Journal of Babylon University/Pure and Applied Sciences/ No.(3)/ Vol.(22): 2014

Main properties of urease partially purified from seeds of Syrian mesquite (*Prosopis farcta*)

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Abstract

Novel sources of urease from leguminosae were selected for activity test, one the them (*Prosopis farcta*) showed high specific activity (36.52 U mg^{-1}). The active uerase extract then subjected to series of purification steps involved salting out, DEAE, CM ion exchange and size exclusion chromatography. Specific activity were 52.44, 193.27, 230.55, 433.33 U mg^{-1} respectively. Main features of urease were detected included estimation of molecular weight (95.500 kDa), optimum pH for activity (8) and stability (7-8), optimum temperature for activity (45^{o}C) and stability (25-30 ^{o}C).

الخلاصة

تم اختبار فعالية اليوريزمن مصادر جديدة لنباتات العائلة البقولية حيث وجدت اعلى فعالية نوعية في نبات الخرنوب (٣٦.٥٢ وحدة/ملغم). نقي المستخلص الفعال بعدة خطوات شملت التمليح الخارجي، المبادل الايوني السالب والموجب والترشيح الهلامي حيث كانت الفعالية النوعية ٥٢.٤٤، ١٩٣.٢٧، ٢٣٠.٥٥ و٤٣٣.٣٣ وحدة/ملغم على التوالي. حددت الخصائص الرئيسية لانزيم اليوريز مثل حساب الوزن الجزيئي (٩٥,٥٠٠ كيلو دالتون)، الاس الهايدروجيني الامثل للفعاليه (٨) والثبات (٧–٨)، درجة الحرارة المثلى للفعاليه (٤٥ م^{o}) والثبات (٢٥–٣٠ م^{o}) .

Introduction

Ureases (EC 3.5.1.5, urea amidohydrolase) are metalloenzymes depend on nickel ($Ni+^{2}$) in its activity. Urease act as catalyzer for breakdown of urea to form two molecules of NH_3 and one of CO_2. (Allison *et al*., 2006; Balasubramanian and Ponnuraj 2008). The rate of reaction is 8×10^{17} faster than the reaction without enzyme (Callahan *et al*., 2005). Ureases are widely distributed in plants especially in legume (*Canavalia ensiformis* and *Glycine max*), fungi, and bacteria (Menegassi *et al*., 2008). In spite of presence of urease in high concentrations in seeds of several species of the leguminosae, Cucurbitaceae, Asteraceae, and Pinaceae (Bailey and Boulter, 1971), also found in lower concentrations in the vegetative organs in other families (Hogan *et al*., 1983; Polacco and Winkler, 1984). In a recent study (Carlini and Polacco, 2008) indicated ability of urease to kill the insects and antifungal activity. Urease also used for determination of urea in blood (Achakzai *et al*., 2003). According to these results, our study focused on finding a new source of urease for extraction, purification and characterization which may be used in future as insecticide, antifungal or in medical applications.

Materials and Methods

Plant Material

Seeds of leguminosae (*Alhagi graecorm, Prosopis farcta, Melilotus indica, Albizia lebbeck,* and *Sesbania)* were collected from its plants distributed in different regions of Babylon province. The seeds then washed many times by distilled water, dried at room temperature and stored at 4^{o}C until use.

Enzyme Extraction

Urease was extracted according to methods of El-Shora (2001). The plant seeds (50 g) were blended with 100 ml of chilled 50 mM phosphate buffer pH 8 containing 10 mM ß-mercaptoethanol. The homogenate was collected by filtration through four layers cheesecloth, and the filtrate was centrifuged at 10000 rpm for 30 min. The precipitate was ignored and supernatant was used for estimation of enzyme activity.

Estimation of Enzymes Activity

The urease was assayed following the method described by Achakzai *et al*. (2003).

Determination of Proteins

Protein concentration in the leguminosae seeds extract was determined by the method of Lowry (1951).

