Screening of some Egyptian plants for milk clotting activity

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ABSTRACT

This study was conducted to evaluate the possible presence of potent milk-clotting enzymes (MCE). Nine plants *(E. millim* Leave, *F. elastica* Leave, *M. oleifera* seeds, *A. lebbeck* seeds, *A. variegata* leaves, *T. foenum-graecum* seeds, *A. Chinesis* fruit, *H. annuus* seeds and F. *vulgare* seeds) were screened in order to get the most promising alternative source for potent (MCA). These results illustrated that MCA Of different extracts were dependent on plant type. The extraction of MCE from *A. lebbeck* seeds gave higher MCA and MCA / proteolytic activity (PA) Ratio compared to that extracted from the other plants. The aqueous extract of *A. lebbeck* seeds was taken as a crude enzyme source. MCE of *A. lebbeck* from prepared seeds dissolved in 0.1 M phosphate buffer pH 5.7 + 5% NaCl that gave the high activity in comparison to other buffers; moreover 20–40 % ammonium sulfate saturation precipitated MCE with 11.83 % yield and 6.16% purification rate. Sephadex G-100 recorded the highest specific activity (SA) and purification fold with 5.98 % yield and 9.84% purification rate. The crude and partial purified enzyme has optimal pH 6 and optimal temperature 40°C. The thermally stability of enzyme exhibited at 30 to 40°C for 10-60 minutes. NaCl inhibited MCA for both crude and partial purified enzyme, even at low concentration (1%). CaCl2 stimulated MCA up to 30 mM. Several metal ions were stimulant on the enzyme like Cd^{2+}, Mn^{2+}, Ba^{2+} NH_4^{2+} and Fe^{2+} while Cu^{2+}, K^{+}, I^{+} and Ni^{2+} were act as inhibitors. Storage at 4°C for 28 days resulted in a 3.6 and-5.3% loss in activity to both partial purified and crude enzyme, also Storage at room temperature for same days resulted in a 22.6 and 24.4% loss in activity to both partial purified and crude enzyme respectively.

Key words: Milk Clotting Enzyme, purification, proteolytic activity, Plant, *A. lebbeck*.

INTRODUCTION

Calf rennet, which consist rennin (EC 3.4.23.4) as the prevalent enzyme component, has been mainly used as a MCE. Several studies interests have been directly towards find (MCE), new sources of MCE in cheese manufacture Cavalcanti, *et al*., (2004). Ahmed *et al.,* (2009) found that the production of microbial rennet used genetic engineering bacteria that was a suitable alternative for animal rennet, but the interest has increased directly toward the natural coagulants extracted from different plant source. In addition, the use of animal rennet has consumer constraints due to religious reasons (e.g. Judaism and Islam), safety (bovine spongiform encephalopathy), and diet (vegetarianism), or bans on genetically engineered food. Overtime, utilize of veal rennet has consumers constraints because of religion (e.g. Judaism and Islam), safety (bovine spongiform encephalopathy), and nutrition (vegetarianism), or bans on genetic engineering food. Due to these reasons, many studies attention has been aroused towards discovering new MCE from vegetable or plant sources which can satisfactorily replace rennet Sanni *et al*., (1999).

(Jacob *et al*., 2011) stated that proteases as milk coagulant must show highest clotting activity and low PA (ratio: milk clotting/PA). This ratio relies on the ability of the enzyme to hydrolysis specifically on κ-casein. Shah, *et al*., (2014) reported that the use of plant enzymes in cheese making since ancient times. Since 1960, vegetable rennet has been widely used in dairy industry. Sbhatu *et al.* (2020) reported that cheeses manufactured with vegetable coagulants can be found mainly in Mediterranean, West Africa, and southern European countries. Spain and Portugal have the large difference produce of cheeses using *Cynara sp*. as the vegetable coagulant. Abdeen, *et al*, (2021) showed that MCE from *Moringa oleifera* PSC could be used as a coagulant in goat cheese making without any disadvantages till the end of storage.

(Otani *et al*., 1991a) produced a MCE from *A. julibrissin*. He found that extracts have a high activity without developing any bitterness in cheese until the end storage. No other research has been conducted to study more widely the MCA of any Albizia species. Searching for alternatives coagulants from readily and locally available sources such as vegetable enzymes is not only feasible but also necessary in order to meet the demand for

MCE for cheese making. Albizia is planted in rural regions and in public gardens of Egypt; it was first introduced in Egypt in1869 (Miyase et al., 2010).Therefore, the purpose of this study is to evaluate the possibility clotting ability of extracts from local plants.

