Original Research Article

Seeds of *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Vigna unguiculata* (L.) Walp. and *Zea mays* L. : chemical characterization of the coagulating solutions

ABSTRACT

The aim of this study was to characterize the amino acids in the seeds of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays*; and the proteins in the coagulating solutions of the seed powders of these 3 plants. Amino acids were separated by ion exchange chromatography and determined by ninhydrin reaction with photometric detection. Proteins were characterized by steric exclusion high-performance liquid chromatography (SE-HPLC) on a Superdex column in the range of 10 kDa to 500 kDa and on a Shodex column in the range of 204 Da to 35 000 Da. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) was also performed. The results obtained showed that the most abundant amino acid in the seeds of the 3 plant species is glutamic acid, a negatively charged side chain amino acid at neutral pH. The respective contents are 6150 mg/100 g in *Citrullus lanatus* seeds; 4030 mg/100 g in *Vigna unguiculata* seeds and 1820 mg/100 g in *Zea mays* seeds. The percentage of protein constituents with a molecular weight less than 10 kDa is 87.3%; 74.1% and 61.6%; *for Citrullus lanatus*, *Zea mays* and *Vigna unguiculata* coagulant solutions respectively. The percentage of protein constituents with a molecular weight between 1000 Da and 1500 Da is 24.9%; 20.4% and 29.2%; respectively for the coagulant solutions of *Vigna unguiculata*, *Zea mays* and *Citrullus lanatus*. A percentage of 25.8%, between 800 and 1000 Da, is also noted for *Citrullus lanatus*. The study of the coagulant activity showed percentages of turbidity reduction higher than 80%.

Key words: *Citrullus lanatus*, *Vigna unguiculata*, *Zea mays*, Seeds, amino acids, Proteins, Coagulants, Coagulating solutions.

1. INTRODUCTION

In rural areas of developing countries, there is a problem of water supply [1,2] and 97% of the population does not have a piped water supply [3,4]. These populations sometimes use water for household chores that may come from rivers, streams, ponds and wells. The quality of these waters can be improved by home treatment with natural plant-based substances with coagulant activity [5-7]. Goal 6, one of the 17 Sustainable Development Goals adopted in 2015 as part of the United Nations 2030 agenda, suggests "ensuring the availability and sustainable management of water and sanitation for all", [8]. As part of the implementation of this goal, we are undertaking investigations on the research of natural coagulants of plant origin, to have scientific data on natural substances of plant origin with coagulant activity; to be able to expand the range of natural coagulants of plant origin and also to contribute to the improvement of water quality for people in rural areas of developing countries [9-11]. Our previous work has shown the coagulant activity of aqueous solutions of *Arachis hypogaea* and *Cucumeropsis mannii* seed powders in the clarification of surface water samples of initial turbidities 131 NTU, 128,60 NTU, 94 NTU, 91,06 NTU, 89.45 NTU, 41,45 NTU and 32,75 NTU [12-14]. They also characterized the proteins in the coagulating solutions of the seed powders in these solutions [13]. In this study, *Citrullus lanatus, Vigna unguiculata*, and *Zea mays* species were studied. This study aims at chemical characterization of the seeds, characterization of proteins in coagulant solutions of the seed powders and evaluation of coagulant activity of seeds of these three plant species in surface water clarification. *Moringa oleifera* Lam., was used as a reference in this study because of its proven coagulant activity in surface water clarification due to cationic polypeptide polyelectrolytes [15,16]. Aluminum sulfate, which is the most widely used mineral coagulant in the treatment of water for human consumption, was also used as a reference in this study [17].

2. MATERIAL AND METHODS

2.1. Plant material

The plant material consisted of seeds of *Citrullus lanatus*, *Vigna unguiculata*, *Zea mays* and *Moringa oleifera*. The seeds of *Citrullus lanatus were* obtained from fresh fruits of *Citrullus lanatus* that were purchased in August of the year 2021, at the Total market located in the district N° 1 of the city of Brazzaville, Republic of Congo. Seeds of *Vigna unguiculata*, *Zea mays* and *Moringa oleifera* were also purchased at the Total market in August 2021.

2.2. Chemical characterization of seeds

2.2.1. Chemical composition of the seeds

The study of the chemical composition of *Citrullus lanatus*, *Vigna unguiculata*, and *Zea mays* seeds involved the determination of the contents of total lipids, total proteins, amino acids, total carbohydrates, total starch, crude ash, and moisture content. Three treatments were performed and the test sample size for each treatment was 100 grams.

2.2.1.1. Total protein and amino acid contents

The crude protein content was obtained from the nitrogen content determined by the Kjeldhal method and the amino acid content according to NF EN ISO 13903 and NF EN ISO 13904. The free amino acids were extracted with diluted hydrochloric acid. The co-extracted nitrogenous macromolecules were precipitated and removed by filtration. The amino acids were then separated by ion exchange chromatography and determined by reaction with ninhydrin with photometric detection.

2.2.1.2. Total lipid content

The lipid content was obtained by Soxhlet extraction with petroleum ether for 6 hours.

2.2.1.3. Total carbohydrate and total starch contents

The total carbohydrates were obtained by computation, according to the formula 100 - [(moisture) + (total proteins) + (total lipids) + (crude ash)]. The starch content was determined according to the NF EN ISO 10520 standard.

2.2.1.4. Crude ash content and moisture content

The crude ash content was obtained after incineration of the organic material and weighing of the residue. For moisture, the 100 g test sample was dried at 70°C and the loss of mass was determined by weighing.

