heavily by β-galactosidase activity, whereas Cu+2reduced the least. Study results confirm the efficacy ofβ-galactosidaseenzymefrom Ziziphusspina-christi leaf extract which can be utilized in food and healthcare industry.

Introduction:-

The enzyme, galactosidase, is sometimes called D-galactohydrolase. It is in the same family as lactase and transglycosylases. It is often used to split lactose into galactose and glucose [1], and it can also split-linked galactose residues in a number of different substances. This enzyme is found in many places in nature, including inside the cells of plants and animals, as well as in many types of germs [2, 3]. β-galactosidases break down structural polysaccharides in plant cell walls, which is one of the biochemical processes they are involved[4]. In healthcare industry, they are used for treating people with lactose intolerance [5, 6]. Two of the most important function of these enzymes are production ofgalactosylated products and removal of lactose from dairy products [5]. The ability of β-galactosidases to break down the non-reducing -D-galactosyl groups at the ends of -D-galactosidases makes them unique [6]. The enzymes are widely present in parts of many plants, which can be extracted and purified for further utilization. It has been extracted from chickpeas [7], almonds [8], apricots [9], Vigna unguiculata [10], and apricot seed [11]. Galactosidase is required for the fruit ripening, they have shown theβ-galactosidase activity during the entire process of fruit growth and ripening in pepper [12], and Arabidopsis [13].Astudy by Asma et al. [14] has found that the production level of mRNAβ-galactosidase rises greatly during the ripening process. Scientists have found β-galactosidases in many parts of plants, like seeds [9], stems [10], and some parts of roots [6], like the meristem zones, trichomes, cotyledons, vascular tissues, and pollens. Plant β-galactosidase is abundant in nature, economical and can be used for different applications, which makes it a great choice for commercial use [5].

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Heavy metals are necessary for plants to grow and are play an important role in the process of making many important chemicals [15]. In addition to making heavy metals, a number of other important chemicals are also produced.Micronutrients like zinc (Zn2+), copper (Cu^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), and cobalt (Co^{2+}) are required by the plants for their growth. On the other hand, the biological roles of metals like Cd^{2+}, Pb^{2+}, and Hg^{2+} are not fully known [16]. Literature evidences that heavy metals are toxic to the environment as they are very poisonous, especially when there is huge quantity [17]. There is evidence that heavy metals can change the rate of chemical reactions and the enzyme properties. In turn, this can cause changes in the energy utilization by plants. Having too many heavy metals in the body can also lead to reactive stress [17].

Ziziphusspina-christi, which is also called Wormwood or al-ba'atharan in Arabic, is a fragrant annual shrub that grow in dry places. They are geographically found in the valley bottoms along the borders between Saudi Arabia, Jordan (Al-Mudawarah) and in Egypt's Sinai Peninsula [18, 19]. People in both Jordan and Egypt have used this plant as medicine for a long time since they bioactivities in artemisia, such as antiviral, antipyretic, antihemorrhagic, anticoagulant, anticancer, anti-anginal, and anti-ulcerogenic properties [20].β-galactosidasepresent in plant extracts of Ziziphusspina-christiwas isolated and a basic investigation was carried out to observe the functions of enzymes, their kinetics, and the effects of heavy metals. This study is the first step towards using β-galactosidase in the future for a wide array of applications in the field of medicine,biotechnology and food industry.

Materials and Methods:-

Collection of plant samples

Fresh specimens of Ziziphusspina-christithat had just been harvested was purchased from the local marketplaces in Baghdad, Iraq.

Preparation of Plant Extracts

β-galactosidase was extracted fromZiziphusspina-christi plant as follows; the plants were macerated with 0.2 M sodium-phosphate buffer (pH 6.0)in a blender for 4 min. After filtering the mixture through a cotton sheet, the homogenate was separated and 0.2M sodium phosphate buffer (pH 6.0) was added and subjected to centrifugation for 20 min at a rate of 10,000 rpm/min. The supernatant containing the extracted galactosidase enzyme was collected and tested for β-galactosidaseactivity [21].