Purification Steps

In order to purify of urease, several crude extracts saturated separately with different amounts of ammonium sulfate on magnetic stirrer to obtain final concentrations of 20, 30, 40, 50, 60, 70, 80 and 90%. After centrifugation (30 min at 6000 rpm and 4^{o}C), the pellet resuspended in 50 mM phosphate buffer pH 8.0 and dialyzed against same buffer. The resulting solution that has activity was chromatographed on DEAE Cellulose column (1.5×30 cm) pre-equilibrated by same buffer used in dialysis at flow rate 25ml/h at 4^{o}C. The unbounded fractions (2ml) have no activity were collected. Urease (purified) fractions were eluted (2ml) from the column with linear gradient of sodium chloride (0-1M) dissolved in equilibrium buffer. The eluted fractions pooled and passed through CM Cellulose column was prepared as in DEAE Cellulose. Urease (purified) in unbounded fractions (2ml) were collected and bounded were eluted (2ml). The fractions of urease were pooled then loaded onto a Sephacyle S-200 (1.5×50cm) pre-equilibrated by same buffer as above except molarity equal to 0.2M and pH7. Fractions were collected (2ml) with flow rate 25ml/h. Activity and proteins were assayed in each step of purification.

Enzyme Properties

Molecular weight of urease was calculated by gel filtration using Sephacyle S-200 prepared as above and different standard proteins. Urease then estimated according its elution compared with elution of standard proteins. Optimum pH and stability of urease using different pH value ranging from 3-11. Optimal temperature and heat stability using different temperature value ranging from 20-80^{o}C were also determined.

Results and Discussion

Test of Urease Activity

Many leguminosae members were used for investigation of urease activity to choose the legume seeds with highest specific activity for later experiments. Data in table 1 showed that the values of specific activity were varied among seeds. Crude extract of *Prosopis farcta* recorded the highest activity (36.73 U mg^{-1}) while *Sesbania* had lowest activity (0.78 U mg^{-1}). Urease extraction without reducing agent resulted in sharp decrease in activity (results not shown) that may indicate an important role of cysteine in enzyme activity. Adams and Rinne (1981) referred to urease in legumes contain considerable cysteine and methonine and so is a sulfur-rich proteins. No, articles are available about the activity of crude extract in our selected legumes, but some researches presented similar studies. Al-Khafaji (2007) used 0.2M phosphate buffer pH7.5 to detect

the activity in different species of leguminose that exploit as food. Legumes seeds (29 species) were examined for their urease production. All of the tested species contained detectable urease activity (Rosenthal, 1973). Another study used seeds of some species of Palmaceae as a source of urease and reported increase of activity at pH8 (Al-Shikirchy, 2004).

Table 1. Urease activity in some species of leguminosae

Legume seeds	Activity (U ml^{-1})	Protein (mg ml^{-1})	Specific activity (U mg^{-1})
Prosopis farcta	124.90	3.42	36.52
Alhagi graecorm	41.64	2.81	14.81
Melilotus indica	14.68	2.03	7.23
Albizia lebbeck	25	6.66	3.75
Sesbania	3.52	4.5	0.78

Urease Purification

Different ways for urease clarification in seeds have been described, especially using chromatography. Ammonium sulfate precipitation used as pre-purification and followed by ion exchange chromatography. Ammonium sulfate usually used the salting out of enzymes, have benefits of the precipitation effect caused by high concentrations of salts. Salting out useful for the fractionations of protein, and its concentration, useful for the prevent growth of bacterial growth (Barros *et al*., 2001). Results of this study revealed that the ammonium sulfate precipitated urease at (50%) produced 52.44 U mg^{-1} specific activity and recovery 51.48% (figure 1 and table 2). The advantages of precipitation are, absence large heat of solution ammonium sulfate result in heat generated is easily dissipated and protects most proteins against denaturation (Deutscher, 1990). Balasubramanian and Ponnuraj (2008) added ammonium sulfate to 30% then raised to 55% as first step for urease purification from extraction of pigeon pea (*Cajanus cajan*).