2.2.2. Solubility of seed proteins

Protein solubility of *Citrullus lanatus*, *Vigna unguiculata*, and *Zea mays* seeds was evaluated on protein suspensions at 2% content at pH 3, 4, 5, 6, 7, and 8. Protein content was determined by the Kjeldahl method on the supernatant after centrifugation at 15 000 G for 10 minutes.

2.3. Characterization of coagulating solutions of seed powders

2.3.1. Characterization of proteins and peptides by SE-HPLC in the range 10 kDa to 500 kDa and 204 Da to 35 000 Da

The steric exclusion high performance liquid chromatography (SE-HPLC) profile of the coagulant solutions of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays* seed powders was performed with an Alliance HPLC (Waters) equipped with a UV diode detector. 2 columns were used: a Superdex 200 column with a lower range of 10 kDa and an upper range of 500 kDa; and an INTERCHIM Shodex Asahipak GF-310HQ column with a lower range of 204 Da and an upper range of 35 000 Da. After solubilization, 30 µL of solution was injected. Separation was performed at a flow rate of 0.4 mL / min. Detection was performed at 214 nm. A calibration curve was performed under the same conditions using standards of known molecular weight. The results are then expressed as relative percentages of proteins in each molecular weight range.

2.3.2. Characterization of proteins by SDS PAGE electrophoresis

Sodium dodecyl sulfate-containing polyacrylamide gel electrophoresis (SDS PAGE) was also performed to characterize proteins in coagulant solutions of *Citrullus lanatus*, *Vigna unguiculata*, and *Zea mays* seed powders. Electrophoresis was performed using BioRad pre-cast 12% polyacrylamide gels under denaturing conditions by addition of sodium dodecyl sulfate. Extracts were diluted in sample buffer according to the LAEMMLI method (277 mM Tris - HCl, pH 6.8). Migration was performed in Tris-Glycine-SDS buffer at pH 8.3 and 180 volts for approximately 45 minutes. BioRad molecular weight standard, conditioned Tris-Tricine 10-20%, from 10 kDa to 250 kDa was used for protein identification. Staining was performed with Coomassie blue, and analyses were performed in duplicate. Further beta-mercaptoethanol treatment was performed to analyze proteins from *Zea mays* seeds.

2.3.3. Characterization of the ionic composition

The ion contents of the coagulating solutions of *Citrullus lanatus, Vigna unguiculata* and *Zea mays* seed powders were determined using a DR 3900 HACH spectrophotometer at the wavelengths indicated in the brackets for the different types of ions. The contents of the following positive ions were determined: aluminum (525 nm), iron III and iron II (510 nm), calcium (423 nm), magnesium (415 nm), zinc (620 nm), sodium (589 nm) and potassium (767 nm). The following negative ions were also determined: nitrates (520 nm), sulfates (650 nm), carbonates (654 nm), bicarbonates (660 nm) and chlorides (480 nm).

2.3.4. Physico-chemical characteristics

The physicochemical parameters that were determined for the coagulating solutions of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays* seed powders are: hydrogen potential (pH), conductivity, general mineralization, density, turbidity and color.

2.3.4.1. Hydrogen potential (pH)

The pH was measured with a HANNA pH meter combined with a reference electrode with a temperature measurement, according to the NF T90-008 standard [18].

2.3.4.2. Conductivity and general mineralization

Conductivity was measured using a HANNA multifunction conductivity meter, with reference to NF EN 27888 and general mineralization was evaluated from the conductivity with reference to NF T 90-111 [19,20].

2.3.4.3. Density, turbidity and color

The density was measured by the densimetric method, using a VWR DURAND densimeter. Turbidity was measured with a Turbiquant 1100 IR turbidimeter, according to the NF EN ISO 7027 standard. Color was determined with a LOVIBOND color comparator, in reference to the NF EN ISO 7887 standard [18, 21].

2.4. Study of the coagulant activity

The study of the coagulant activity consisted in evaluating first the coagulant activity of the solutions of the powders of the seeds of *Citrullus lanatus, Vigna unguiculata and Zea mays,* on samples of raw water of respective turbidities 77,46 NTU and 95,42; 231,8 NTU and 105,2 NTU; 106,4 NTU and 153,9 NTU. Then, we conducted a comparative study of the coagulant activity of the solutions of the powders of the seeds of these three plants with those of the seeds of *Moringa oleifera* and aluminum sulfate; the analysis was carried out on a sample of raw water of turbidity 68.29 NTU.

2.4.1. Description of the sampling area

The seven raw water samples in this study were collected from the Djoué River, a tributary of the Congo River, located south of Brazzaville. Geographic coordinates indicate: 04°18' 34'' South latitude, 015°13' 36'' East longitude and 270 m above sea level. Raw water samples of turbidity 77.46 NTU and 95.42 NTU were collected in April 2021; those of turbidity 231.8 NTU, 105.2 NTU, 106.4 NTU, 153.9 NTU and 68.29 NTU were collected in September and October 2021.

2.4.2. Preparation of the solutions

Seeds of *Citrullus lanatus, Vigna unguiculata, Zea mays* and *Moringa oleifera were* dehulled, dried and ground. For each plant, 100 g of the resulting product was dispersed in 1000 mL of distilled water. The aluminum sulfate solution, was prepared at the concentration of 10 g/L.