MATERIALS AND METHODS

Material:

All chemicals used in the present study were purchased from Sigma-Aldrich, VWR International Ltd, S D Fine-Chem Limited, and Merck Company. Plant sources *Euphorbia millim, Ficus elastica* Leave, *Moringa oleifera* seeds, were collected from Faculty of Agriculture, Cairo. Al-Azhar University farm. *A. lebbeck* seeds, was collected from trees grown in Al-qalyubia, Governorate, Egypt. *Aloe variegata* leaves, Sunflower seeds, Fenugreek *(Trigonella foenum-graecum)* seeds, kiwi fruit (*Actinida Chinesis)* and fennel (*Foeniculum vulgare*) seeds, were purchased from Agriculture Research Center, Dokki, Egypt. Fresh whole buffalo's milk was obtained from the farm of Faculty of Agriculture, Al-Azhar University, Mostorod, Cairo, Egypt. Microbial rennet powder Chy-Max from (Chr Hansen., Holding A/S, Boege, 2970 Hoersholm, Danmark, was used for cheese production at the rate of 1g /100 l. Veal rennet Liquid was prepared from fourth stomach as described by **Amer, (2017)**.

Methods

Preparation of crude enzyme extracts with different buffers

The crude extracts were prepared according to the protocol described by Abdalla *et al.,* (2011). A set of preliminary experiments was carried out in order to identify highest MCA. Seeds were air dried at room temperature for 2 weeks. The seeds were closely cleaned and then coarse grind using an electric blender and stored in polyethylene bags at (4-5°C) even being used for enzyme extraction. The leaves and fruit were prepared by washing with distilled water, then cutting them into small pieces and homogenize. Ten g from above plants were soaking in 100 ml in distilled water and three different buffers with or without 5% (w/v) NaCl (0.1 M acetate buffer pH 5.0, 0.1 M acetate buffer pH 5.0 with 5% NaCl, 0.1 M sodium phosphate buffer pH 5.7, 0.1 M sodium phosphate buffer pH 5.7 with 5% NaCl, 0.1 M Tris–maleate buffer pH 5.2 and 0.1 M Tris–maleate buffer pH 5.2 with5% NaCl). The mixtures were stirred at (4°C) for 24 h. The extracts were filtrated through cheese cloth and centrifuged at 5000 rpm / 15 min. to obtain crude extracts.

Determination of MCA

The MCA of the enzyme preparation was assessed according to Otani, *et al,* (1991b). The MCA was calculated by Kawai and Mukai (1970) as: SU = 2400×5×D/T×0.5 (1), where T is milk-clotting time (s) and D is dilution of the enzyme.

PA determination

Protease activity of supernatant was determined by the method of Chopra and Mathur, (1983).

Determination of protein content

Protein content was determined colorimetrically at 595 nm using Coomassie brilliant blue G-250 (CBBG) and bovine serum albumin (BSA), according to Bradford (1976).

Buffers preparation

All types buffer used were prepared according to Gomori (1955).

Purification of crude enzyme extracts

Step 1: Precipitation by ammonium sulfate

Crude extract was sedimentation by various concentrations of ammonium sulfate (20, 40, 60, 70, 80 and 100% saturations), according to Colowick and Kaplan (1955).

Step 2: Gel Filtration by Using Sephadex G-100

Enzyme extract was purified by gel filtration method reported by Dioxn and Webb (1968).

Characterization of crude and partial purified:

Optimum pH

The optimal pH of the purified and crude extract was determined by replacing 0.1 M phosphate buffer pH 5.7 in the milk clotting assay with the following buffer: 0.1 M citrate buffer (pH 3.5- 5.5), 0.1 M phosphate buffer (pH 5.7-7.5), and 0.1 M Tris-HCl buffer (pH 8-9). The reaction was carried out using the milk clotting assay procedure according to Ahmed *et al*, (2012).

Optimum temperature

The optimal temperature of the purified and crude extract was determined by the method of Ahmed *et al* (2012) incubating the reaction mixture of the milk clotting evaluate

at various temperatures ranging from 20 to 100 °C for 10 min.

Thermal stability

Aliquots of enzyme extract to (0.2 ml) were heat treated for 10, 20, 30, 40, 50 and 60 minutes in water baths set at various temperatures of 30 to 80 °C then quickly cooling to 37°C and measured instantly for residual enzyme activity according to El-Bendary *et al.* (2007).

Effect of some salts

Effect of the presence of 1 mM of Ni So_4. xH_2o, $CaCl_2$.$6H_2o$, $CdCl_2$, Ba $Cl_2.2H_2O$, $(NH_4)_2So_4.FeSo_4.6H_2o$, NH_4 HSo_4, Mn $So_4.H_2O$, KCl, Cu $So_4.5H_2O$, Ki, Fe $So_4.7H_2o$, and Fe $Cl_2.6H_2O$ effect on enzyme activity was studied. Cui *et al*. (2007).

Effect of calcium chloride

Effect of the presence of various concentrations ranged from (0-50 mM) of $CaCl_2$ according to Ahmed *et al* (2012).

Effect of sodium chloride

Effect of the presence of different concentrations of NaCl ranged from (0-16%) according to Ahmed *et al* (2012).

Effect of enzyme storage stability

Plant MCE was stored by a refrigerator and at room temperature (20-22 °C) for 28 days and the residual activity was evaluated according to Ahmed *et al*, (2012).

