Cameroun Journal of Biological and Biochemical Sciences, 2026, 34, 10-19.

DOI: https://doi.org/10.63342/cjbbs2026.34.25024.en

CAMEROON JOURNAL OF BIOLOGICAL AND BIOCHEMICAL SCIENCES
Published by the Cameroon Biosciences Society (CBS)

Original research article

Extraction and characterization of urease from *Centrosema pubescens* Benth.

BARTHELEMIE GUEBOTO, FREDERIC NICO NJAYOU* & PAUL FEWOU MOUNDIPA

Laboratory of Pharmacology and Toxicology, Department of Biochemistry, Faculty of Science, University of Yaoundé I, PO Box 812, Yaoundé, Cameroon

Corresponding Author: njayou@yahoo.fr

Received: 17 Jul 2025, Reviewed: 03 Sep 2025, Revised: 22 Sep 2025, Accepted: 03 Sep 2025, Published: 09 Jan 2026.

ABSTRACT

Urease is an industrially important enzyme whose production remains limited due to the low availability of the raw material (urease source). Controlling the production of this enzyme can, in view of its many applications, promote the development of importing countries such as Cameroon. This study aimed to extract and characterize urease from *Centrosema pubescens* for its future valorization. This work is part of the search for local sources of this enzyme through its purification and characterization from *Centrosema pubescens*. Seeds were germinated at 2, 4, 6, 8, and 10 days, and crude extracts of the enzyme were prepared in an extraction solvent consisting of a mixture of phosphate buffer (pH 7; 0.1 M)/acetone 80/20 v/v to obtain homogenates at 25% w/v, and then centrifuged. Protein content and urease activity were assessed spectrophotometrically. The day of germination on which the germinated seeds showed high enzymatic activity was selected. The crude extract was partially purified using ammonium sulphate precipitation followed by desalting through exclusion chromatography. The presence of urease in the mixture of active fractions was verified by denaturing gel electrophoresis (SDS-PAGE). The kinetic parameters (Vmax and Km) of urease were determined using the kinetic method. The results showed that the 4th day of germination showed the highest specific activity (0.140±0.004 µmol NH4+/min/mg protein) and was chosen as the germination day for the rest of the work. The crude extract from the 4th day was selected for having a purification percentage of 18.78 and a degree of purification of 27.79. The kinetic parameter values for urease under these conditions were: 0.052 ± 0.01 µmol NH4+/min and 0.48 ± 0.153mM for Vmax and Km, respectively, and those for Jack Bean urease were 0.53 ± 0.01 µmol NH4+/min and 0.030 ± 0.01 mM. These observations show that *Centrosema pubescens* may be a good source of urease.

Key words: Urease, *Centrosema pubescens*, kinetic parameters, plant.

RÉSUMÉ

L'uréase est une enzyme d'importance industrielle dont la production reste limitée en raison de la faible disponibilité de la matière première (source d'uréase). Compte tenu de ses nombreuses applications, la maîtrise de la production de cette enzyme peut favoriser le développement des pays importateurs comme le Cameroun. Le but de cette étude était d'extraire et de caractériser l'uréase de *Centrosema pubescens* en vue d'une valorisation future. Ce travail s'inscrit dans le cadre de la recherche des sources locales de cette enzyme à travers la purification et la caractérisation de l'uréase de *Centrosema pubescens*. Des extraits bruts ont été préparés dans un mélange de tampon phosphate (pH 7; 0.1 M)/acétone 80/20 v/v en vue d'obtenir un homogénat à 25% m/v pour chaque jour de germination des graines (2, 4, 6, 8 et 10 jours) par centrifugation. La teneur en protéines et l'activité uréasique ont été évaluées par spectrophotométrie. Le jour de germination au cours duquel les graines germées ont montré une activité enzymatique élevée a été sélectionné. L'extrait brut préparé a été utilisé pour la purification partielle de l'uréase par précipitation au sulfate d'ammonium suivie d'une chromatographie d'exclusion après dessalage. La présence d'uréase dans le mélange de fractions actives a été vérifiée par électrophorèse sur gel dénaturant (SDS-PAGE). Les paramètres (Vmax et Km) de l'uréase ont été déterminés par la méthode de cinétique enzymatique. Les résultats ont montré une activité spécifique plus élevée (0,140±0,004 µmol NH4+/min/mg de protéine) au 4e jour de germination et les graines à cette période physiologique ont été utilisées comme matériel pour la suite du travail. L'uréase a été purifiée dans les graines germées au jour 4 avec un pourcentage et un degré de purification respectifs de 18,78 et de 27,79. Les valeurs des paramètres cinétiques pour l'uréase dans ces conditions étaient : 0,052 ± 0,01 µmol NH4+/min et 0,48 ± 0,153 mM pour Vmax et Km respectivement, et celles de

l'uréase de Jack Bean étaient de 0,53 ± 0,01 µmol NH4+/min pour la Vmax et 0,030 ± 0,01 mM pour le Km. Ces observations montrent que *Centrosema pubescens* peut être une bonne source d'uréase.

Mots-clés : Uréase, *Centrosema pubescens*, paramètres cinétiques, plante.