2.4.3 Clarification tests on raw water samples of 231.8 NTU, 153.9 NTU, 106.4 NTU, 105.2 NTU, 95.42 NTU and 77.46 NTU

The clarification tests of raw water samples with turbidity 231.8 NTU, 153.9 NTU, 106.4 NTU, 105.2 NTU, 95.42 NTU and 77.46 NTU were performed by Jar-Test (16). For the Jar-Test, 1000 mL of raw water sample was introduced into the beakers of a Lovibond ET 740 flocculator, followed by the addition of different increasing volumes of *Citrullus lanatus* seed powder solutions, for raw water samples of 95.42 NTU and 77.46 NTU ; *Vigna unguiculata*, for raw water samples of 231.8 NTU and 105.2 NTU and *Zea mays* for raw water samples of 153.9 NTU and 106.4 NTU. After rapid agitation of 180 rpm for 3 minutes and slow agitation of 18 rpm for 20 minutes, the treated water samples were subjected to decantation. After 30 minutes of settling, turbidity was measured; turbidity was again measured after filtration of the treated and settled water samples. Three Jar-Tests treatments were performed for each raw water sample treated with the different doses of *Citrullus lanatus, Vigna unguiculata and Zea mays* solutions.

2.4.4 Comparative study of the clarification tests of the raw water sample of 68.29 NTU

The Jar-Test treatment, for the comparative study of the clarification tests of the raw water sample of 68.29 NTU with the solutions of *Citrullus lanatus*, *Vigna unguiculata*, *Zea mays*, *Moringa oleifera* seeds powders and aluminum sulfate was carried out under the operating conditions previously described.

2.5. Statistical analysis

Statistical analysis was performed on the clarification test results of the 68.29 NTU raw water sample by calculating standard deviation using Microsoft Excel 2013 software.

3. RESULTS AND DISCUSSION

3.1 Chemical characterization of the seeds

3.1.1. Chemical composition of the seeds

The results of the study of the chemical composition of *Citrullus lanatus, Vigna unguiculata and Zea* *mays* seeds are presented in Tables 1 and 2. The mean values of crude protein, total lipid, total carbohydrate, total starch, moisture and crude ash contents are presented in Table 1; those of amino acid contents in Table 2. Observation of Table 1 reveals that *Citrullus lanatus* seeds have the highest crude protein content (32.64 g/100 g); they also contain a high total lipid content (50.06 g/100 g); their starch content is less than 0.2 g/100 g. *Vigna unguiculata* seeds have a fairly high protein content (23.49 g/100 g); total starch is the major component (43.50 g/100 g) and the total lipid content is very low (2.50 g/100 g). *Zea mays* seeds have the lowest protein content (10.01 g/100 g); they have the highest starch content (55.20 g/100 g); the total lipid content is low (6.10 g/100 g). These results corroborate the literature data which indicate that total lipids and crude proteins are the major constituents of *Citrullus lanatus* seeds. In contrast, total carbohydrate and crude protein are the major constituents of *Vigna unguiculata* and *Zea mays* seeds; the total starch content is significant in the seeds of these two plant species [22].

Table 1: Chemical composition of *C. lanatus, V. unguiculata* and *Z. mays* seeds

Parameters (g/100g)	*C. lanatus*	*V. unguiculata*	*Z. mays*
Crude protein	32,64	23,49	10,09
Total Lipids	50,06	2,50	6,10
Total carbohydrates	10,26	64,71	76,91
Total starch	<0,2	43,50	55,20
Crude ash	3,87	4,27	1,40
Humidity	3,17	5,03	5,50

Observation of Table 2 shows that the most abundant amino acid in the seeds of the 3 plant species is glutamic acid, a negatively charged side chain amino acid at neutral pH. The respective contents are 6150 mg/100 g in *Citrullus lanatus* seeds; 4030 mg/100 g in *Vigna unguiculata* seeds and 1820 mg/100 g in *Zea mays* seeds.

Aspartic acid, the other negatively charged side chain amino acid at neutral pH, was identified in the seeds of the 3 plant species at contents of 2810 mg/100 g; 2850 mg/100 g and 627 mg/100 g, respectively in the seeds of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays*. Of the amino acids with positively charged side chains at neutral pH identified (Arginine, Lysine and Histidine), Arginine is the most abundant with a content of 5090 mg/100 g in *Citrullus lanatus*, 1800 mg/100 g in *Vigna unguiculata* and 456 mg/100 g in *Zea mays*. The results on amino acid contents in *Citrullus lanatus* seeds are similar to those obtained by Ketevi and al where arginine (15.69%); glutamate (13.10%) and aspartate (9.41%) are the most abundant amino acids [23]. The average value of lysine content that we observed in *Vigna unguiculata* seeds, is close to that quoted in the literature (1591 mg/100 g), [22]. For *Zea mays*, the lysine content obtained is low; this result corroborates that reported by Semassa and al who indicated low lysine contents in *Zea mays* seeds [24].

Table 2: Amino acid contents of *C. lanatus, V. unguiculata* and *Z. mays* seeds

Amino acids (mg/100g)	*C. lanatus*	*V. unguiculata*	*Z. mays*
Amino acids with positively charged side chains at neutral pH (basic amino acids)			
Histidine	835	725	292
Lysine	963	1650	245
Arginine	5090	1800	456
Amino acids with negatively charged side chains at neutral pH (acidic amino acids)			
Aspartic acid	2810	2850	627
Glutamic acid	6150	4030	1820
Amino acids with uncharged side chains at neutral pH but polar			
Serine	1630	1240	482
Threonine + cystine	1460	917	346
Asparagine	503	223	217
Glutamine	1030	760	354
Amino acids with uncharged side chains at neutral pH and non-polar			
Glycine	1970	918	350
Alanine	1510	1010	731
Valine	1390	1150	467
Leucine	2180	1820	1240
Isoleucine	1220	982	319
Methionine	872	321	200
Proline	1160	970	872
Phenylalanine	1720	1310	481
Tryptophan	518	271	72,4