RESULTS AND DISCUSSION

Screening (MCE) in different samples

Table (2) represents the results of screening experiments for the existence of MCA of the several plants extracts in distilled water containing NaCl (5%) for 24 hours at 4ºC. The obtained results showed that MCA of several extracts were dependent on plant type that was varied with plant type. The extraction of MCE from *A. lebbeck* seeds gave higher MCA and MCA / PA ratio among other plants extracts other plants. Similar results were obtained by (Ahmed *et al*., 2009; Naz *et al*., 2009; Elmazar *et al*., 2012; Néstor *et al*., 2012 and Bajulge, *et al*., 2018) who found a MCE of several plant seeds extracts. The aqueous extract of *A. lebbeck* seeds was taken as a crude enzyme source.

Activity of *A. lebbeck* extract enzyme under different buffering conditions

The results of the extraction methods on the MCA and PA are presented in Table (3). It shows that the results of MCA of *A. lebbeck* seeds crude extracts as affected by buffer type and sodium chloride addition. Extraction of *A. lebbeck* plant seeds with 0.1 M phosphate pH 5.7 + 5% NaCl gave the highest MCA and SA of MCE and decrease in PA and Specific PA. Also the results gave the highest ratio Specific MCA/ Specific PA compared to other buffering solutions. The results obtained agree with Kholif, (2017) who reported that, phosphate buffer pH 5.9 gave the high parameters compared to other solution. Results indicated also that crude extracts prepared using 5%Nacl in buffers solutions had the highest clotting activities as assessed by shorter clotting time, and higher ratio MCA/ PA compared to extracts without 5% NaCl. This is due to NaCl increase the ionic strength of the extraction solution and release of the maximal of enzyme substance (Lopez *et al*, 1998). Several investigators reported similar results whereas Ahmed *et al.* (2009); Talib *et al.* (2009); Guiama *et al.*, (2010) and Ramadan, *et al.* (2019) recommended the use of 5% NaCl in sodium acetate buffer as an effective extracting in the preparation of crude extracts.

Purification of MCE

The results in Table (4) stated that 20-40 % saturation showed the high MCA, specific activity, total activity, yield (%) and rate purification. Thus, the range of 20-40 % was chosen for possibility purification of the MCE and PA for seeds of *A. lebbeck*. With increasing the ammonium sulfate, concentration of the MCA was gradually decreased. The implementation of ammonium sulfate at saturation (20 – 40 %) in the present work is superior to highest concentrations that generally used for the purification of MCE from different sources Abdeen, *et al*, (2021) and Derso and Dagnew (2019). Sharma *et al.* (2018) found that the highest activity, yield and purification rate were found with 40-60 % concentration of ammonium sulphate. The partially purified MCE was chromatographed in a column of Sephadex G-100 and the purification showed only one peak with MCE (at fraction 18, 19, 20 , 21 and 22) and one peak with protein concentration as cleared in (Table 5 and Figure 1). This was obtained when the dialysis pooled fraction was carried on Sephadex G-100 column equilibrate with 0.1 M sodium phosphate buffer pH 5.7. Purification results of the enzyme from *A. lebbeck* seeds using different purification means resulted in 9.836 rate purification with a yield of 5.98 %

and specific activity of 14360. Results are in agreement with Kholif, (2017) **who** showed only one peak from extract of *S. elaeagnifolium* seeds. Aya M. Abd-ElKhalek, *et al.* (2020) found that the use of Sephadex G-200 gave the better activity.

Characterization of the purified and crud enzyme

Optimum pH

The effect of different pH values on MCA for both the crud and purified extract were shown in Fig. (2). The enzyme retains its activity in the pH range of 3.5 to 6.5, and highest MCA of *A. lebbeck* seeds were observed at pH 6. Activity gradually decreased as a result of increased pH. At higher pH value of 8.5, the enzyme completely lost its activity due to denaturation of the enzyme protein. These data indicated that the activity of the enzyme is greatest under acidic conditions. Similar behavior of optimum pH was reported (Aya Abd El-Khalek, 2021). Al-Dhabi *et al.* (2020) studied the enzyme showed high activity at pH 9. Ramadan, *et al*. (2019) found that the optimum pH for artichoke protease was 5.0.

Optimum temperature

The results (Fig. 3) showed that MCA of different extracts increased when temperature increased from 20 to 40 C°. The optimal temperature for the MCA was also investigated. The maximal MCA of our purified and crud MCE was at 40 C°. The activity rapidly decreased as the reaction temperature increased above 60C° because of the thermal damage of the enzyme protein. The decrease in clotting activity as a result of increasing temperature over certain limit could be explained by the changes in salt equilibrium (Balcones *et al.*, 1996).This finding was conformed with the findings obtained by (Amer, 2017) and (Siddig *et al*. 2018.)

Effect of $CaCl_2$ concentrations

Calcium chloride is necessary for second stage of enzyme coagulation (Ahmed *et al*, 2010). In the current study, the concentrations ranged from 5 mM to 50 mM of $CaCl_2$ were evaluated. Results cleared an increase in MCA with $CaCl_2$ concentration up to 30 mM for both the curd and purified enzyme (Fig. 4). However, MCA reduced when concentrations of $CaCl_2$ were highest than 30 mM. Similar results trend were noticed by (Wang *et al*. 2015); (Kholif, 2017) and (Amer, 2017). Increasing Ca ion concentration led to improvement of the MCA of all samples (Kumar, *et al*., 2005).