1. INTRODUCTION

Urease (urea amidohydrolase, E.C. 3.5.1.5) is a nickel-containing metalloenzyme that falls within the hydrolase class. It plays a key role in nitrogen metabolism, particularly by breaking down urea, a process that produces ammonia (NH_3) and carbonic acid (H_2CO_3) (Mobley et *al*. 1995). Urease is widely distributed in nature. Microbial ureases are found in many bacteria of the genus Lactobacillus and Helicobacter, where they are produced as a virulence factor in patients with gastric ulcers (Wassermann et *al*. 2010), as well as certain microscopic fungi such as *Rhizopus oryzae*. However, the use of these microorganisms as a source of urease remains limited due to their low availability, the high cost, and the sophisticated equipment required to purify the enzyme (Javadi et *al*. 2018). In plants, urease is a ubiquitous enzyme (Witte and Medina-Escobar 2001) and is very abundant in the seeds of many cucurbits and legumes such as *Canavalia enciformis* (Jack Bean), whose urease is the most extensively studied (Polacco et *al*. 1982). Urease is produced during the germination of these seeds. As a result, the germinating seeds of the above-mentioned plant species are considered a source of this enzyme. In organisms of this kingdom, the main function of urease is linked to the use and recycling of endogenous nitrogen or nitrogen derived from external sources (Follmer 2008).

Urease is an enzyme of industrial importance because of its multiple applications. In the medical field, it is used in the development of haemodialysis machines and diagnostic kits for Helicobacter pylori infections (Witte et *al*. 2002). In agriculture and biotechnology, urease is used as a soil fertiliser (Hamdan et *al*. 2013) and as an ingredient in the manufacture of bio-cement (Javadi et *al*. 2018).

However, industrial production of this enzyme remains limited because of the low availability of the raw material (urease source), due to the fact that the main source of this enzyme (Jack-Bean or white bean) is consumed by the population. Hence, it is important to seek out and develop new local sources of this enzyme. Therefore, the present work was undertaken to determine whether *Centrosema pubescens* could be used as a good source of urease through the extraction and characterization of the enzyme.

2. MATERIALS AND METHODS

2.1. Biological material

The biological material consisted of *Centrosema pubesens* (CP) seeds harvested in Yaoundé and identified at the National Herbarium of Cameroon (41654/HNC). Once harvested, the plant material was further dried under sunlight for two days, pulped, and stored in a dry place for later use.

2.2. Effect of germination time on enzymatic activity

2.2.1. Germination and preparation of the crude extract

Dried *C. pubesens* seeds were sorted, weighed, and soaked in distilled water for 6 h. The seeds were then spread out on paper, covered with cotton, and left to germinate for 2, 4, 6, 8, and 10 days in a moderately well-lit area. The crude extract was prepared according to the method of Zusfahair et *al*. (2018). After each day of germination, the germinated seeds were ground using a mortar in an extraction solvent consisting of a mixture of phosphate buffer (pH 7; 0.1 M)/acetone 80/20 v/v to obtain homogenates at 25% m/v. The homogenates thus obtained were kept at 4°C for 3 h, then filtered and centrifuged (3000g, 15 min, 4°C). The supernatant obtained was recovered, stored at -20°C, and treated as a crude extract.

2.2.2. Estimation of the protein content of the crude extract

The protein content of each crude extract was determined using the Biuret method (Gornall et *al*. 1949). Calibration of bovine serum albumin (BSA) was carried out using a stock solution with a concentration of 3mg/ml. The absorbance was read at 540 nm against the blank, and the amount of protein was determined using the BSA calibration line.

2.2.3. Determination of the urease activity of the various enzyme extracts

Urease activity was determined using the indophenol method initially described by Weatherburn and modified by Mahmood et *al*. (2012). The assay was performed by introducing 0.05 mL of enzyme extract and 1.450 mL of phosphate buffer (pH 7.4; 100 mM) into each test tube. After pre-incubation (5 min, 37°C) in a water bath, 0.5 mL of urea at a final concentration of 25 mM (Pervin et *al*. 2013) was added, and the resulting mixture was incubated for various times (0, 5, 10, 15, 20, 25, and 30 min). After this time, 0.5 mL of alkaline solution (1% w/v sodium hydroxide and 0.082% v/v active sodium hypochlorite) and 0.5 mL of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) were added to each test tube containing the hydrolysates. After 60 min incubation at 37°C and development of the blue colour, the absorbance of the solution was read at 630 nm against the blank. The calibration line established from an ammonium sulphate solution with an initial concentration of 0.1mg/ml was used to convert absorbance into the quantity of ammonium ions formed. The initial velocities were obtained by plotting a slope on the straight part of the hyperbolic graphs obtained by representing the number of moles of ammonium ions formed after hydrolysis as a function of incubation time for each day of germination. The specific activity was calculated using the formula:

$$\text{Specific activity} = \frac{V0}{CP}$$

Where:V_0 = initial speed of the enzyme (in µmol of NH_4^+ formed/min), CP = final quantity of protein in the test tube (mg)

2.3. Purification of urease

2.3.1. Germination and preparation of the crude extract

The seeds were germinated for 4 days, and the procedure was carried out as previously described in paragraph 1.2.1. The crude extract was prepared according to the method of Dikhtyarev et *al*. (1983) after four (4) days of germination. The homogenate obtained was centrifuged at 10,000g for 20 min at 4°C, and the supernatant was the crude enzyme extract (EB).