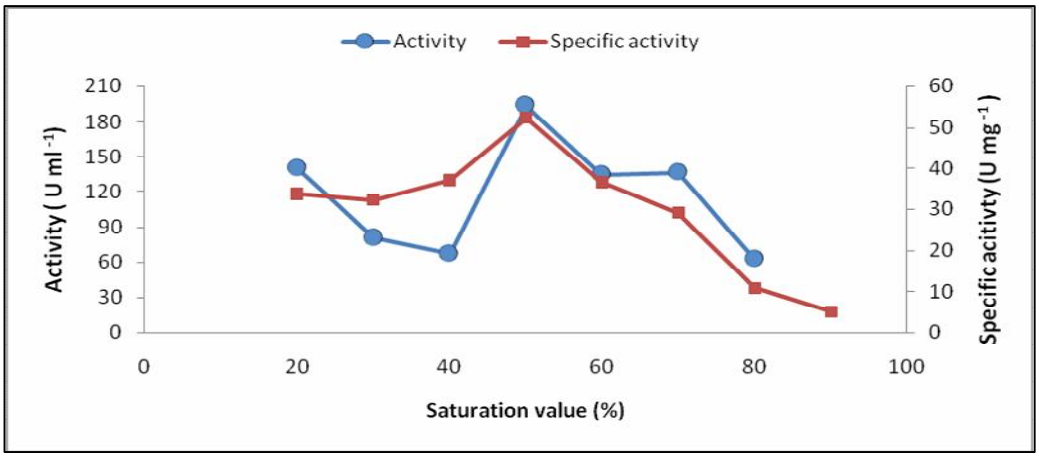

Fig 1. Activity and specific activity of urease of *Prosopis farcta* using salting out by ammonium sulfate

The choice of two kinds of ion exchange chromatography was due to unknown *pI* of extracted urease. Using anion exchanger (DEAE Cellulose), results (figure 2) showed three peaks of proteins absorbance without urease activity in washed fractions while in elution showed five peaks of absorbance with urease activity in first peak (fractions 51-63). According to these results, urease perhaps has negative charge and *pI* lower than its pH. To confirm this presume the eluted fractions that have activity were subjected to cation exchanger (CM Cellulose), results showed presence of urease activity in washed fractions.

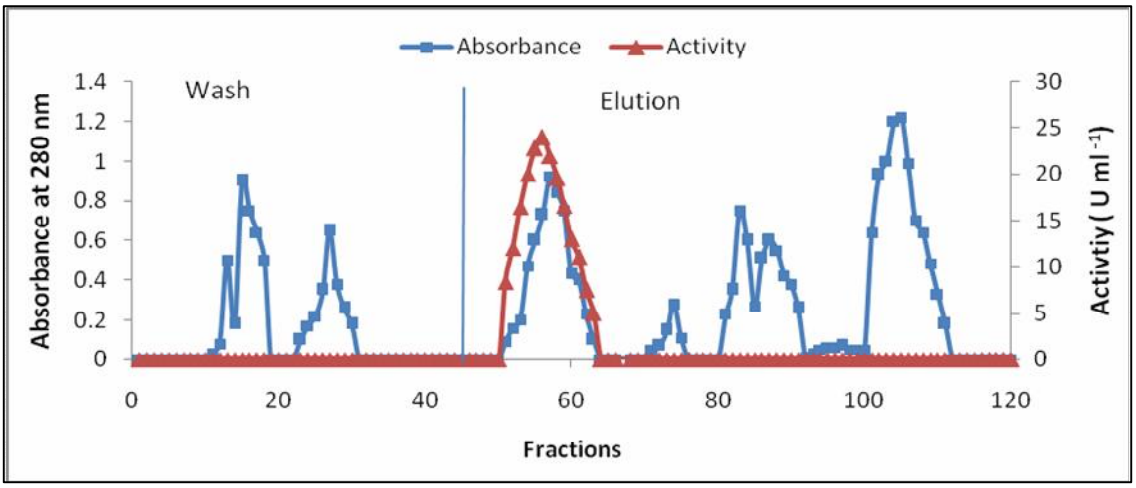

Fig 2. DEAE Cellulose chromatography of urerase of *Prosopis farcta* with dimension 1.5×30 cm, pre-equilibrated by 50 mM phosphate buffer pH 8.0 at flow rate 25ml/h.

The specific activity and recovery of washed urease were 230.55 U mg^{-1} and 24.35% respectively (Table 2). Scopes, (1994) and Bollag *et al,* (1996) recommended using both anion and cation exchange matrix that may lead to remove most other contaminated proteins.

Final step of purification was determined by Size exclusion Sephacryl S-200 technique to obtain more enzyme purity. Three peaks of proteins were recorded and activity was fixed and matched with fractions 60-65 of third peak (Figure 3). Specific activity, purification factor and recovery were 433.33, 11.79 and 8.32 respectively (table 2). References that deal with anions exchange and Size exclusion chromatography for urease purification reviewed by (Al-Shikirchy, 2004) and (Al-Khafaji, 2007).