Effect of NaCl concentrations

Different concentrations of NaCl from (0-16 % NaCl) on the crude and purified MCA, showed that a gradual reduction in MCA were studied. A gradual reduction was noticed with increasing sodium chloride ratio up to 16% (Fig. 5). Similar trend were found by several researchers like proteases from *M. pusillus* QM 436 El-Tanboly *et al*. (2013) and *S. elaeagnifolium* fruit seeds Kholif, (2017). Gradual increase up to 10% of NaCl resulted in increase of MCA, and then the activity decreased as a result of the increase NaCl level Abdeen, *et al*, (2021).

Thermal stability

The crude and purified enzyme was incubated on the temperature from 30-80°C for (10-60 min). The enzyme activity for the crude and purified enzyme is stable at 30 to 40°C on the incubation times from (10-60 min) and then slight reduced in enzyme activity on times of the (10-60 min) at 50°C. Then it began a sharp decrease in enzyme activity on incubation at 60C° at all times of incubation and the disappearance of enzyme activity completely at 80°C (Fig. 6 ,7).

El-Bendary *et al*. (2007) illustrated that the MCE was remained in stable activity until 40°C for more than 30 minutes, and then lose 35% of its activity after ten minutes incubation at 60°C. At 40°C for 60 minutes, the bacterial coagulants lost 26.6% of its activity, while the plant enzymes were still fully active.

Effect of different metal ions and other materials

Several metal ions were added in concentrations (1 mM) as shown in (Fig. 13). As shown, Cd^{2+}, Mn^{2+}, Ba^{2+} NH_4^{2+} and Fe^{2+} were activators, while Cu^{2+}, K^{+}, I^{+} and Ni^{2+} were act as inhibitors for the enzyme activity. Lakshmi *et al*. (2018) reported that ions Cu^{2+}, Ni^{2+}, Fe^{2+}, Ca^{2+} were act as activators ,while Mn^{2+},Ba^{2+}, Co^{2+} were considered as inhibitors to the protease from *Bacillus cereus strain S8*. Wang *et al,* (2009). The metal ions Mg^{2+}, Ca^{2+}, Ba^{2+}, Mn^{2+}, Al^{3+}, Fe^{2+} had a very show chantries to acceleration coagulation of milk however Na+ and K+ decelerated the MCA slightly.

Effect of storage temperature on MCA

As shown in (Fig. 9) about 96.4 and 94.7 % still retained of purified and crude enzyme activity and was observed for refrigerator temperature respectively; in contrast, MCE still retained 77.4% and 75.6% of purified and crude enzyme activity at room temperature

respectively after being kept 28 days. Ahmed and Helmy, (2012) reported that the plant enzymes were more stable during storage period compared to bacterial enzymes. About 90.39 and 75.39 % still retained of activity and was observed for planet MCE after being kept for 15 days at the refrigerator and the room temperature respectively (Kholif, 2017). Esawy and Combet-Blanc (2006) reported that *B. licheniformis* 5A5 MCE retained complete activity until the end storage.

CONCLUSION

MCE from *A. lebbeck* was successfully extracted by soaking seeds in 0.1 M phosphate buffer pH 5.7 + 5% NaCl. A simple and economical extraction method was developed, in addition to the availability of the source of vegetal. The extraction of MCE from *A. lebbeck* seeds gave higher MCA and MCA / proteolytic activity (PA) Ratio compared to that extracted from the other plants. The crude and partial purified enzyme has optimal pH 6 and optimal temperature 40°C. The thermally stability of enzyme exhibited at 30 to 40°C for 10-60 minutes. In conclusion, *A. lebbeck* MCA Can be used as a coagulant in cheese making.

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Table 1: MCA, PA and MCA / PA Ratio crude extracts of selected samples

No.	Name of plants	MCA(U/ml)	PA(U/ml)	MCA / PA Ratio
1	*Euphorbia millim Leave*	78.7	0.498	158
2	*Ficus elastica* Leave	195.4	0.778	251.1
3	*Moringa oleifera* seeds	154.8	0.885	174.9
4	*A. lebbeck* seeds	548.2	0.579	946.8
5	*Aloe variegata* leaves	207.6	0.787	263.8
6	Sunflower seeds	112.6	0.602	185.4
7	Fenugreek seeds	101.9	0.914	111.5
8	kiwi fruit	153.2	0.487	314.6
9	fennel seeds	35.4	0.678	52.2

Table 2: Activity of *A. lebbeck* extracted enzyme under different buffering conditions

Type of buffers (0.1 M)	pH	MCA (U/ml)	PA (U/ml)	PC (mg/ml)	Specific MCA	Specific PA	Ratio specific MCA/ Specific PA
Phosphate	5.7	453	0.661	0.489	926	1.35	685
Phosphate (5% salts)	5.7	600	0.578	0.411	1459	1.4	1042
Tris–maleate	5.2	312	0.782	0.501	623	1.56	399
Tris–maleate (5% salts)	5.2	358	0.870	0.477	751	1.8	417
Acetate	5.0	320	0.740	0.454	705	1.6	440
Acetate (5% salts)	5.0	462	0.867	0.448	1031	1.94	531

MCA= unit of milk clotting activity, PA= Proteolytic activity, PC = Protein content, Specific MCA = MCA / PC, Specific PA = PA / PC,

Table 3: A preliminary ammonium sulphate saturation fractionation on MCA from *A. lebbeck* seeds fruit.