Protein Quantification

Quantification of the protein content was estimated using spectrophotometry at a wavelength of 595 nm using the Bradford method [22], and Bovine Serum Albumin (BSA) was used as the reference standard.

Enzymes analysis

The rate at which β-galactosidase degrades ortho-Nitrophenyl-β-D-galactoside(ONPG) was quantified using the procedure described by Hussein et al. [23].In the presence of β-galactosidase, ONPG is hydrolyzed to D-galactose and o-nitrophenol (ONP). The β-galactosidase protocol required 0.4 mL of 0.1M acetate buffer (pH 4.0), 0.5 mL of substrate solution (2 mM/L), and 0.1 mL of enzyme solution. After 15 min of incubation at 37 °Celsius, 1 mL of solution containing 0.1mm of sodium carbonate was added to inhibit the reaction, and the solution was read at 420 nm to observe the efficiency of the enzyme. According to the parameters of the experiment, a "unit" of enzyme activity is the quantity of enzyme that catalyses the production of 1 micromole of ONP in one minute. That quantity of enzyme is equivalent to one "unit".

The Impact of pH on Enzyme Activity

According to Gulzar and Amin [9], the optimal pH of β-galactosidases was determined by incubating the enzyme at a temperature of 50 °Cin a number of buffers whose pH values ranged from 2.0 to 9.0. The results of the experiment were recorded. Each buffer system was tested independently for its enzyme activity. A calculation was made to determine the proportion of relative activity.

Influence of Temperature on Enzymatic Activity

After putting the reaction mixtures through a series of temperature changes ranging from 25to 80 °C, the ideal temperature for galactosidases was determined. The enzyme activity was measured as a percentage of its relative activity in order to get an accurate reading.

Kinetic Parameters Determination

In the context of the document, the kinetic parameters of the beta-galactosidase enzyme from Ziziphusspina-christi was determined by measuring the initial reaction rates at different concentrations of ONPG (ortho-Nitrophenyl-β-galactoside), which served as the substrate. When employing ONGP as the substrate, both the maximum velocity of β-galactosidases, denoted by Vmax, and the Michaelis-Menten constant, denoted by Km, has to be measured. At a pH of 6.0 and a temperature of 50 °Celsius, the effect of substrate concentration on enzyme activitywas evaluated. Different concentrations of ONGP in the range of 1 mM to 9 mM was used. The absorbance of the reaction was measured at a wavelength of 420 nm.The Line-Weaver Burk plot, also known as the reciprocal plot, was used to analyze the data. The plot involved plotting the reciprocal of the velocity (1/V) against the reciprocal of the substrate concentration (1/[S])[24].

Effect of metal ions and heavy metals on enzyme activity

The enzyme activity was analyzed in the presence of various metal ions and inhibitors like $HgCl_2$ and KCN.0.2 M sodium-phosphate buffer (pH 6.0) was used to prepare the stock solutions of $FeSO_4$, $NiCl_2$, $MnCl_2$, $CuSO_4$, $FeCl_3$, KCN, and $CaCl_2$. Each of these solutions was added one at a time to the reaction mixture until it reached a final concentration of 0.001 mol/Liter. The pH of the buffer was then changed to the level that worked best for each β-galactosidase extract.Beta-mercaptoethanol and ethylene-diaminetetracetic acid (EDTA), two inhibitors, were also used in the test. EDTA was present at a concentration of 0.0001 mol/L and beta-mercaptoethanol at a concentration of 0.01 mol/L. The enzyme activity was observed in the presence of the above-mentioned metal ions and inhibitors.

Statistical analyses

Each experiment was performed in triplicates, and the results are expressed as Mean ± SDanalysed in Graph pad Prism 5.0.

Results & Discussion:-

Protein Concentration

Using the Bradford method and BSA as the reference protein, the amount of protein was quantified in the extract of (Ziziphusspina-christi). According to the results of the investigation, the protein content in the Ziziphusspina-christi extract was 0.56±0.75 mg/ml.