2.3.2. Ammonium sulphate precipitation

Precipitation was performed according to the protocol described by Rajendran et *al*. (2019), 50% salt saturation. The mixture was homogenized for 15 min at 4°C until a precipitate formed. The mixture was then centrifuged (10000g, 20 min, 4°C), and the pellet obtained was re-suspended in the working buffer, and aliquots of the supernatant (S1) were stored for future use.

2.3.3. Gel filtration desalting of the supernatant

Gel exclusion chromatography was used for desalting according to the protocol described by Altin et *al*. (2017). The supernatant obtained in the previous step (2 mL) was desalted by gel exclusion chromatography on Sephadex G-50 previously stabilised on the column (4cm x 2) for 24 hours. A fluorescein marker was added to the sample to monitor the elution. Five hundred (500) µL of the sample was gently placed on top of the gel. Elution was carried out using phosphate buffer (pH 7; 100 mM), and 1 mL fractions were collected in test tubes.

2.3.4. Protein assay in each fraction

The amount of protein in each fraction collected was measured by reading optical densities at 280 nm.

2.3.5. Detection of ammonium ions in each fraction

Ammonium ions were monitored by the indophenol method as previously described.

2.3.6. Determination of enzymatic activity

Enzyme activity was assayed in fractions not containing fluorescein by the indophenol method. Fractions showing urease activity were considered as the enzymatic extract (EE), collected and homogenized, and then aliquots were stored at -20°C for later use.

2.3.7. Establishment of the purification profile

The purification profile was established by measuring the amount of protein and urease activity in the crude enzymatic extract (EB), the supernatant obtained after ammonium sulphate precipitation (S1), and in the mixture of enzymatic fractions collected (EE). The purification percentage and degree of purification were calculated as follows:

$$\text{Purification percentage } = \frac{\text{Total proteins of the fraction}}{\text{Total proteins of the crude extract}} \times 10$$

$$\text{Degre of purification} = \frac{\text{Specific activity of the fraction}}{\text{SA of the crude extract}} \times 100$$

2.3.8. Detection of urease in the different fractions by electrophoresis

Electrophoresis is a technique used to separate molecules based on their charge, as charged molecules migrate through a supporting medium such as a polymer matrix, polyacrylamide, or agarose gel under the influence of an electric field (Laemmli 1970). The process begins with the preparation of the gels: a 10% acrylamide separation gel and a 5% acrylamide stacking (or concentration) gel, which are cast between two glass plates. The separation gel is poured first, typically about 5 mL, and then covered with ethanol to ensure uniform solidification at room temperature for 20 minutes. After the separation gel has set, the ethanol is removed, and 2 mL of the stacking gel solution is added. A comb of 1 mm thickness is then inserted to create wells for sample loading. The proteins were diluted in 5-fold concentrated Laemmli buffer and heated to 96°C for 10 min in a water bath. Ten microlitres of the resulting mixture containing 50 µg of protein (for all the samples to be analyzed) were added to the wells. Electrophoresis was then carried out in the presence of a control consisting of Jack Bean urease (90 kDa per subunit). In the concentration gel, migration was carried out at 70 V for 1 hour, followed by 120 V for 1 hour and 30 minutes for the separation gel, using a conductive electrophoresis buffer.

2.3.9. Determination of the kinetic parameters of the enzymatic extract (EE)

The enzymatic hydrolysis of urea by the enzyme extract (EE) and the determination of the ammonium ions released were carried out using the indophenol method as previously described using six (06) urea concentrations tested: 25, 15, 10, 2.5, 1, and 0.1 mM. After determining the initial velocities (V_0) by calculating the slopes of the tangent to the linear part of the curves representing the number of moles of product formed as a function of time for each urea concentration, the graph $1/V_0 = f(1/[urea])$ was constructed. The exact values of the urease kinetic parameters were determined from the graph of the double inverses. The points of intersection between the straight line and the y-axis (1/Vmax) and between the extension of the straight line and the x-axis (-1/Km) were identified and used to calculate the kinetic constants Vmax and Km.

2.4. Statistical analysis

Statistical analyses of the initial velocity and the specific activity obtained were carried out using Graphpad Prism 8.0.1 software. The results were expressed as mean ± standard deviation, and the different values were compared using the analysis of variance test “one-way ANOVA” followed by the multiple comparison test (Turkey). Differences were considered significant for $p \leq 0.05$.

3. RESULTS

3.1. Effect of germination time of *Centrosema pubescens* seeds on enzyme activity.

Enzyme activity varies as a function of seed germination time as well as the concentration of the enzyme involved in enzyme catalysis. In this study, the germination times were 2, 4, 6, 8, and 10 days, and the final protein concentration was 0.13 mg/ml. Table I below shows the initial velocity (V_0) and specific activity values obtained as a function of germination time (days). The initial velocities (V_0) vary from 0.004 to 0.018 µmol of ammonia formed/minutes, and Specific activities (SA) ranged from 0.031 to 0.140 µmol ammonia formed/min/mg protein. The difference in urease activity in terms of specific activity was significantly

(P<0.05) higher on day 4 of germination compared with the other days, reflecting increased production of the enzyme on day 4 of germination compared with the other days, as shown in Figure 1.