3.1.2. Solubility of seed proteins

The results of the protein solubility study of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays* seeds are presented in Figure 1. The proteins of *Zea mays* and *Citrullus lanatus* seeds are poorly soluble between pH 3 and pH 8; 10% of the proteins are soluble, which corresponds to metabolic proteins of the albumin type and peptides. *Vigna unguiculata* seeds have a high protein solubility at pH 7 and 8 (60%). Only 18 to 20% of the proteins are soluble at pH 4 and 5. This study showed that pH has little influence on the degree of protein extraction from *Zea mays* and *Citrullus lanatus* seeds. In contrast, protein extraction from *Vigna unguiculata* seeds is sensitive to pH. Adjusting the pH to 7-8 could improve the degree of protein extraction for *Vigna unguiculata* seeds. The results obtained with *Vigna unguiculata* seeds are similar to those obtained for *Arachis hypogaea* seeds where the solubility of seed proteins reaches 70% at pH 7 and 8 [14]. The native pH values for solutions of the powders of these seeds are 5.60; 5.55 and 5.38 for *Zea mays*, *Citrullus lanatus* and *Vigna unguiculata* respectively. In the literature, we distinguish according to their solubility properties, 4 major families of seed proteins: albumins, globulins, prolamins and glutelins [25, 26].

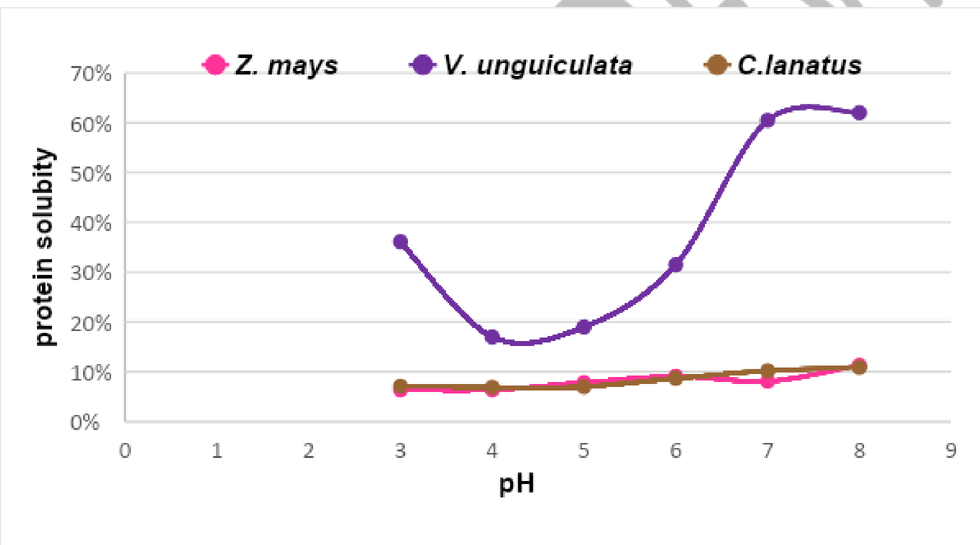


Figure1. Protein solubility of *C. lanatus*, *V. unguiculata* and *Z. mays* seeds at different pH values.

3.2 Characterization of coagulating solutions of seed powders

3.2.1. Characterization of the protein and peptide composition

The results of the steric exclusion high performance liquid chromatography (SE-HPLC) analyses are presented in Tables 3 and 4 and Figures 2 and 3. Observation of Table 3 shows that the percentage of protein constituents with molecular mass less than 10 kDa is 87.3%; 74.1% and 61.6%; respectively for *Citrullus lanatus*, *Zea mays* and *Vigna unguiculata* coagulant solutions. Table 4 shows that the percentage of protein constituents with a molecular weight between 1000 Da and 1500 Da is 24.9%; 20.4% and 29.2%; respectively for the coagulant solutions of *Vigna unguiculata*, *Zea mays* and *Citrullus lanatus*. A percentage of 25.8% is also noted, between 800 and 1000 Da, for *Citrullus* *lanatus*. Figure 2 shows that in the range of 10 kDa to 500 kDa, little protein is observed in the aqueous extract of *Zea mays*. 3 peaks are observable at 42 min, 48 min and 65 min for the aqueous extract of *Vigna unguiculata* seeds. They could correspond respectively to analogues of legumins (hexamers of 250 - 300 kDa), vicilin (trimer of 150 kDa) and peptides (~ 10 kDa). Two peaks are observable at 60 minutes and 65 minutes for the aqueous extract of *Citrulus lanatus* seeds. The majority peak corresponds to small peptides. Figure 3 shows that in the range of 204 Da to 35000 Da, relatively few peptides are observed for *Zea mays* and *Vigna unguiculata*. A larger peak is observed for *Citrullus lanatus* around 42 minutes, that is about 1000 Da.

The SE-HPLC results are in agreement with those of SDS PAGE electrophoresis of *Vigna unguiculata*, *Citrullus lanatus*, *Zea mays* seeds and aqueous extracts of the seeds of these 3 plants obtained after decantation and centrifugation presented by Figure 4. The seeds of *Vigna unguiculata* possess a set of protein subunits typical of legumes; vicilin analogues (~ 50 kDa) and legumins (30 - 35 kDa) are observed. *Citrullus lanatus* seeds have a different profile, with protein subunits present around 45 kDa, 35 kDa, 20 - 25 kDa and a large proportion of small proteins and peptides (<10 kDa). Large subunits are not very present in the aqueous extracts obtained after decantation and centrifugation. Small proteins and peptides with molecular masses less than 10 kDa are predominantly extracted. For *Zea mays*, the different *Zea mays* proteins (zeins) around 50 kDa, 20 - 25 kDa, 17 kDa and 10 kDa are observed in the seeds. These proteins are very poorly soluble in water and are absent from aqueous extracts.

