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# Chemical characterization of the coagulating solutions of the powders of the seeds of *Arachis hypogaea* L., *Cucumeropsis mannii* Naud. and *Moringa oleifera* Lam.

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## Abstract

*The aim of this study is to characterize the proteins in the coagulating solutions of the powders of the seeds of Arachis hypogaea L., Cucumeropsis mannii Naud. and Moringa oleifera Lam. Analyzes were performed using high performance size exclusion liquid chromatography (SE-HPLC) on a Superdex column in the range of 10kDa to 500kDa and on a Shodex column in the range of 204Da to 35000Da. Electrophoresis on polyacrylamide gel containing sodium dodecyl sulfate (SDS PAGE) was also performed. The results obtained showed that the coagulating solutions of C. mannii and M. oleifera mainly contain small proteins, of which 75.4% and 94.4% have molecular masses of less than 10kDa. The coagulating solution of A. hypogaea, on the other hand mainly contains large proteins, of which 25.4% have molecular masses between 100kDa and 300kDa, 16.8% between 300kDa and 500kDa and 16.8% have molecular masses greater than 500kDa.*

**Keywords:** *Arachis hypogaea*, *Cucumeropsis mannii*, *Moringa oleifera*, Seeds, Coagulants, Proteins.

## Introduction

In water treatment, coagulation is the destabilization of indecantable colloidal particles, contained in raw water, by neutralizing their negative charges by the addition and rapid dispersion of a chemical reagent providing positive charges and called coagulant[1]. The coagulation step is followed by the flocculation step which is carried out with the aim of allowing the agglomeration of the destabilized colloids into voluminous and settable flakes. Coagulation / flocculation processes eliminate turbidity from the water, but also microbiological germs that can hide in colloidal particles[2]. The coagulant can be of mineral or organic origin[3,4]. Apart from synthetic organic coagulants, several studies have demonstrated the coagulant activity of natural substances of plant origin[5-8]. In rural areas of developing countries, the problem of water supply arises[9,10] and 97% of the population does not have piped water supply[11,12]. These populations sometimes use water that can come from rivers, streams, ponds and wells for household chores. The quality of these waters can be improved by treatment at home with natural substances of plant origin with coagulant activity[13-15]. Objective 6, one of the 17 Sustainable Development Goals adopted in 2015 as part of the United Nations 2030 agenda, suggests "to ensure the availability and sustainable management of water and sanitation for all"[16]. As part of the implementation of this objective, we are undertaking investigations on the search for natural coagulants of plant origin, to obtain 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 contribute to improving water quality for populations in rural areas of developing countries[17-19]. In this study, the species *Arachis hypogaea* L. and *Cucumeropsis mannii* Naud. have been studied. *Moringa oleifera* Lam., Was used as a reference in this study because of its coagulant activity proven in the clarification of surface water, due to cationic polyelectrolytes of the polypeptide type[20,21]. Aluminum sulfate, which is the most common mineral coagulant in the treatment of water intended for human consumption, is also used as a reference in this study[22]. Our previous work has shown the coagulant activity of the powder solutions of the seeds of *A. hypogaea* and *C. mannii* in the clarification of surface water samples of initial turbidities 89.45NTU, 94NTU and 128.60 NTU[23]. This study aims to characterize the proteins in the coagulating solutions of the powders of the seeds of these two plants.

## Materials and methods

**Plant material:** The plant material consists of the seeds of *A. hypogaea*, *C. mannii*, and *M. oleifera*. The harvest was carried out in November of the year 2018. The seeds of *A. hypogaea* and *C. mannii* were collected near the village Mboulankio located 45 kilometers north of Brazzaville and those of *M. oleifera*, in the district n ° 1 of the city of Brazzaville.

**Preparation of solutions:** The seeds of *A. hypogaea*, *C. mannii* and *M. oleifera* were shelled, dried and crushed. For each plant, 100g of the product obtained was dispersed in 1000mL of distilled water. The aluminum sulphate solution was prepared at a concentration of 10g/L.

**Study of coagulant activity: Collection of surface water samples:** The 131 NTU and 32.75 NTU turbidity surface water samples were collected respectively from the Djoué River in November 2018 and from the Congo River in June 2019. The geographic coordinates indicate 04°18'34" South latitude, 015°13'36" East longitude and 270 m altitude above the sea, for the Djoué River and 04°18'55" South latitude, 015°12'34" East longitude and 256m of altitude above the sea for the Congo river.