Table I: Initial velocity (V0) and specific activity (SA) as a function of germination day

Germination day	2	4	6	8	10
V0 (µmol of ammonia/min)	0.004±0.001	0.018± 0.001*	0.011±0.001	0.012±0.001	0.009±0.001
SA (µmol of ammonia/min/mg of protein)	0.031±0.004	0.140±0.004*	0.092±0.008	0.094±0.007	0.070±0.006

Values are mean ± standard deviation of 3 replicates. (*) *Significant differences between groups at p≤ 0.05, Turkey test.*

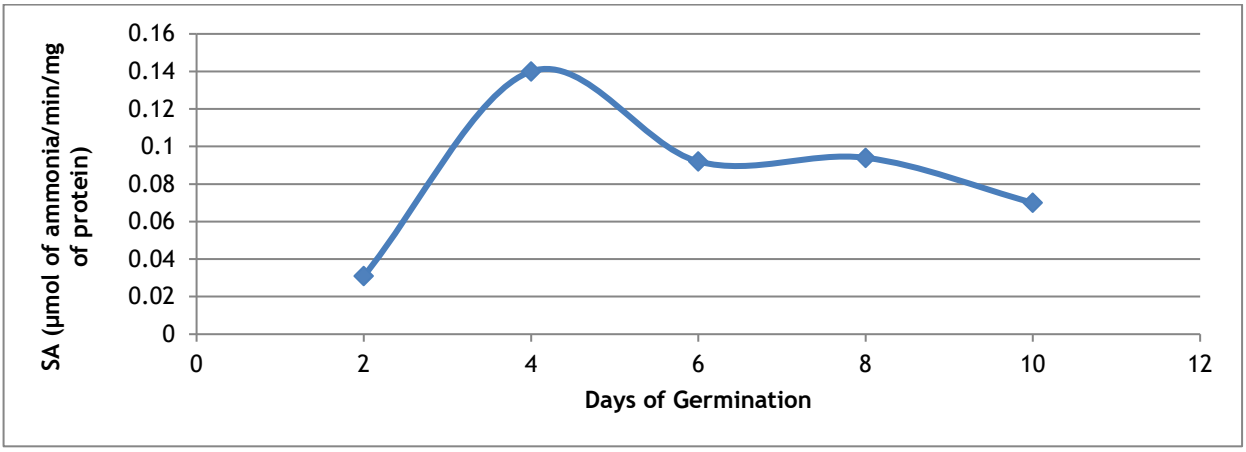

Figure 1: Specific activity (SA) as a function of the days of germination

3.2. Purification profile

When proteins are purified by precipitation using a saturated neutral salt solution, it is very often necessary to carry out desalting. In our work, desalting was carried out using gel exclusion chromatography with protein separation, a technique that enables an elution profile to be established for the various constituents of the mixture to be separated. Figure 2 shows the elution profile for proteins, salts, and urease. The maximum amount of protein was obtained in tubes 6 to 8, and the maximum amount of salt (ammonium sulphate) was obtained in tube 39. Urease activity was found in tubes 1 to 5, and the maximum was obtained in tubes 2 and 3 (Figure 3).