Influence of pH on the activity of β-galactosidase

At a particular pH, each enzyme operates at its most efficient level, and these optimal pH levels vary amongst enzymes. Any change in pH will cause changes in the three-dimensional structure of enzymes, which will in turn have an effect on the activity of the enzymes. When the pH level either increases or decreases, certain amino acids go through a process called deprotonation or protonation. This process causes changes to occur in the structure and function of proteins [24]. As shown in Figure 1, there was a discernible shift in the level of β-galactosidase activity that occurred in response to varying pH values.The activity of the enzyme was at its highest at a pH of 5.5, and maintained its stability throughout a wide range of pH values, from 2.5 to 8.5. When exposed to varied pH levels, the β-galactosidase enzyme demonstrated a range of variable levels of activity. At pH 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6, 6.5, 7.0, 7.5, 8.0, and 8.5, the activities were measured to be 26.29%, 35.34%, 46.83%, 80.15%, 91.75%, 100%, 96.94%, 84.21%, 54.77%, 33.82%, and 19.45%, respectively.

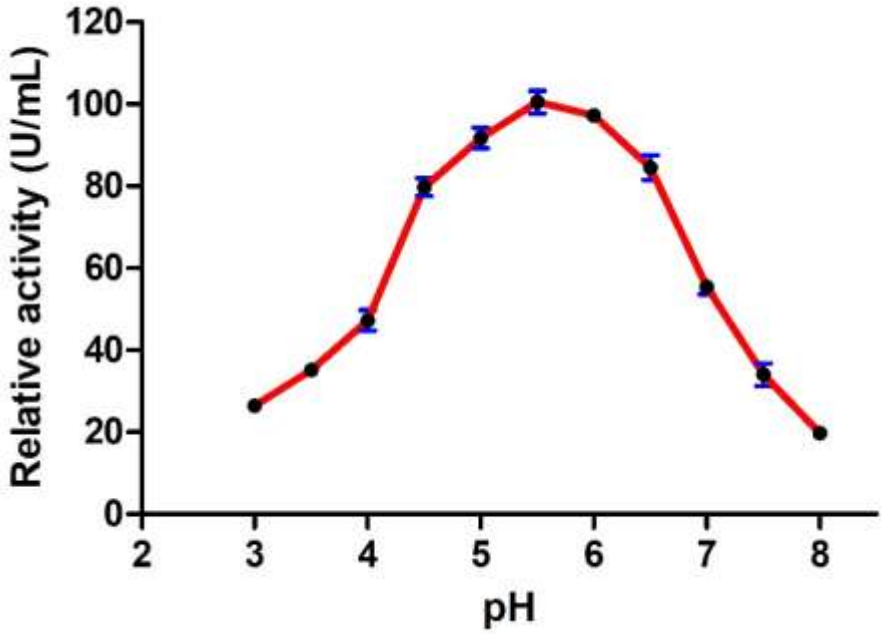

Figure 1:- Relative activity of the enzyme β-galactosidasefrom Ziziphusspina-christiextract investigates at different pH levels.

Mean ± SD of triplicates

The findings were in line with earlier findings [3], which suggested that an acidic pH is ideal for plant β-galactosidase. The results were compatible with these findings. It was discovered that a pH range of 4.0 to 6.0 was ideal for the extraction of three isoenzymes of β-galactosidase from apricots [11]. Almonds have a pH of 5.5, which has been proven to be the ideal range [8]. On the other hand, researchers discovered that the ideal pH level for β-galactosidase derived from peaches was 3.0 [25], while the that for Hymenaeacourbaril is 3.5 [26], and kidney beans is 4.0 [27]. According to the results of the study, the enzyme is effective in reducing the amount of lactose that is present in whey or milk when the pH level is between 4.5 and 6.8 [5].