**Clarification testing of 131 NTU and 32.75 NTU turbidity surface water samples:** Clarification testing of raw water samples with 131 NTU and 32.75 NTU turbidity was performed by Jar-Test[22]. For the performance of the Jar-Test, 1000mL of raw water sample was introduced into the beakers of a Lovibond ET 740 flocculator, followed by the addition of different increasing volumes of the powder solutions of the seeds of *A. hypogaea*, *C. mannii*, *M. oleifera* and aluminum sulfate. After stirring rapidly at 180 rotations per second for 3 minutes and slowly stirring at 18 rotations per second for 20 minutes, the treated water samples were subjected to decantation. After 30 minutes of decantation, followed by filtration, the residual turbidity was measured for each beaker with a Turbiquant 1100 R turbidimeter. Three Jar-Test treatments were carried out for each sample of raw water treated with the different doses of the solutions. Powder from the seeds of *A. hypogaea*, *C. mannii*, *M. oleifera* and aluminum sulfate.

**Characterization of coagulant solutions: Characterization of the protein and peptide composition:** The protein and peptide compositions of the coagulating solutions of the powders of the seeds of *A. hypogaea*, *C. manni* and *M. oleifera* were determined using high performance size exclusion liquid chromatography (SE-HPLC). The molecular mass profiles of the solutions of the powders of the seeds of the 3 plants were characterized by SE-HPLC on a Superdex column allowing the separation of proteins between 10kDa and 500kDa; and on a Shodex column allowing the separation of peptides between 204 Da and 35000Da. Electrophoresis on polyacrylamide gel containing sodium dodecyl sulfate (SDS PAGE) was also performed to characterize the proteins of the coagulating solutions of the powders of the seeds of the 3 plants.

**Characterization of proteins by SE-HPLC in the range 10 kDa to 500kDa:** The profile of the high performance size exclusion liquid chromatography (SE-HPLC) of the coagulant solutions of the powders of the seeds of the 3 plants, in the range 10kDa to 500kDa, was carried out with an HPLC Alliance (Waters) equipped with a UV diode detector. A Superdex 200 column with a lower range of 10 kDa and an upper range of 500 kDa, was used. After solubilization, 30µL of solution were injected. The separation was carried out at a flow rate of 0.4mL /min. Detection was performed at 214nm (peptide bond). A calibration curve was performed under the same conditions using standards of known molecular weights. The results are then expressed as relative percentages of proteins of each range of molecular weights.

**Characterization of peptides by SE-HPLC in the range 204 Da to 35000Da:** The profile of the high-performance size exclusion liquid chromatography (SE-HPLC) of the coagulant solutions of the powders of the seeds of the 3 plants, in the range 204Da to 35,000Da was carried out with an HPLC Alliance (Waters) equipped with a UV diode detector. An INTERCHIM Shodex Asahipak GF-310HQ column with a lower range of 204Da and an upper range of 35000Da was used. After solubilization and filtration, 30µL of solution were injected. The separation was carried out at a flow rate of 0.4mL / min. Detection was performed at 214nm (peptide bond). A calibration curve was performed under the same conditions using standards of known molecular weights. The results are expressed as the relative percentages of protein of each molecular weight range.

**Characterization of proteins by SDS PAGE electrophoresis:** Electrophoresis on polyacrylamide gel containing sodium dodecyl sulphate (SDS PAGE) was also performed to characterize the proteins of the coagulating solutions of the powders of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera*. The electrophoresis was carried out using 12% acrylamide gels of pre-cast BioRad polyacrylamide under denaturing conditions, by addition of sodium dodecylsulfate. The extracts were diluted in sample buffer according to the LAEMMLI method (277mM Tris-HCl, pH 6.8). Migration was carried out in Tris-Glycine-SDS buffer at pH 8.3 and voltage 180 volts for approximately 45 minutes. The BioRad molecular weight standard, conditioned 10-20% Tris-Tricine, 10 kDa to 250 kDa was used for protein identification. Staining was performed using Coomassie blue and analyzes were performed in duplicate.

**Characterization of the ionic composition:** The ion contents of the coagulating solutions of the powders of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera* 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 (525nm), fer III and fer II (510nm), calcium (423 nm), magnesium (415nm), zinc (620nm), sodium (589nm) and potassium (767nm). The contents of the following negative ions were also determined: nitrates (520nm), sulfates (650nm), carbonates (654nm), bicarbonates (660nm) and chlorides (480 nm).

**Physico-chemical characteristics:** The physicochemical parameters which have been determined for the coagulating solutions of the powders of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera* are: hydrogen potential (pH), conductivity, general mineralization, density, turbidity and color.