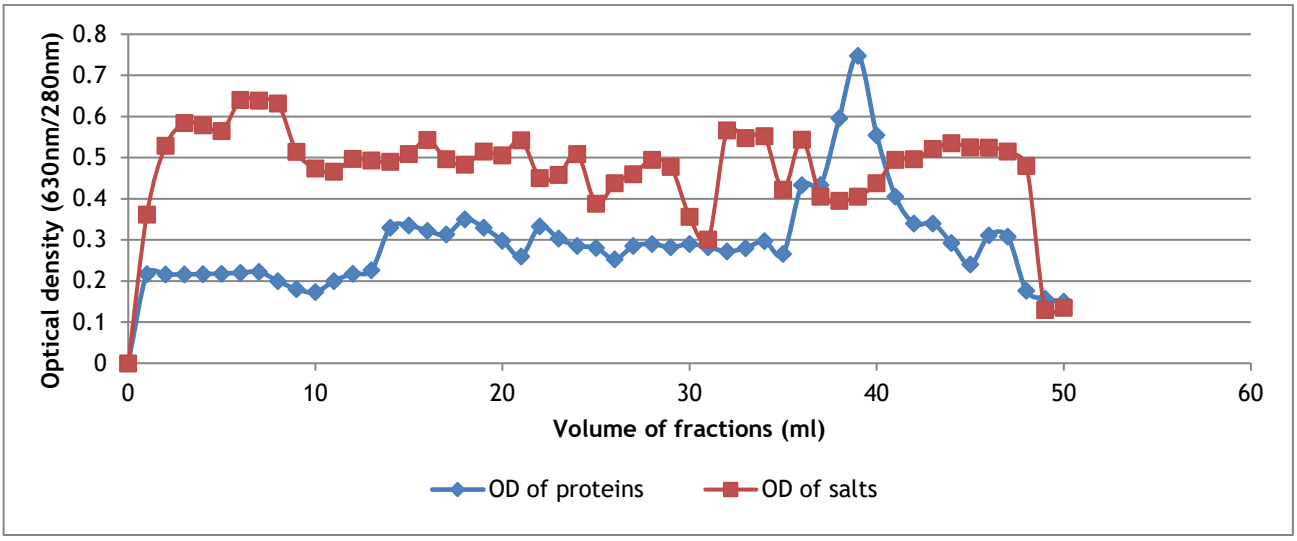

Figure 2: Elution profile of proteins (a) and ammonium ions (b) from the fractions collected as a function of the tubes during exclusion chromatography.

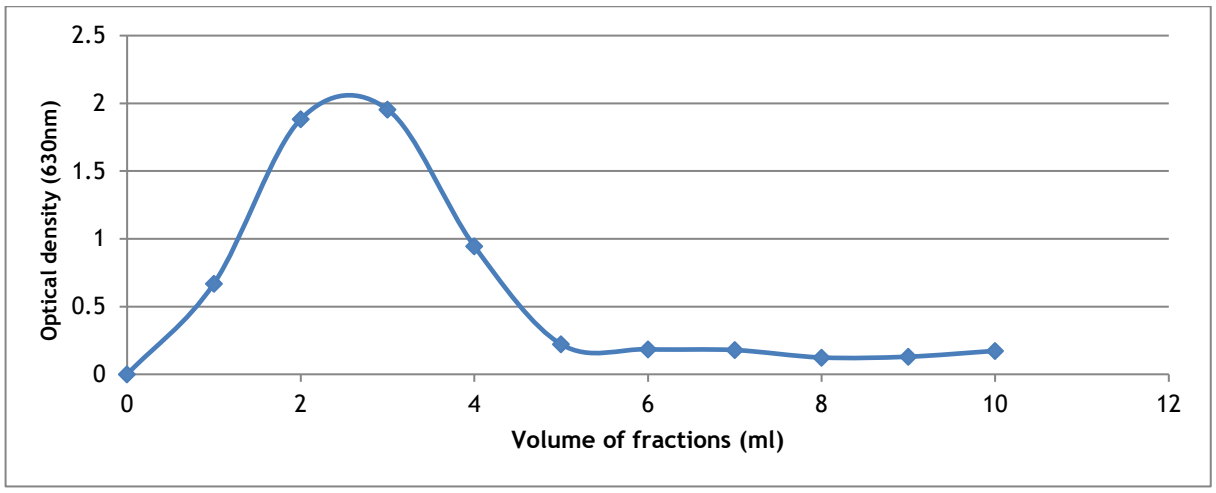

Figure 3: Exclusion chromatography elution profile of enzyme activity as a function of volume of fractions tubes.