The Effect of Temperature on β-galactosidase Activity

Vmax represents the temperature at which a certain enzyme reaction occurs at its maximum rate. The rate of the reaction accelerates with increasing temperature until it attains the maximum velocity, at which point the greatest quantity of molecules possessing adequate energy to surmount the energy barrier and generate the reaction products are involved[24]. The rate of the reaction will diminish due to the denaturation of the enzyme, which occurs when the naturally folded structure of proteins is altered. The denatured structure induces the proteins to unravel into an arbitrary arrangement.Increased temperatures cause the disruption of hydrogen bonds, resulting in a subsequent modification of the enzyme's molecular structure.

To ascertain the impact of temperature on the β-galactosidase activity in the plant extract, enzyme activity was quantified over a range of temperatures including 25°C and 75°C. The ideal temperature was determined to be 50°C, as depicted in Figure 2. The stability of enzymes was noted throughout the complete range of temperatures, which extended from 25°C to 60°C. Conversely, upon reaching 80°C, the enzyme demonstrated complete inactivation, operating at a mere 0% of its optimal capacity. In this study, the thermal stability of β-galactosidase was evaluated between 25°C and 80°C. The relative activities were as follows: 0% at 25°C, 16.02±0.92% at 30°C, 34.63±0.64% at 35°C, 57.47±0.77% at 40°C, 82.15±0/93% at 45°C, 100±0.90% at 50°C, 86.66±0.55% at 55°C, 46.62±0.69% at 60°C, 22.73±0.75% at 65°C, 13.43±0.42% at 70°C, 6.78±0.39% at 75°C, and 0% at 80°C.

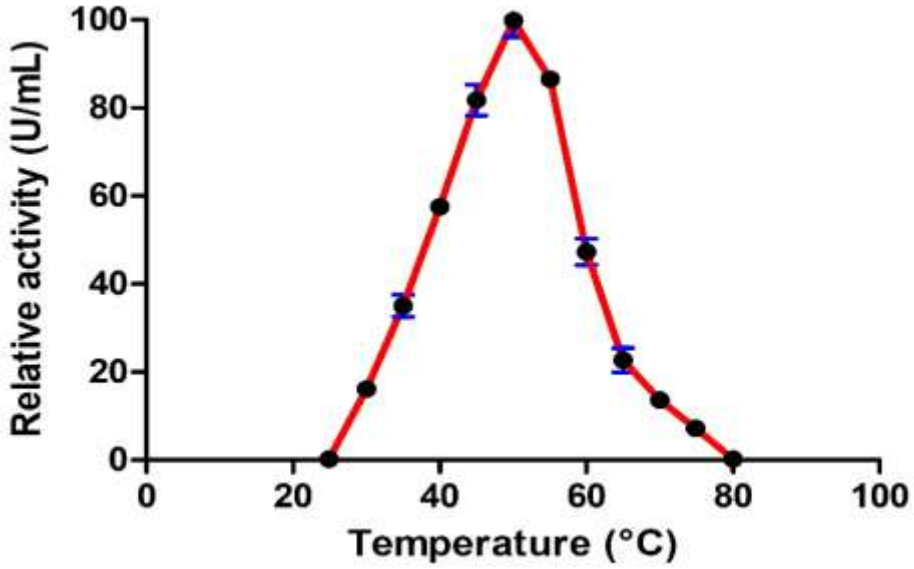

Figure 2:- The percentage of relative activity of β-galactosidase in the crude extract of Ziziphusspina-christiextracts at different temperature levels