Ammonium Sulfate Saturation (%)	Volume (ml)	(MCA/ml)	PC (mg/ml)	Total (MCA)	Total PC	Sp.MCA	Yield (%)	Rate purification
Crude enzyme Homogenate	50	600	0.411	30000	20.55	1459.85	100	1.00
0-20	5	125	0.126	625.00	0.63	922.06	1.2	0.632
20-40	5	710	0.079	3550	0.395	8987.3	11.83	6.16
40-60	5	172	0.094	860.0	0.47	1829.7	2.8	1.253
60-80	5	78.11	0.071	390.55	0.355	1100.1	1.3	0.753
80-100	5	23.56	0.038	117.8	0.190	620.0	0.3	0.425

MCA = unit of milk clotting activity; PC= Protein content; Sp=Specific activity (activity / protein content);Total activity =activity ×volume of fraction; Yield = total activity of the fraction / total activity of crud enzyme × 100; Rate purification = Specific activity of fraction / specific activity of the crude fraction

Table 4: Purification steps of milk clotting enzyme from ***A. lebbeck*** using ammonium sulfate and gel filtration (Sephadex G-100)

Purification step	Volume (ml)	MCA/ml	PC (mg/ml)	Total Activity (MCA)	Total PC	Sp. (MCA)	Yield (%)	Rate Purification
Crude enzyme Homogenate	50	600	0.411	30000	20.55	1459.85	100.0	1.00
Ammonium sulfate saturation (20-40%)	5	710	0.079	3550	0.395	8987.3	11.83	6.16
Sephadex G -100	5	359	0.025	1795	0.125	14360	5.98	9.84

MCA = unit of milk clotting activity; PC= Protein content; Sp.=Specific activity (activity / protein content); Total activity =activity ×volume of fraction
Yield = total activity of the fraction / total activity of crud enzyme × 100; Rate purification = Specific activity of fraction / specific activity of the crude fraction

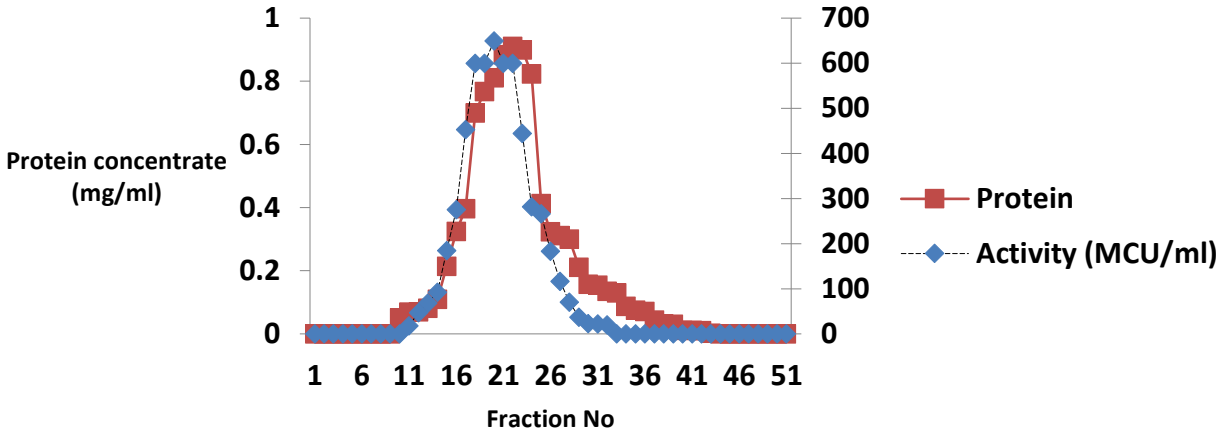

Figure 1: Gel filtration for the chromatography of Milk clotting activity on a Sephadex G-100 column (40 x 2.5 cm) the column was equilibrated with 0.1 M phosphate buffer, pH 5.7 at a flow rate of 0.7 ml min^{-1} and 5 ml fractions