In the literature, the work of some authors has shown the coagulant activity of proteins extracted from plant species, in the clarification of surface water [27,28]. Bodlund and al, have highlighted coagulating proteins of molecular masses 6.5 kDa and 9 kDa in Mustard seed extracts [29]. Arunkumar and al, isolated a protein with coagulant activity of 12 kDa from the seeds of *Strychnos potatorum* [30].

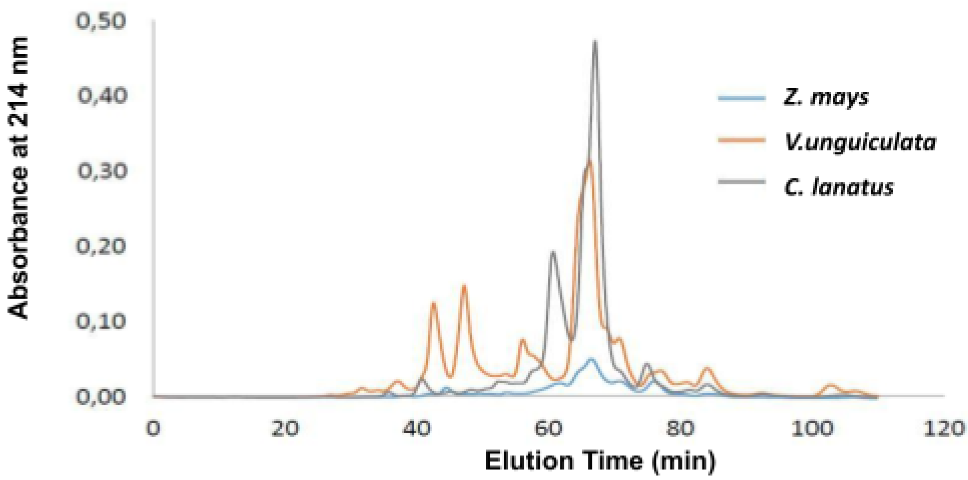


Figure 2. HPLC profiles of aqueous extracts - 10 kDa to 500 kDa

Figure 3. HPLC profiles of aqueous extracts 204-35000 Da

Table 3. Relative distribution (%) of protein molecular masses between 10 kDa and 500 kDa

Molecular weights (kDa)	*Z. mays*	*V.unguiculata*	*C. lanatus*
> 500	5,1	2,6	0,3
300 - 500	0,8	1,6	0,3
100 - 300	6,0	10,3	2,4
80 - 100	1,2	3,2	0,3
60 - 80	1,4	6,2	0,5
35 - 60	2,7	3,7	1,2
18 - 35	3,9	4,5	3,0
10 - 18	4,7	6,3	4,6
< 10	74,1	61,6	87,3

Table 4. Relative distribution (%) of molecular masses of proteins between 204 and 35 000 Da

Molecular weight (Da)	*Z. mays*	*V.unguiculata*	*C. lanatus*
35 000 - 18 000	6,5	1,0	4,0
18 000 - 10 000	4,6	2,5	4,3
10 000 - 6 000	4,0	3,4	4,9
6 000 - 3 000	8,8	5,3	3,9
3 000 - 2 500	5,3	3,2	1,0
2 500 - 2 000	10,3	7,8	1,7
2 000 - 1 500	14,7	14,7	2,4
1 500 - 1 000	20,4	24,9	29,2
1 000 - 800	7,8	7,0	25,8

800 - 500	17,1	19,6	19,0
500 - 204	0,4	10,6	3,7

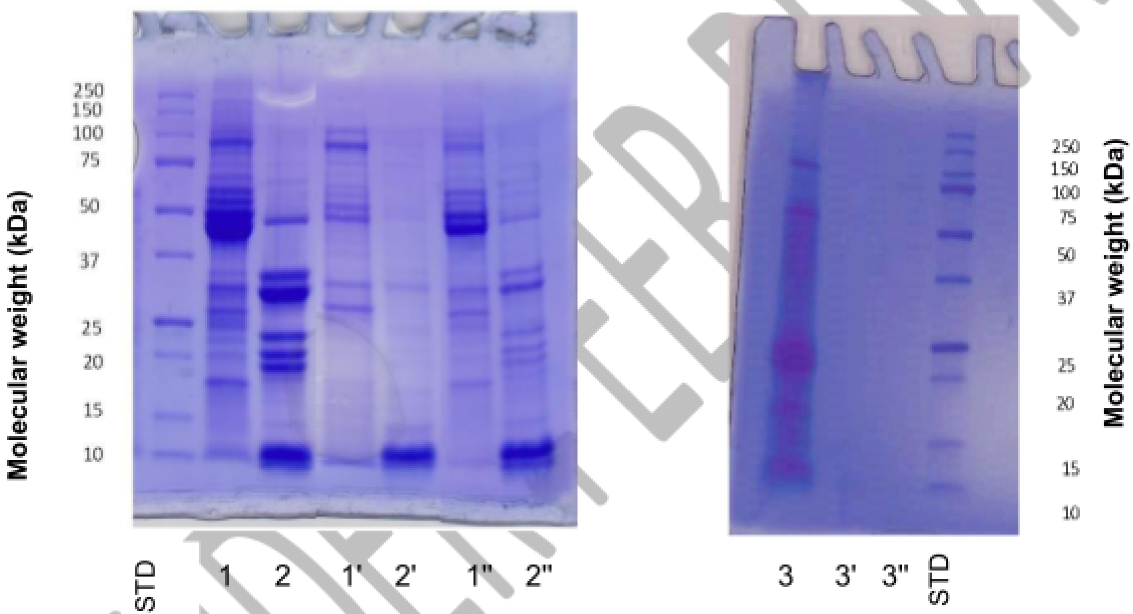


Figure 4. SDS PAGE electrophoresis profiles of aqueous extracts

1: Seeds of *V. unguiculata*	2: Seeds of *C. lanatus*	3: Seeds of *Z. mays*
1' : Decanted extract of *V. unguiculata*	2' : Decanted extract of *C. lanatus*	3' : Decanted extract of *Z. mays*
1" :Centrifugal extract of *V. unguiculata*	2" : Centrifuged extract of *C. lanatus*	3" : Centrifuged extract of *Z. mays*