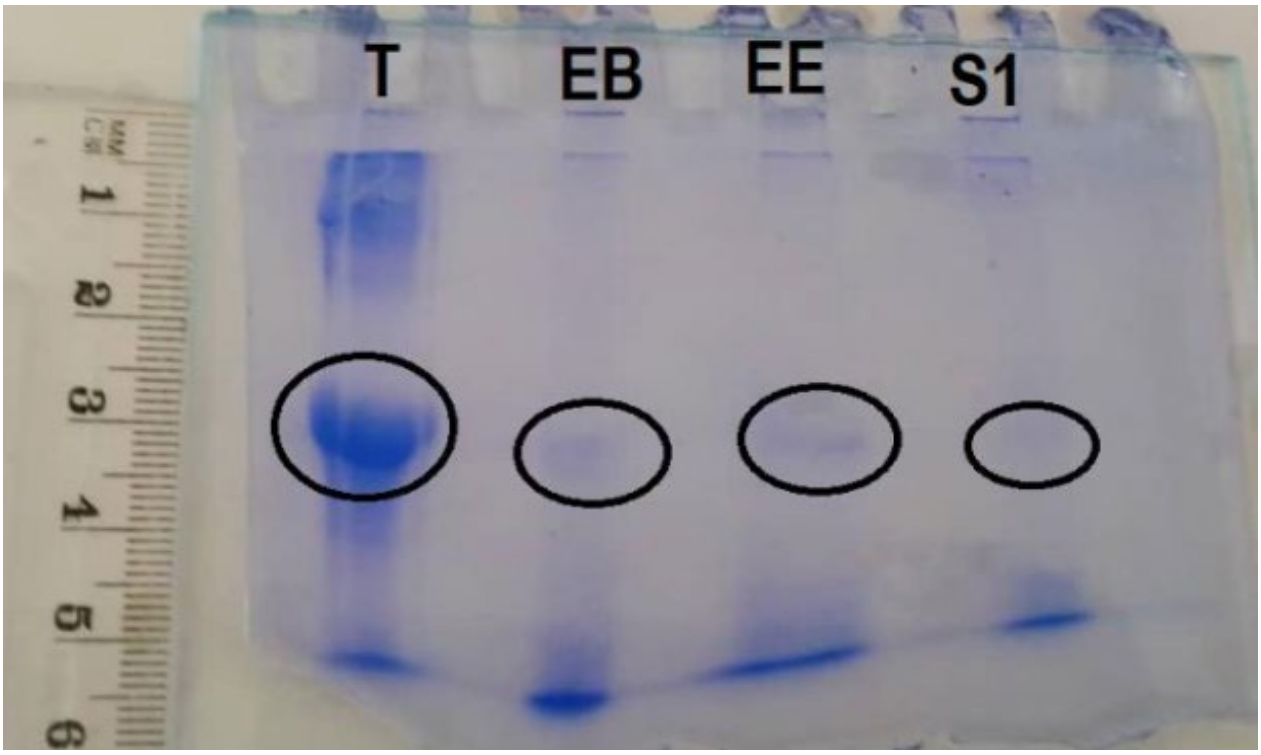

Figure 4: Electrophoresis gel of the different extracts EB, EE, and S1

T= control (Jack Bean urease), EB= crude extract, EE= enzymatic extract obtained after desalting by gel exclusion chromatography, S1= supernatant obtained after precipitation of proteins from the crude extract with ammonium sulphate and centrifugation.

Table II below summarizes the purification steps of urease from the selected wild bean species. This table shows a decrease in the percentage of purification (75.16% for the S1 supernatant and 18.79% for the final enzyme extract) and an increase in the degree of purification (27.78% for the final enzyme extract) during purification. The presence of urease in our different fractions (S1, EB, and EE) was verified by electrophoresis under denaturing conditions (SDS-PAGE). The results showed that for the supernatant (S1), the enzymatic crude extract (EB) and the enzymatic extract (EE) tested, we obtained a single band located at the same distance as the band obtained with Jack Bean urease (control), a light and dark band for the purified fraction EE, a less visible band for the crude extract and no band for the supernatant S1 figure 4.

Table II: Purification table for urease from *Centrosema pubescens* species

Steps	Crude extract	Supernatant obtained after precipitation	Enzymatic extract
Total volume (mL)	91	80	7
[P] (mg/mL)	49.72	42.52	12.15
Total protein (mg)	4524.52	3401.6	85.05
Activity (V0) (µmol of ammonia/min)	0.0475	0	0.07445
Total activity (V0*VT)	4.323	0	0.5212
SA (µmol of ammonia/min/mg of protein)	0.0191	0	0.1225
Percentage of purification	100	75.16	18.79
Degree of purification	1	0	27.78

3.3. Enzyme kinetic parameters

Determining the kinetic parameters of an enzyme is important because these parameters determine the ability of an enzyme to perform enzymatic catalysis. This determination is based on the graphical representation of (initial velocities = f (substrate concentrations)), which requires the initial velocities to be determined. These curves enabled us to obtain initial velocities for each urea concentration tested, and the results obtained have been presented in Table III below.

Table III: Initial velocities and their inverse for each urea concentration tested

Urea concentration in (S) (mM)	0.1	1	2.5	5	10	15	20
Initial velocity (V_0) (µmol of ammonia/min)	0.0072	0.0021	0.0397	0.05	0.062	0.0642	0.0648
1/S (mM^{-1})	10	1	0.4	0.2	0.1	0.067	0.04
$1/V_0$ (µmol d'ammoniac^{-1} *min)	138.889	47.619	25.189	20	16.129	15.576	15.432

Based on these values, the Michaelis-Menten representation was produced (Figure 5). From this curve, it can be seen that the intersection of the horizontal asymptote of the curve with the y-axis gives an approximate value of Vmax = 0.0726 µmol NH4+/min, for which the abscissa of Vmax/2 gives an approximate value of Km = 2.150 ± 0.367. From the values obtained for the inverses, the Lineweaver-Burk representation ($1/V_0$ = f (1/[S]) (Figure 6) was constructed. From this representation, we can see a straight line that intersects the x-axis and y-axis at points representing -1/Km and 1/Vmax respectively. The exact values of Vmax and Km obtained were as follows: Vmax = 0.052 ± 0.01 µmol NH_4^+/min and Km = 0.48 ± 0.153 mM.