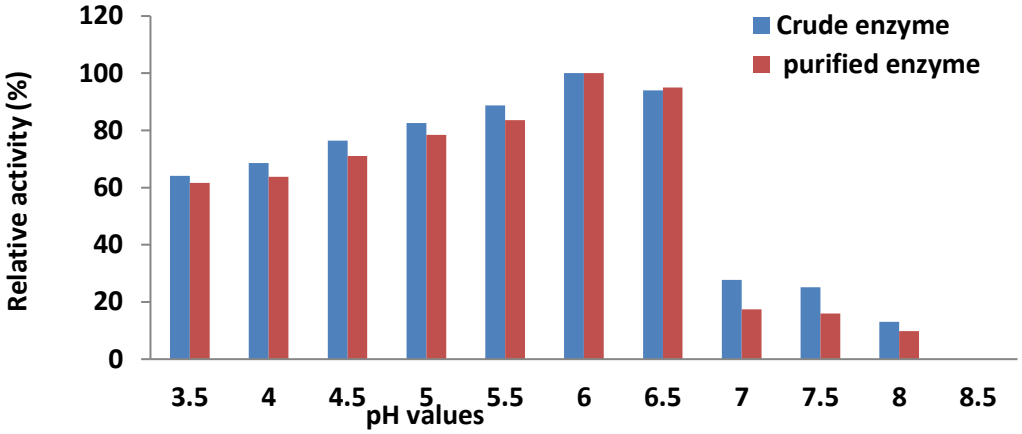

Figure 2: Effect of varying pH values on activity of the crude and purified MCE from *A. lebbeck* seeds plan

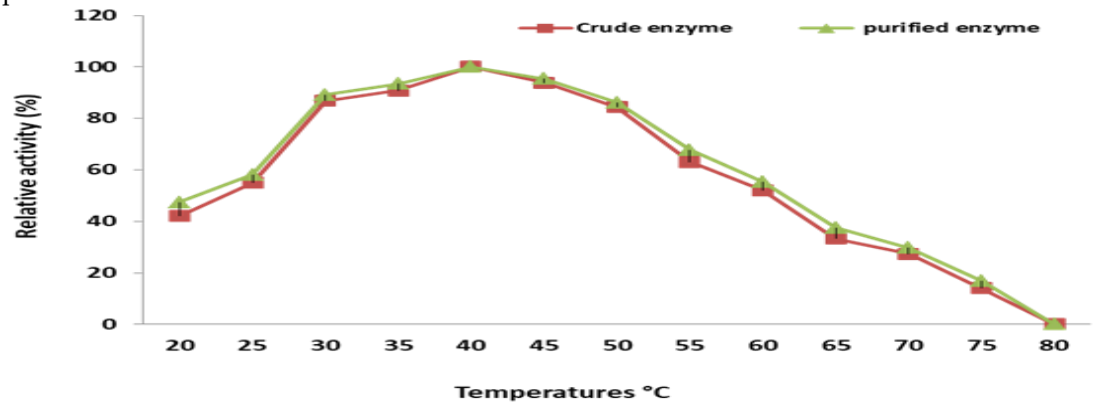

Figure 3: Effect of varying Temperatures on activity of the crude and purified MCE from *A. lebbeck* seeds plant

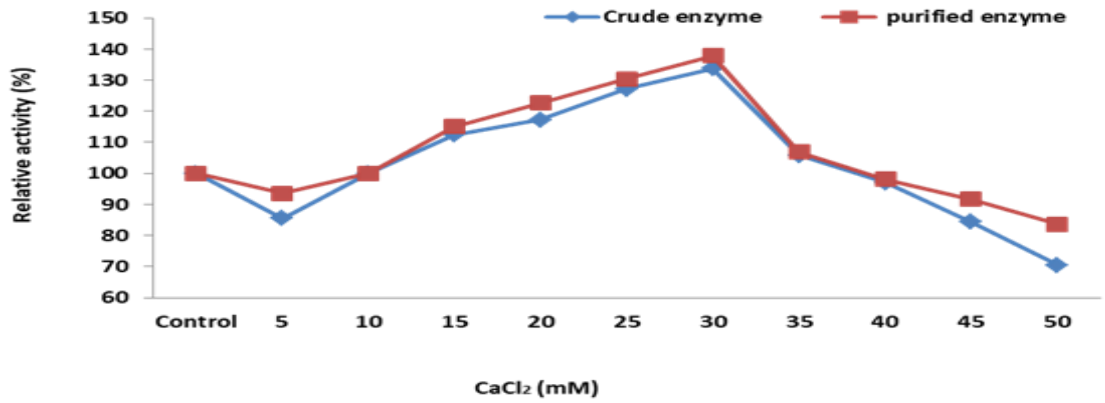

Figure 4: Effect of $CaCl_2$ concentrations on activity of the crude and purified MCE from *A. lebbeck* seeds plant