3.2.2. Characterization of the ionic composition

The results of the ionic composition of the solutions of *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays* seed powders are presented in Table 5 . The abundant positive ions in the 3 solutions are Calcium and Sodium ions, with the respective values of 213 mg/L and 96.87 mg/L, for *Citrullus lanatus*; 246.09 mg/L and 125.37 mg/L, for

Vigna unguiculata ; 124.64 mg/L and 50.96 mg/L for *Zea mays*. Aluminum ion contents are negligible and are 0.20 mg/L; 0.26 mg/L and 0.10 mg/L, respectively for *Citrullus lanatus, Vigna unguiculata* and *Zea mays* coagulant solutions. Iron III ion contents are also negligible and 0.03 mg/L; 0.10 mg/L and 0.15 mg/L in *Citrullus lanatus*, *Vigna unguiculata* and *Zea mays* coagulant solutions. These results are similar to those obtained for the coagulating solution of *Moringa oleifera* seeds, where the contents of aluminum and iron III ions are negligible and 1.52 mg/L and 0.26 mg/L, respectively [13].

Table 5: Ionic composition of aqueous extract of *C. lamatus*, *V. unguiculata* and *Z. mays*

Ion composition	*C. lanatus*	*V. unguiculata*	*Z. mays*
	Composition of positive ions		
Aluminium	0,20	0,26	0,10
Iron III	0,03	0,10	0,15
Iron II	0,14	0,40	0,60
Calcium	213	246,09	124,64
Magnesium	43,12	49,82	25,23
Zinc	0,54	0,70	0,28
Sodium	96,87	125,37	50,96
Potassium	17,51	22,65	9,21
	Composition of negative ions		
Nitrate	0,0	0,0	0,0
Sulfate	26,08	33,75	13,72
Carbon	0,0	0,0	0,0
Bicarbonate	323,30	732	3,05
Chloride	309,24	291,61	106,30

3.2.3. **Physico-chemical characteristics**

Table 6 gathers the results of the physico-chemical parameters, of the solutions of powder of seeds of Citrullus lanatus, Vigna unguiculata and Zea mays. The examination of this table reveals that the 3 solutions are acidic, with pH values of 5.55; 5.38 and 5.60 respectively for the solutions of Citrullus lanatus, Vigna unguiculata and Zea mays seeds powder. Conductivity and general mineralization are higher for the powder solution of Vigna unguiculata seeds , with respective values of 2411 µS/ cm and 2.285 g / L.

Table 6. Physico-chemical parameters of extact of *C. lanatus*, *V. unguiculata* and *Z. mays*

Physico-chemical parameters	*C.lanatus*;	*V.unguiculata*	Z.mays
pH at 20°C	5,55	5,38	5,60
Conductivity at 20°C	1863	2411	980
General mineralization ($g.L^{-1}$)	1,76	2,285	0,93
Turbidity (NTU)	92,61	37,10	38,02
Color (mg/LPto)	10	2,50	1,25
Density at 15°C	1,012	1,013	1,003

3.3. Study of the coagulant activity

3.3.1. Identification of the coagulant activity

The addition of different doses of the solutions of the three plants caused a decrease in turbidity, for each treatment that was performed. The decrease in turbidity is 231.8 NTU to 4.99 NTU and 105.2 NTU to 4.94 NTU, a reduction in turbidity of 97.84% and 95.30% respectively for *Vigna unguiculata* (Table 7b). For *Zea mays*, the decrease is 153.9 NTU to 4.39 NTU and 106.4 NTU to 4.95 NTU, a reduction in turbidity of 97.14% and 95.34% respectively (Table 7a). For *Citrullus lanatus*, the turbidity reduction was 95.42 NTU to 18.11 NTU and 77.46 NTU to 19.36 NTU (Table 7c). After filtration of the treated and decanted water samples, the turbidities of 4.99 NTU, 4.94 NTU, 4.39 NTU, 4.95 NTU, 18.11 NTU, and 19.36 NTU decreased to 2.45 NTU, 3.56 NTU, 2.91 NTU, 2.78 NTU, 7.11 NTU, and 8.36 NTU respectively. For *Vigna unguiculata* and *Zea may*, these residual turbidity values are consistent with the WHO recommended turbidity value for drinking water, which is a residual turbidity value less than or equal to 5 NTU. For *Citrullus lanatus*, the residual turbidity values obtained are close to the value recommended by the WHO.

These results show an elimination of the turbidity of water by the solutions of seed powder of *Vigna unguiculata*, *Citrullus lanatus* and *Zea mays*. This elimination of turbidity can be explained by the fact that the solutions of the seed powders of the three plants caused coagulation, which is the neutralization of colloidal particles, responsible for the turbidity of water [31]. These results therefore highlight the coagulant activity of the seeds of *Vigna unguiculata*, *Citrullus lanatus* and *Zea mays* in the clarification of surface water. The coagulant activity of *Citrullus lanatus* has already been reported by Singh [32]; that of *Vigna unguiculata* by Jayalakshmi [33] and that of *Zea mays* by Sasikala [34].