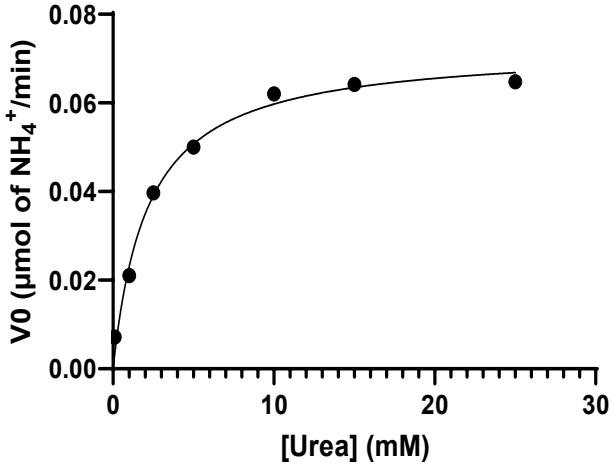

Figure 5: Initial velocities as a function of urea concentration

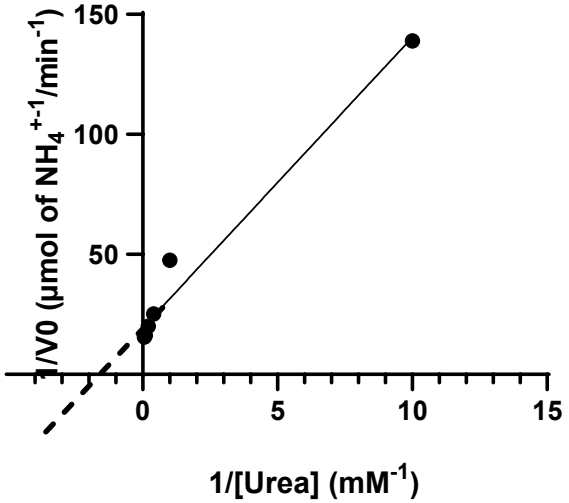

Figure 6: Representation of inverses of initial velocities as a function of inverses of urea concentration (Lineweaver-Burk)

4. DISCUSSION

In view of the importance of urease in everyday human life and its many applications, new local plant-based and available sources need to be sought. This study aimed to extract and characterize urease from *Centrosema pubescens*.

Urease is an enzyme involved in the metabolism of nitrogen in germinating plants. The production of this enzyme depends on the germination period. In the first part of the work, we determined the day of germination at which urease is abundant in germinating seeds of *Centrosema pubescens*. The specific activities in germinated seed extracts at different days of germination (DG) ranged from 0.031 to 0.140 mol ammonia formed/min/mg protein (Table III). The difference in urease activity in terms of specific activity was significantly higher on day 4 of germination compared with the other days. Our results are similar to those obtained by Zusfahair et *al*. (2018), who worked on the determination of the biochemical properties of urease in asparagus (*Vigna unguiculata ssp sesquipedalis* L.) and found that urease was predominantly produced in these seeds on day 8 of germination. The production of hydrolytic enzymes in plants that contain them depends on the species of plant used, the growing region, and the plant's growth metabolism.

In order to determine the kinetic parameters of an enzyme, it is necessary to purify it first. After gel electrophoresis in denaturing conditions, the purified enzyme showed the same migration distance as that of the control (Jack bean). According to the literature, this means that the sample tested contained urease, with a molecular weight of 90 kDa, as confirmed by Weber et *al*. 2008, who worked on optimizing the isolation and purification of jack Bean urease for crystallization. The intensity obtained for the enzymatic extract compared to the other two fractions may be because the urease in this fraction has been concentrated, and proteins other than urease have been eliminated. The purification profile revealed a purification percentage of 18.78% and a degree of purification of 27.79, indicating the effectiveness of the purification methods used. Our results are in agreement with those of El-Hefnawy et *al*. (2014), who worked on the extraction, purification, kinetics, and thermodynamic properties of urease extracted from germinated seeds of *Pisum Sativum* L. and found a purification percentage of 42% and a degree of purification of 12.85.

The kinetic parameters of an enzyme indicate its efficiency in catalysing the reaction. With regard to these kinetic parameters, we found a Vmax of 0.052 ± 0.01 and 0.030 ± 0.004 µmol NH_4^+/min and a Km of 0.48 ± 0.153 and 0.53 ± 0.01 mM, respectively, for *Centrosema pubescens* urease and Jack Bean urease (positive control). These results are close to those found by Lim and Leverenz. (2019) who have worked on the characterization of urease from *Citrillus lanatus* (watermelon) seeds with a view to estimate total nitrogen in human urine. In their work, they found a urease activity of 12.2 and 52.6 U/mg protein and a Km of 3 and 3.6 mM, respectively, for *Citrillus lanatus* urease and Jack Bean urease. The effectiveness of the enzyme is highly dependent on the plant source used.

5. CONCLUSION

Based on all those results, it is clear that *Centrosema pubescens* Benth. is a potent source of urease with important enzyme activity.

ABBREVIATIONS: Km: Michaelis-Menten constant. SA: specific activity. SDS-PAGE: Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis. Vo: initial velocity. Vmax: maximal velocity.

ACKNOWLEDGMENTS. None.

FUNDING. None.

DISCLOSURE. None

CONFLICT OF INTEREST. The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS. BG and FNN carried out all experiments reported in the manuscript. FNN and PFM designed the study. All authors read and approved the final manuscript.

ETHICAL STATEMENT. No biological material from animal origin was used in this study.

DATA SHARING STATEMENT. No additional data are available.

REFERENCES

Altın S, Tohma H, Gülçin İ, & Köksal E. (2017). Purification, characterization, and inhibition sensitivity of peroxidase from wheat (*Triticum aestivum* ssp. Vulgare). *International Journal of Food Properties* https://www.tandfonline.com/doi/abs/10.1080/10942912.2016.1225308

Biglar M, Soltani K, Nabati F, Bazl R, Mojab F, & Amanlou M. (2012). A Preliminary Investigation of the Jack-Bean Urease Inhibition by Randomly Selected Traditionally Used Herbal Medicine. *Iranian Journal of Pharmaceutical Research* 11(3), 831–837.