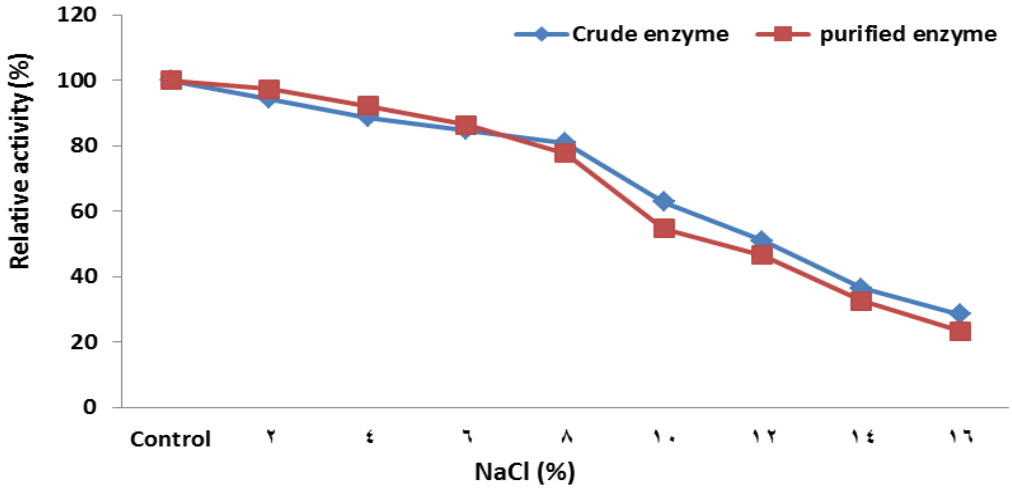

Figure 5: Effect of NaCl concentrations on activity of the crude and purified MCE from *A. lebbeck* seeds plant

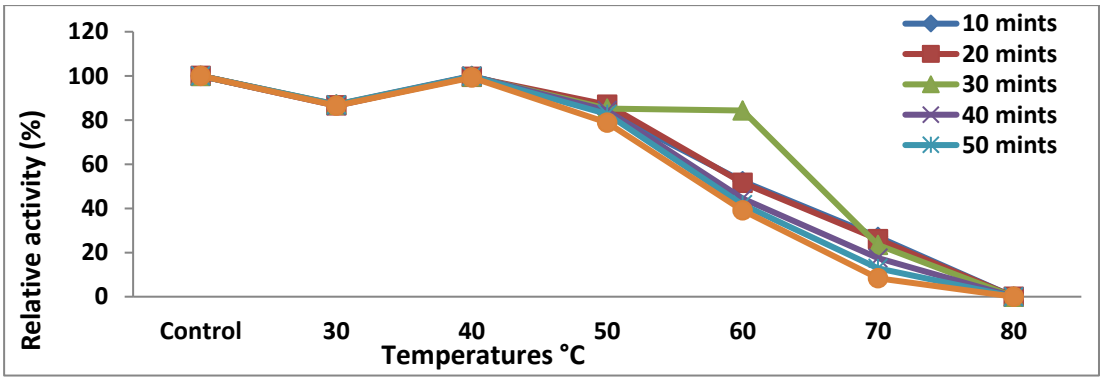

Figure 6: Effect of Thermal stability on activity of the crude MCE from *A. lebbeck* seeds plant

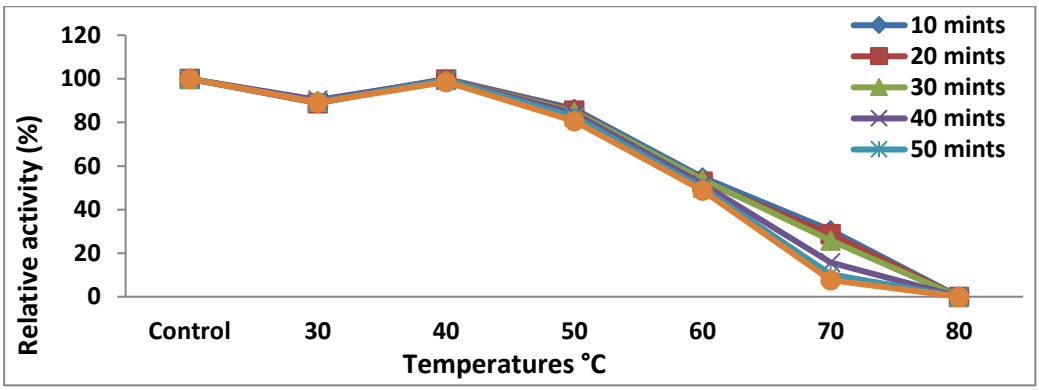

Figure 7: Effect of Thermal stability on activity of the purified MCE from *A. lebbeck* seeds plant

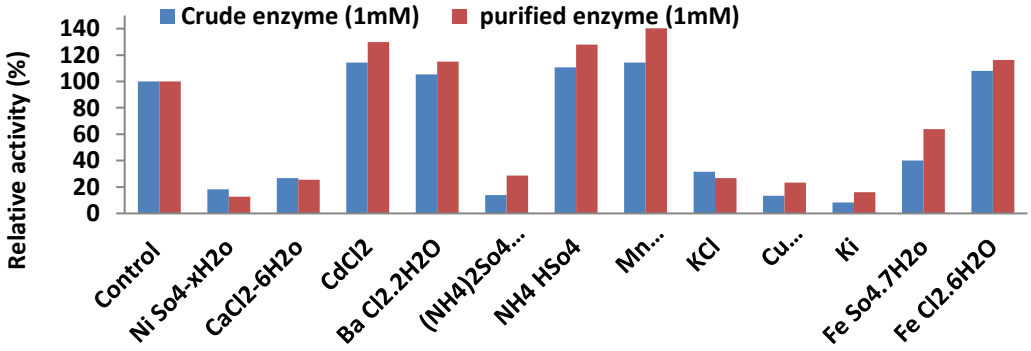

Figure8): Effect of different metal ions on activity of the crude and purified MCE from *A. lebbeck* seeds plant