3.3.2. Comparative study of coagulant activity

The results of the raw water clarification tests of turbidity 68.29 NTU with the solutions of *Citrullus lanatus*, *Vigna unguiculata*, *Zea mays*, *Moringa oleifera* and aluminum sulphate are presented in figure 5. The observation of this figure shows the variation of turbidity with different doses of coagulant solutions. The optimal dose for each coagulant corresponds to the minimum of the curve obtained. It is 4 mg/L for aluminum sulfate (Fig. 5 e), 400 mg/L for *Moringa oleifera* (Fig. 5 d), 3000 mg/L, 3800 mg/L and 7000 mg/L for *Citrullus lanatus* (Fig.5 a), *Vigna unguiculata* (Fig. 5 b) and *Zea mays* (Fig.5 c) respectively.

Table 7. Turbidity reduction percentages, residual turbidities and optimal doses of coagulant solutions

(a)

Initial turbidities (NTU)	Treatments with *Zea mays* powder solution				
	Residual turbidities		% turbidity removal		Optimal dose (mg/L)
	Coagulated and decanted water	Coagulated, decanted and filtered water	Coagulated and decanted water	Coagulated, decanted and filtered water	
106,4	4,95	2,78	95,34	97,38	10000
153,9	4,39	2,91	97,14	98,10	11000

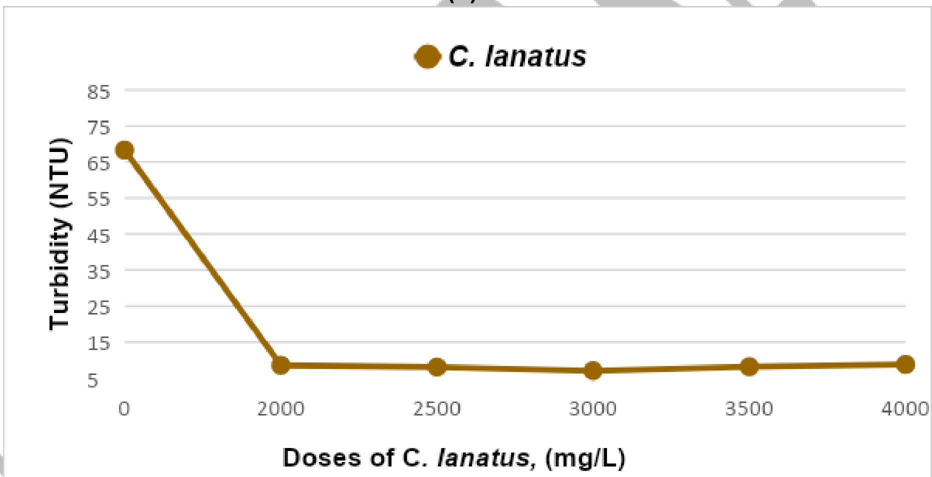
(b)

Initial turbidities (NTU)	Treatments with the solution of *Vigna unguiculata* powder				
	Residual turbidities		% turbidity removal		Optimal dose (mg/L)
	Coagulated and decanted water	Coagulated, decanted and filtered water	Coagulated and decanted water	Coagulated, decanted and filtered water	
231,8	4,99	2,45	97,84	98,94	5000
105,2	4,94	3,56	95,30	96,61	3800

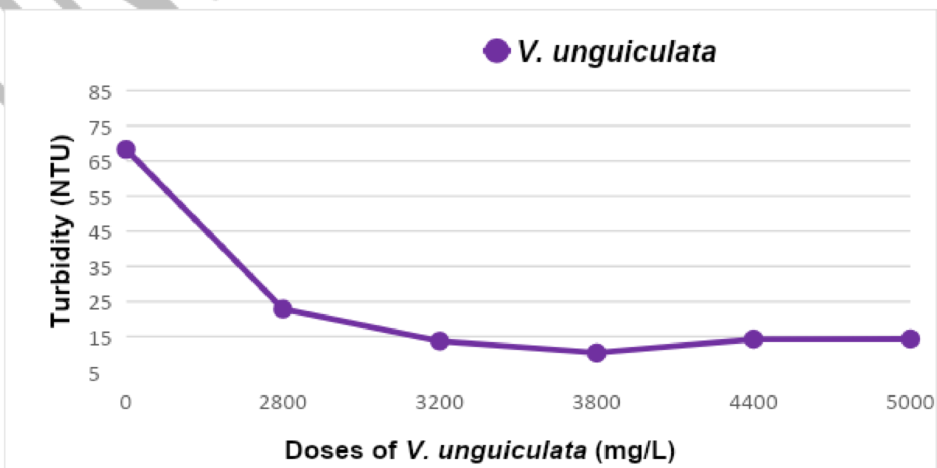
(c)

Initial turbidities (NTU)	Treatment with *Citrullus lanatus* powder solution				
	Residual turbidities		% turbidity removal		Optimal dose (mg/L)
	Coagulated and decanted water	Coagulated, decanted and filtered water	Coagulated and decanted water	Coagulated, decanted and filtered water	
77,46	19,36	8,36	75,01	89,21	4500
95,42	18,11	7,11	81,02	92,55	4500

(a)



(b)



(c)