Burbank M, Weaver T, Lewis R, Williams T, Williams B, & Crawford R. (2013). Geotechnical Tests of Sands Following Bioinduced Calcite Precipitation Catalyzed by Indigenous Bacteria. *Journal of Geotechnical and Geoenvironmental Engineering* 139(6), 928–936. https://doi.org/10.1061/(ASCE)GT.1943-5606.0000781

Dikhtyarev SI, Yanina MM, Kuznetsova RG. (1983). Isolation of a urease from watermelon seeds and the study of its properties. *Chem Nat Compd* 19, 587–591. https://doi.org/10.1007/BF00576090

EL-Hefnawy ME, Sakran M, Ismail AI, & Aboelfetoh EF. (2014). Extraction, purification, kinetic and thermodynamic properties of urease from germinating Pisum Sativum L. seeds. *BMC Biochemistry* 15(1), 15. https://doi.org/10.1186/1471-2091-15-15

Follmer C (2008). Insights into the role and structure of plant ureases. *Phytochemistry* 69(1):18-28. doi: https://10.1016/j.phytochem.2007.06.034

Gornall AG, Bardawill CJ, & David MM. (1949). Determination of serum proteins by means of the biuret reaction. *The Journal of Biological Chemistry* 177(2), 751–766.

Hamdan N, Kavazanjian E, & O'Donnell S. (2013). Carbonate cementation via plant-derived urease: 18th International Conference on Soil Mechanics and Geotechnical Engineering, ICSMGE 2013. 18th International Conference on Soil Mechanics and Geotechnical Engineering, 3, 2489–2492.

Javadi N, Khodadadi H, Hamdan N, & Kavazanjian E. (2018). EICP Treatment of Soil by Using Urease Enzyme Extracted from Watermelon Seeds. *Geotechnical Special Publication* 2018-March (GSP 296), 115-124. https://doi.org/10.1061/9780784481592.012

Laemmli UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259): 680-685. https://doi.org/10.1038/227680a0

Lim K, Leverenz H. Characterization of Urease Derived from Citrullus lanatus (Watermelon) Seeds to Estimate Total Kjeldahl Nitrogen in Human Urine. (2019). *International Journal of Environmental Analytical Chemistry* 99(5), https://doi.org/10.6084/m9.figshare.7998416

Lineweaver H, & Burk D. (1934). The Determination of Enzyme Dissociation Constants. *Journal of the American Chemical Society,* 56(3), 658–666. https://doi.org/10.1021/ja01318a036

Mobley HL, Island MD, Hausinger RP (1995). Molecular biology of microbial ureases. *Microbiol Rev*. 59(3):451-80. https://doi.org/10.1128/mr.59.3.451-480

Pervin S, Jahan GS, & Rana AYK, Rana M, Sana NK, Rahman H, Shaha RK (2013). Effects of some environmental variables on urease in germinating chickpea (*Cicer arietinum L.*) seed. *Journal of Stress Physiology and Biochemistry* 9(3), 345-356.

Polacco JC, Sparks RB. (1982). Patterns of urease synthesis in developing soybeans. *Plant Physiol*. 70(1), 189-94. https://doi.org/10.1104/pp.70.1.189

Rajendran R, Pandi A, Ramchary A, Thiagarajan H, Panneerselvam J, Niraikulam A, Kuppuswami GM, Ramudu KN. (2019). Extracellular urease from Arthrobacter creatinolyticus MTCC 5604: scale up, purification and its cytotoxic effect thereof. *Mol Biol Rep*. 46(1):133-141. https://doi.org/10.1007/s11033-018-4453-8

Wassermann GE, Olivera-Severo D, Uberti AF, Carlini CR. (2010). *Helicobacter pylori* urease activates blood platelets through a lipoxygenase-mediated pathway. *J Cell Mol Med*. 14(7), 2025-34. https://doi.org/10.1111/j.1582-4934.2009.00901.x

Weber M & Jones M & Ulrich J. (2008). Optimisation of isolation and purification of the jack bean enzyme urease by extraction and subsequent crystallization. *Food and Bioproducts Processing* - FOOD BIOPROD PROCESS 86, 43-52. https://doi.org/10.1016/j.fbp.2007.10.005

Witte CP, Medina-Escobar N. (2001). In-gel detection of urease with nitroblue tetrazolium and quantification of the enzyme from different crop plants using the indophenol reaction. *Anal Biochem*. 290(1):102-7. https://doi.org/10.1006/abio.2000.4933

Witte CP, Tiller SA, Taylor MA, Davies HV. (2002). Leaf urea metabolism in potato. Urease activity profile and patterns of recovery and distribution of (15)N after foliar urea application in wild-type and urease-antisense transgenics. *Plant Physiol*. 128(3), 1129-36. https://doi.org/10.1104/pp.010506

Zusfahair, Ningsih D, Fatoni A & Pertiwi, D. (2018). Determination of Urease Biochemical Properties of Asparagus Bean (*Vigna unguiculata* ssp Sesquipedalis L.). *IOP Conference Series: Materials Science and Engineering*. 349. 012073. https://doi.org/10.1088/1757-899X/349/1/012073