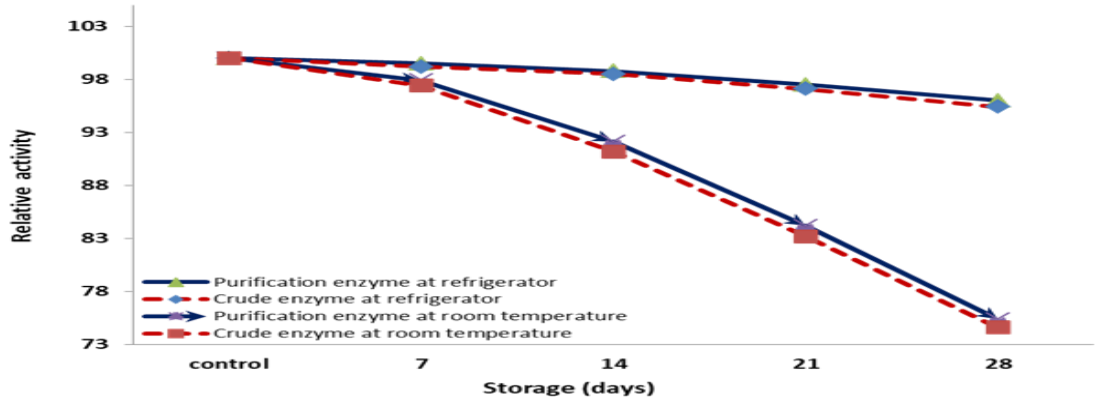

Figure 9: Effect of storage crude and purified MCE from *A. lebbeck* seeds at a refrigerator and room temperature

فحص بعض النباتات المصرية لنشاط تجبن اللبن

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الملخص العربي

أجريت هذه الدراسة بهدف الحصول على إنزيم مجبن للبن من بعض النباتات المتاحة فى البيئة المصرية. حيث تم اختيار تسع نباتات هى *(E. millim* Leave*, F. elastica* Leave, *M. oleifera* seeds, *A. lebbeck* seeds, *A. variegata* leaves, *T. foenum-graecum* seeds, *A. Chinesis* fruit*, H. annuus* seeds and F. *vulgare* seeds) أظهرت النتائج أن *A. lebbeck* seeds كان الأفضل من حيث معامل القوة التجبنية الى القدرة التحليلية للإنزيم المنقى والخام مقارنة بالنباتات الأخرى. أدى استخدام محلول فوسفات الصوديوم المضاف إليه 5% كلوريد صوديوم الى زيادة نشاط الإنزيم مقارنة بالمحاليل المنظمة الأخرى. أدى استخدام الترسيب للإنزيم بملح كبريتات الأمونيوم على تركيز 20 الى 40 % تشبع ثم التقنية باستخدام أعمدة الفصل الكروماتوغرافي Sephadex 100 إلى زيادة نشاط التجبن وعدد مرات التنقية للإنزيم والنشاط نوعي بدرجة نقاوة 9.84 ومحصول 5.98٪. كانت درجة الحرارة المثلى للإنزيم 40 م ^{O} ، وكان ثبات نشاط الإنزيم من 30 إلى40 م ^{O} مع أوقات تحضين من 10 إلى 60 دقيقة. أظهرت النتائج أن الـ (pH 6) هو الأمثل للإنزيم. أعطى تأثير كلوريد الكالسيوم عند تركيز 30 ملليمول أعلى نشاط نسبي للـ MCE النقي والخام. كلوريد الصوديوم أدى الى خفض نشاط الأنزيم تدريجيا. أظهرت النتائج أيونات المعادن بتركيز 1 Mm بالنسبة لـ Cd^{2+}, Mn^{2+}, Ba^{2+} NH_4^{2+},Fe^{2+} كانت منشطة بينما Cu^{2+}, K^{+}, I^{+} and Ni^{2+} كانت مثبطة لنشاط إنزيم تجبن اللبن النقى والخام. أدى التخزين على 4 م ^{O} لمدة 28 يومًا إلى فقد حوالى 3.6 و 5.3٪ في النشاط لكل من الإنزيم المنقى الجزئي والخام، كما أدى التخزين على درجة حرارة الغرفة لنفس الأيام إلى فقدان 22.6 و 24.4٪ في النشاط لكل من الإنزيم المنقى الجزئي والخام على التوالي.

الكلمات الاسترشادية : انزيم مجبن للبن، التنقية، نشاط التحلل، نشاط التحلل، البيزيا