Mean ± SD of triplicates

The unfolding of the enzyme and the consequent loss of its active site, both of which are induced by denatured proteins, are responsible for the reduction in enzyme activity that occurs at higher temperatures [27].It was discovered that nasturtium, peach, andHymenaeacourbaril all thrive at an ideal temperature of 50°C [28, 26, 2].It was reported that the temperature range of 50°C to 53°C is the best for the extraction of three isoforms of β-galactosidase from mung bean seedlings [29]. The ideal temperature for growing each type of plant was considerably different.For instance, the ideal temperature for chickpeas, cowpeas, and almonds was 60°C [7, 30, 3], but the ideal temperature for apricots was 40 °C [18]. On the other hand, the optimum temperature for apricot seeds was determined to be 70 °C [20]. According to the findings of the vast majority of the studies that were discussed earlier, the ideal temperature for the majority of β-galactosidase enzymes is somewhere in the range of 40-60 °C.When selecting enzymes for use in industrial processes, biotechnological processes, or medical processes, one of the most important considerations is finding the temperature at which they function at their optimum.The majority of commercial enzymes are said to achieve their maximal reaction rate, or Vmax, at temperatures ranging from 40 to 50 °C, according to reports [31]. Because Artemisia judaica achieves its maximum level of effectiveness at a temperature of 50 °C, it is well suited for application in both industrial and therapeutic settings [32].

Kinetics Analysis refers to the study and examination of the rates at which chemical reactions occur.
Kinetic Parameters Determination refers to the process of determining the kinetic properties of an enzyme, specifically the Km (Michaelis-Menten constant) and Vmax (maximum velocity) values. In order to determine the kinetic properties of the enzyme, namely the Km and Vmax values of β-galactosidase, the initial reaction rates were measured at a variety of concentrations of ONPG, ranging from 1 mM to 9 mM. The Line-Weaver Burk plot was utilised in order to do the analysis on the data. This plot involved plotting the reciprocal of the velocity (1/V) against the reciprocal of the substrate concentration (1/[S]). After that, the kinetic parameters were figured out by looking at the graph that was produced.

As observed from Figure 3, the Km and Vmax values of the enzyme were determined to be 3.65 mM and 0.18 mol/min, respectively.