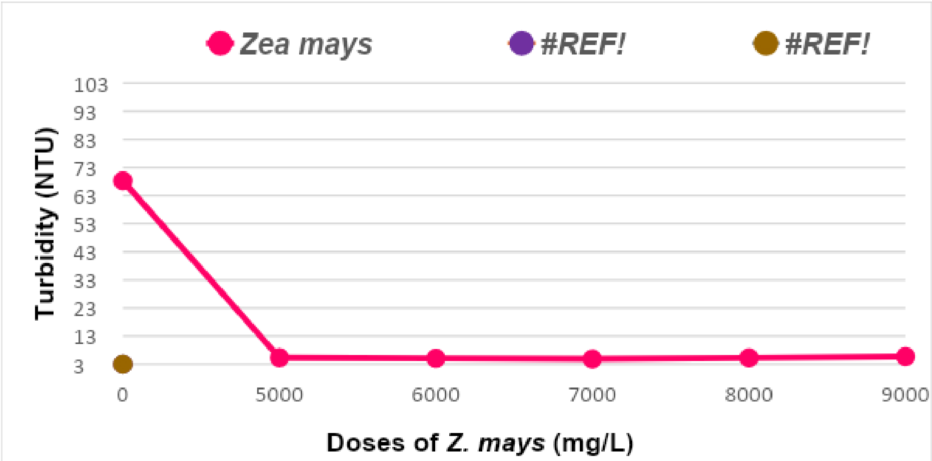
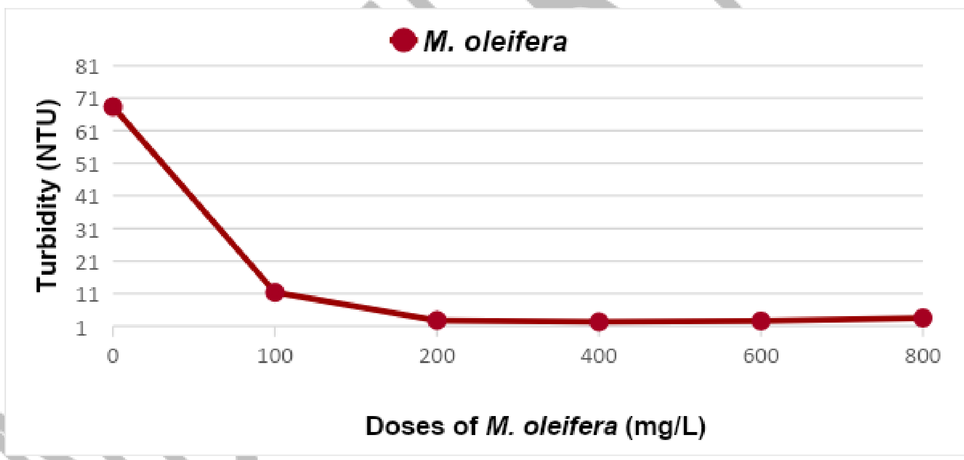


Figure 5: Turbidity variation of the raw water sample of 68.69 NTU as a function of coagulant solution doses

(d)



(e)

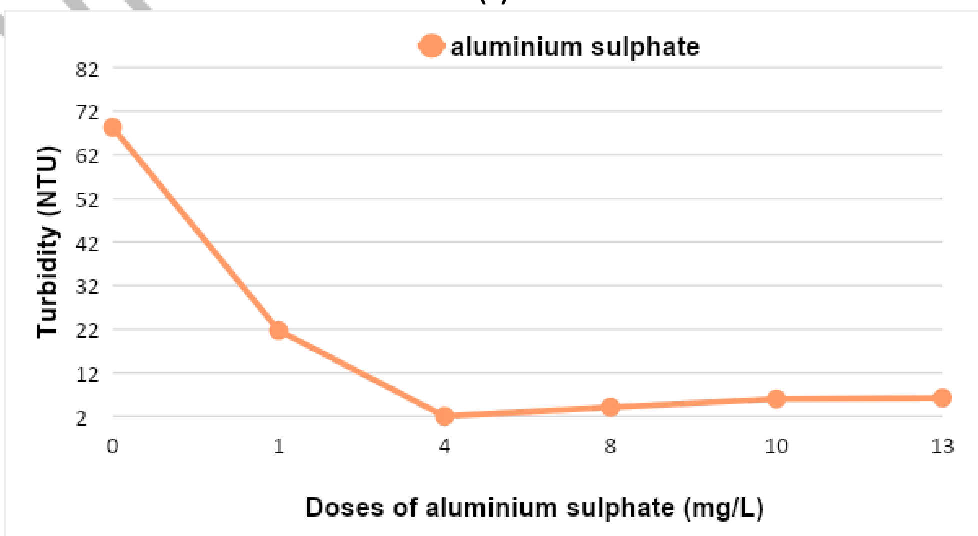


Figure 6: Turbidity variation of the raw water sample of 68.69 NTU as a function of coagulant solution doses

3.4. Statistical analysis

Statistical analysis of the results of the clarification tests of raw water samples of turbidity 68.29 NTU showed for *Citrullus lanatus* a dispersion of 0.02; for *Vigna unguiculata* and *Zea mays* a dispersion is 0.01 for turbidity.

4. CONCLUSION

The results of this study showed that the most abundant amino acid in *Citrullus lanatus*, *Vigna unguiculatas* and *Zea mays* seeds is glutamic acid, a negatively charged side chain amino acid at neutral pH. The respective contents are 6150 mg/100 g in *Citrullus lanatus* seeds; 4030 mg/100 g in *Vigna unguiculata* seeds and 1820 mg/100 g in *Zea mays* seeds. The results of SE-HPLC analyses showed that the percentage of protein constituents with molecular mass less than 10 kDa is 87.3%; 74.1% and 61.6%; respectively for the coagulant solutions of *Citrullus lanatus*, *Zea mays* and *Vigna unguiculata*. The study of the coagulant activity showed percentages of turbidity reduction higher than 80%.

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