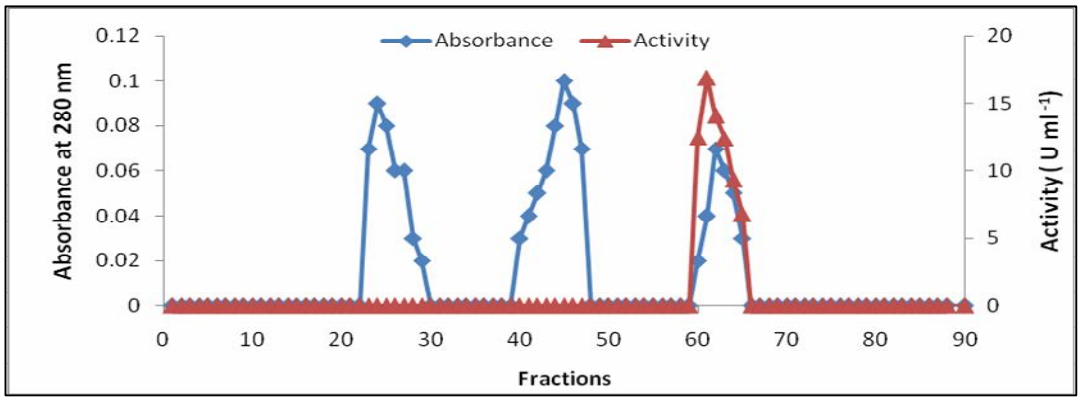

Fig 3. Size exclusion chromatography of urease of *Prosopis farcta*, with dimension 1.5×50cm pre-equilibrated by 0.2M phosphate buffer pH7 at flow rate 25ml/h.

Table 2. Purification summary of urease of *Prosopis farcta*

	Total proteins (mg)	Total activity (U)	Specific activity (U mg^{-1})	Purification factor	Recovery (%)
Raw Urease	51	1874.4	36.75	1	100
50% $(NH_4)_2SO_4$	18.25	965	52.44	1.42	51.48
DEAE Cellulose	2.86	552.76	193.27	5.25	29.48
CM Cellulose	1.98	456.5	230.55	6.27	24.35
Sephacryl S-200	0.36	156	433.33	11.79	8.32

Urease properties

After purification of urease characterization, was achieved involving different features, the important one was molecular weight. Figure 4 showed that urease had molecular weight equal to 95.500 kDa. This result disagreed with Gorin *et al*. (1962) who revealed that urease was very specific to urea and had relatively large molecular weight of 473 kDa, but in another physicochemical study of urease isolated from *Canavalia ensiformis, Glycine max* and *Gossypium hirsutum* indicated that molecular were weights 90.7, 93.6, 98.3 respectively (Menegassi *et al*., 2008).

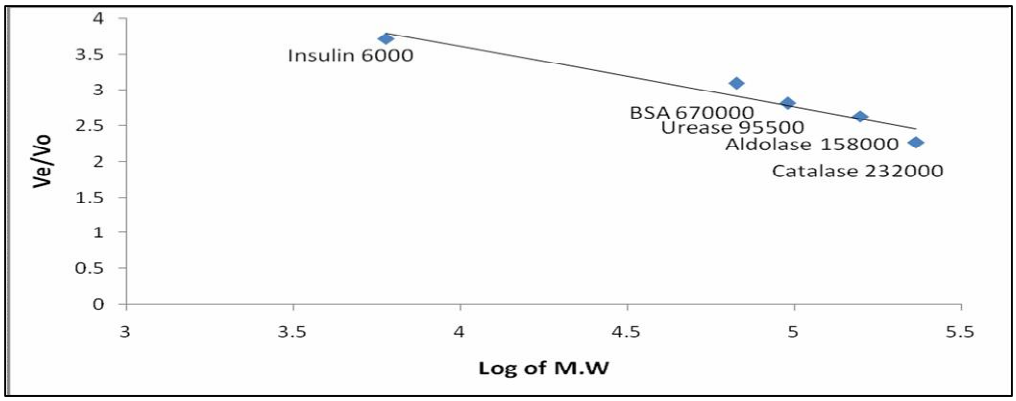

Fig 4. Molecular weight of urease of *Prosopis farcta*

In the present study, optimal pH showed urease had highest activity at pH8 (12.97 U ml^{-1}) while at extreme pH value activity was absent. Another interest activity was 11.2 and 12.06 U ml^{-1} falls in pH 7.5 and 8.5 respectively (figure 5). In fact, alteration in pH perhaps alter the distribution of charges at active site and in the whole surface of the protein molecule. Urease may found polar amino acid residues at its active site whose charge depends on pH. Activities of enzyme tend to decrease at extremes of pH usually exhibiting increase at some intermediate values (Illanes, 2008).