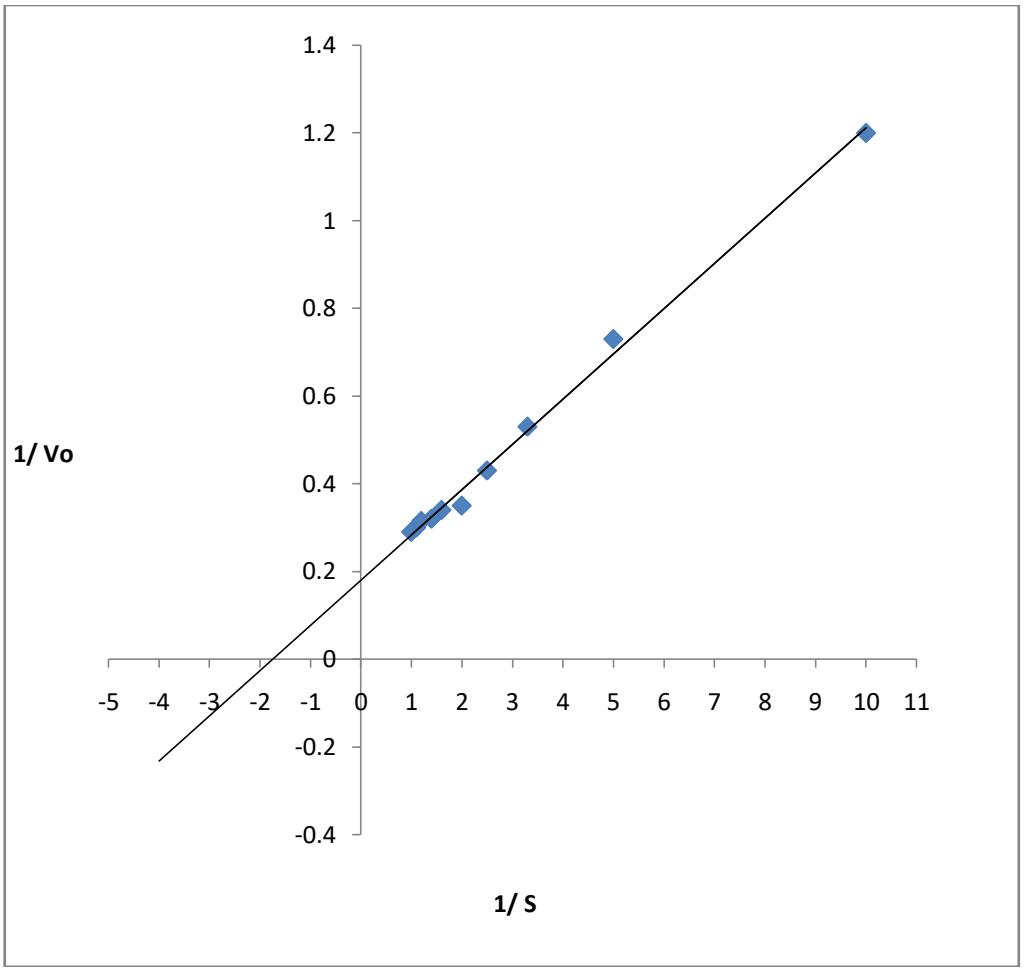

Figure 3:- Ziziphusspina-christiextracts were tested using ONPG as a substrate to determine the Vmax and Km values of β-galactosidase.

The number Km expresses how much of the substrate there is at which the reaction rate is 50% of its highest level. It shows not only how well the substrate binds to the enzyme but also how quickly the substrate is changed into the product during the catalytic activity. Because of this, the Km value can be seen as a basic way to measure how well the substrate binds to the enzyme [31].The enzyme's Km value was higher than what had been recorded for tomato fruit (1.77 mM) [33], apricot β-galactosidase I (1.85 mM) [9], chickpea (1.73 mM) [7], and radish seed (1.19 mM) [30]. Other plants, like peach (5.16 mM) [30] and almond (10.53 mM) [8], had higher Km values than this plant.The rate of a reaction, which is also called its velocity (V), is the number of source molecules that are changed into product molecules in a certain amount of time.When the concentration of the substrate goes up, the speed of an enzyme-catalyzed reaction also goes up. This keeps happening until the reaction reaches its top speed (Vmax). Saturation happens when all of the binding spots on the enzyme molecules are filled with substrate. This is shown by the reaction rate stop growing when there is a more substrate [31].Although, there was a higher Vmax number for the enzyme β-galactosidase in the crude extract than there had been before.β-galactosidase I, β-galactosidase II, and β-galactosidase III were all found to have Vmax values of 0.52, 0.70, and 0.38 mol/min [29]. However, these values were much lower than those of other plants, like rice, which had a Vmax value of 5.2 mol/min [34].These values provide insights into the substrate binding affinity and catalytic efficiency of the beta-galactosidase enzyme from Ziziphusspina-christi.

Changes in the activity of β-galactosidase in a sample of Ziziphusspina-christi caused by metal ions and inhibitors

Figure 4 shows how different metal ions and inhibitors changed the activity ofβ-galactosidase, which was found in the plant species ofZiziphusspina-christi.The results show that both $HgCl_2$ and KCN can totally inhibit the activity

ofβ-galactosidase enzyme extracted from Ziziphusspina-christi plant. $CaCl_2$, $NiCl_2$, and $CuSO_4$ were added, the activities of β-galactosidase were found to drop by 2.7±0.75%, 55.9±0.98%, and 31.5±0.55%, respectively. By the addition of either $FeCl_3$ or $FeSO_4$, the activity of β-galactosidaseincreased by 49.4±0.38% or 52.4±0.62%.