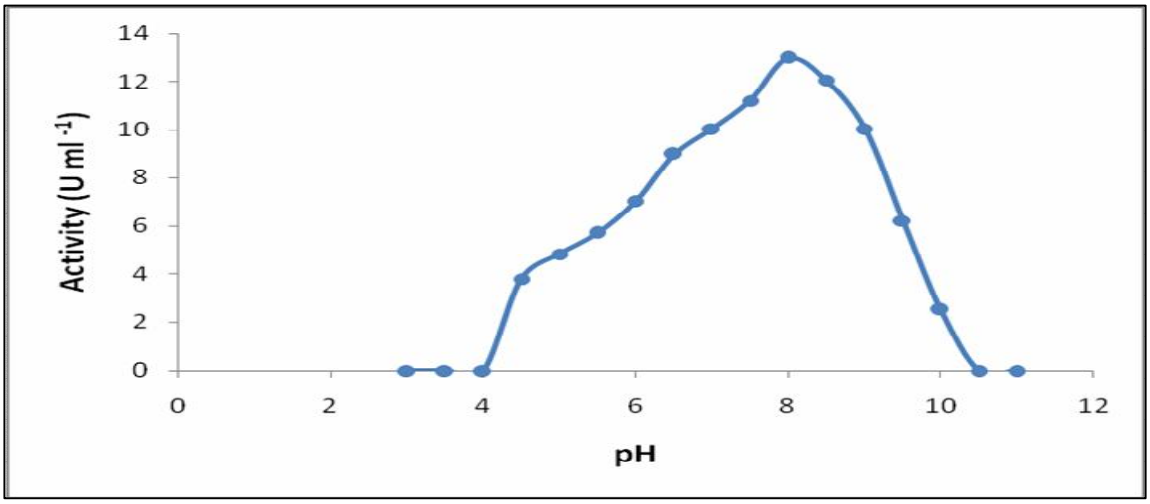

Fig 5. Optimum pH of urease activity of *Prosopis farcta*

The stability of urease presented is in figure 6 after 60 minutes of incubation. The enzyme maintained its activity (100%) at pH ranging from 7-8 where as retained activity decreased at below and above this range. Change in incubation pH may lead to change in protonation of amino acid at active site, protonation is often reversible processes may not influence the urease activity at pH 7-8. Otherwise, change in pH also results in change the charge of structurally important groups often cause irreversible change to the native structure of urease subsequently decreasing in activity in another's pH value of incubation (Bisswanger, 2008).

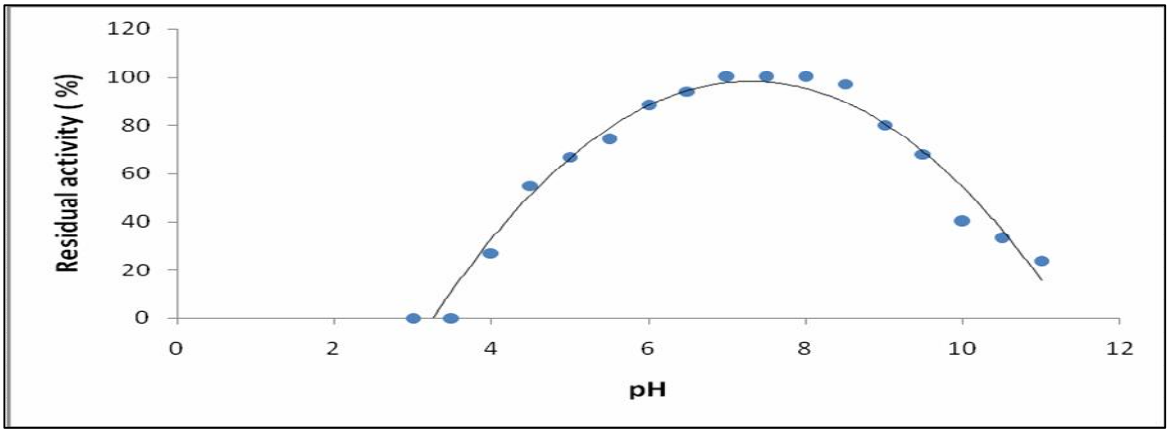

Fig 6. Stability pH of urease of *Prosopis farcta*

Thermal influence on urease activity (figure 7) showed increase of enzyme activity with increase of temperature until it reach to maximum at 45°C (22.94 U ml^{-1}), after this value activity declined gradually until reach to minimum at 80°C (1.15 U ml^{-1}). Loss of considerable activity of urease at high temperature (70-80°C) may be due to the fact that most of enzyme denatured when exposure to high heat resulting in change in enzyme conformation (Leskovac, 2004).