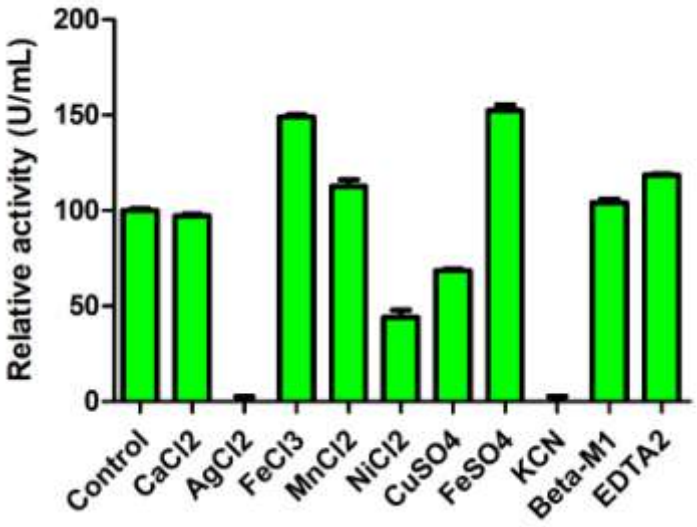

Figure 4:- Effects on relative activity of β-galactosidaseby metal ions
Mean ± SD of triplicates

With the addition of $MnCl_2$, the activities of β-galactosidase from Ziziphusspina-christienhanced by 12.8±0.70%.It was found that adding divalent ions like Fe^{2+} and Mn^{2+} to the reaction mixture made the β-galactosidase enzyme fromLactobacillus crispatus work better [35]. There are two types of Kluyveromycesmarxianus used to separate the β-galactosidase: IFoo541 and var. lactase 1-2. Researchers discovered that Mn^{2+} was the main mineral that aided the crude enzyme from K. marxianus IFoo541 work, while Ca^{2+}inhibited the reaction.Still, the enzyme from K. marxianusvar. lactis 1-2 that has not been improved showed a rise in activity when it was exposed to Ca^{2+} ions, while that of Mn^{2+} ions was only minimal [36]. β-galactosidaseextracted from mung beans lost 15.1% of its activity when it was introduced to Fe^{2+}, Ca^{2+}, Cu^{2+}, and Mn^{2+}. Still, the Hg^{2+}resulted in decreasing it to 42%.β-galactosidase from Bacillus coagulans RC53 had 17.1% less activity when Fe^{2+} was present. It had 91.1% less activity when Cu^{2+} was present, 76.3% less activity when Ni^{2+} was present, and 65.7% less activity when Hg^{2+} was present. But the presence of Ca^{2+} (7.5%) and Mn^{2+} (19.6%) improved the enzyme activity [37].The study by [38] found that Fe^{2+} and Mn^{2+} enhancedβ-galactosidase activity of Bifidobacteriuminfantis HL96, however, Ca^{2+} lead to reduced functionality. It was also found that, both Hg^{2+} and Cu^{2+} totally inhibited the enzyme function.According to the study by [39], β-galactosidase from Bacterium pseudoalteromonas worked 40% better when Mn^{2+} was present, but the activity was minimal when Cu^{2+} was present.

The results showed that the presence of beta-mercaptoethanol in Ziziphusspina-christi did not have a big impact on the enzyme activity of β-galactosidase.When EDTA was added to Ziziphusspina-christi, its enzyme activity dropped by 18.5% and 4.3%, showing that EDTA diminished β-galactosidaseactivity.There was no change in the enzyme activity of β-galactosidase from chickpeas when EDTA [10] was present at any dose.A lesserβ-galactosidase activity was found in persimmon fruit that had been treated with EDTA.

Conclusion:-

The medicinal plant Ziziphusspina-christiwas the subject of the research, which aimed to isolate and characterise the beta-galactosidase enzyme. Temperature and pH were determined to be the critical factors determining the enzyme's optimum activity. Enzyme parameters including Km and Vmax were found to be 3.65 mM and 0.18 mol/min, respectively. Several metal ions and inhibitors were found to influence the enzyme's activity; for example, $HgCl_2$ and KCN completely blocked the enzyme, whilst $FeCl_3$ and $FeSO_4$ enhanced it. While beta-mercaptoethanol alone had no discernible effect on enzyme activity, EDTA considerably reduced it. According to the results of the research work, the β-galactosidase enzyme fromZiziphusspina-christi may have potential applications in the food and pharmaceutical industries.

Conflict of interest
The authors declare any conflict of interest

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