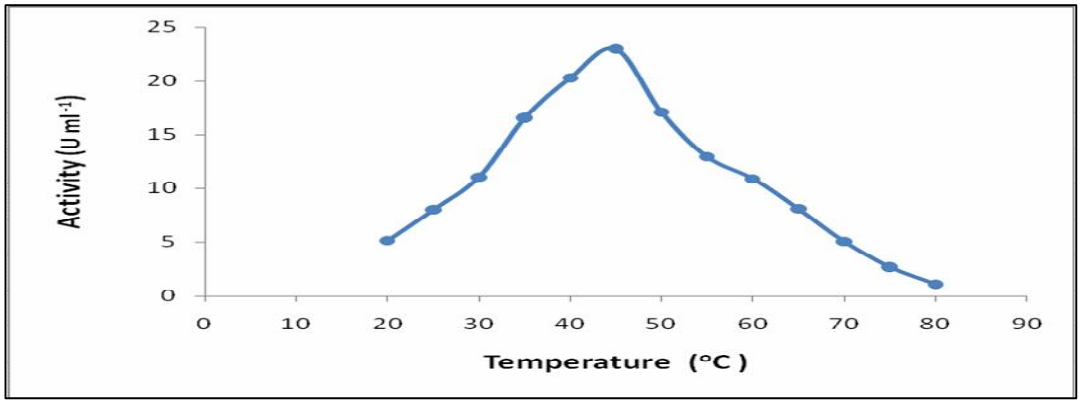

Fig 7. Thermal activity of urease of *Prosopis farcta*

Results of thermal stability of urease when incubated for 40 minute showed that the activity proportion was unaffected at temperatures ranged between 20-35°C and decreased slightly (5%) at 40°C, while the minimum residual activity located at 80°C (Figure 8). Most plant enzyme are semi-stable at temperature ranged from 30 - 40°C, and able to stand temperatures up to 40°C, but above 40°C protein composition begin to distorted (Bonner, 2007).

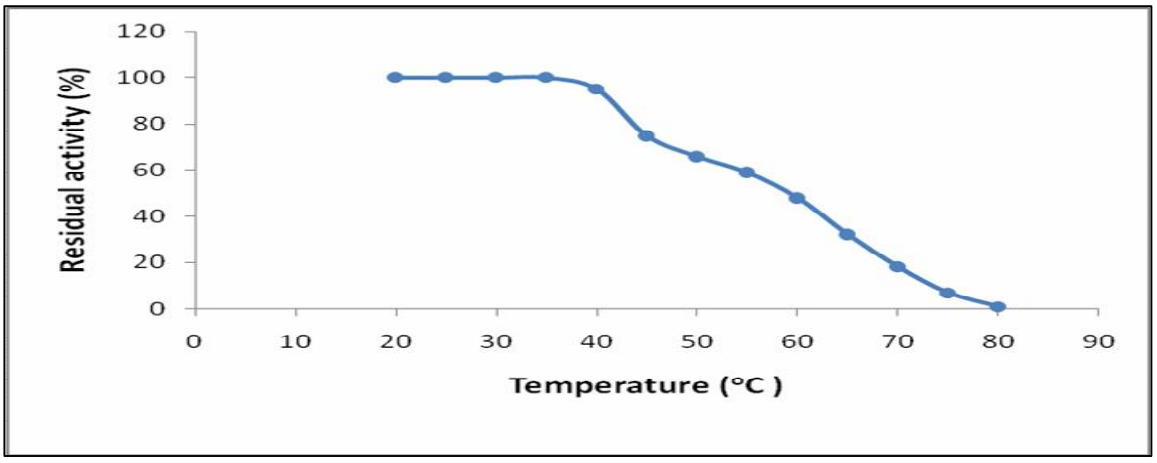

Fig 8. Thermal stability of urease of *Prosopis farcta*

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