**Hydrogen potential (pH):** The pH was measured with a HANNA pH meter combined with a reference electrode with a temperature measurement, according to standard NF T90-008[24].

**Conductivity and general mineralization:** The conductivity was measured using a HANNA multifunction conductivity meter, with reference to standard NF EN 27888 and general mineralization was evaluated from conductivity with reference to standard NF T 90-111[2,25].

**Density, turbidity and color:** The density was measured by the densimetric method, using a VWR DURAND densimeter. Turbidity was measured using a Turbiquant 1100 IR turbidimeter, according to standard NF EN ISO 7027. The color was determined using the LOVIBOND color comparator, with reference to standard NF EN ISO 7887[24,26].

**Statistical analysis:** Statistical analysis was performed on the clarification test results of 131NTU and 32.75NTU turbidity surface water samples, by the standard deviation calculation using Microsoft Excel 2013 software.

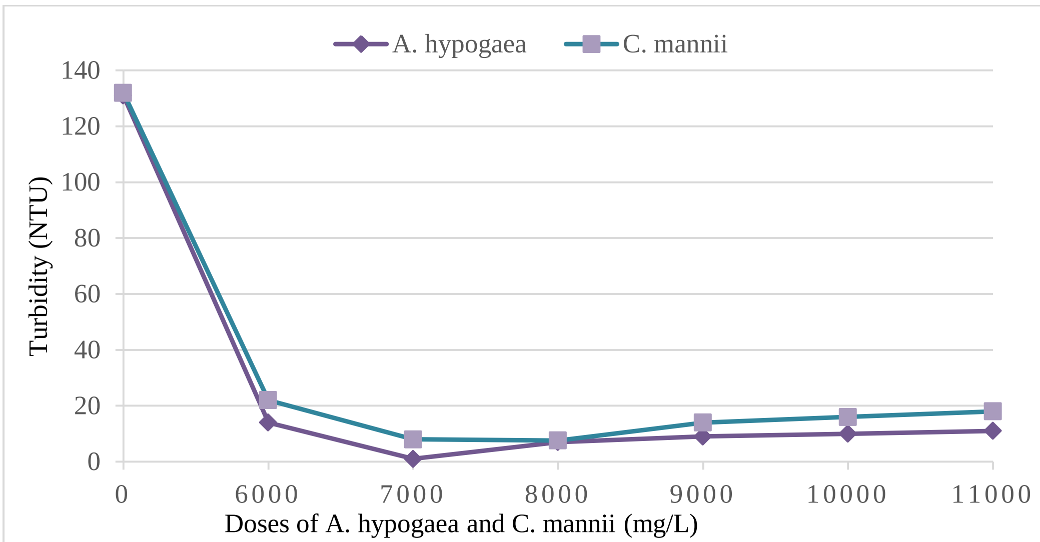
## Results and discussion

**Study of coagulant activity:** The results of the Jar-Test clarification tests of raw water samples with 131NTU and 32.75NTU turbidity with the seed powder solutions of *A. hypogaea* and *C. mannii* are shown in Figures 1a and 2a. Observation of these figures shows a decrease in turbidity from 131NTU to 1NTU; and from 32.75NTU to 0.15NTU for treatment with the *A. hypogaea* powder solutions. Treatment with *C. mannii* powder solutions shows a decrease in turbidity from 131NTU to 0.6NTU; and from 32.75NTU to 0.25NTU. The percent reduction in turbidity for the *A. hypogaea* seed powder solution was 99.23% and 99.54% for the raw water samples of 131NTU and 32.75NTU, respectively. For the *C. mannii* seed powder solution, the percent reduction in turbidity was 99.54% and 99.24%, for the raw water samples of 131 NTU and 32.75NTU, respectively. The optimal doses for each coagulant correspond to the minimum of each of these curves. They are 7000mg/L for the seed powder solution of *A. hypogaea* and 8000mg/L for the seed powder solution of *C. mannii* (Figures-1a and 2a). Treatment with *M. oleifera* powder solution gave a reduction in turbidity of 98.36% and 99.54%, respectively for the raw water samples of 131 NTU and 32.75 NTU; the respective optimal doses are 1400mg/L and 1800mg / L (Figures-1b and 2b). Treatment with the aluminum sulphate solution gave a reduction in turbidity of 99.90% and 99.79%, respectively for the raw water samples of 131NTU and 32.75 NTU; the respective optimal doses are 14mg/L and 35mg/L (Figures-1c and 2c).The residual turbidity values obtained in this study comply with those recommended by WHO, for drinking water, have residual turbidity values less than or equal to 5 NTU.

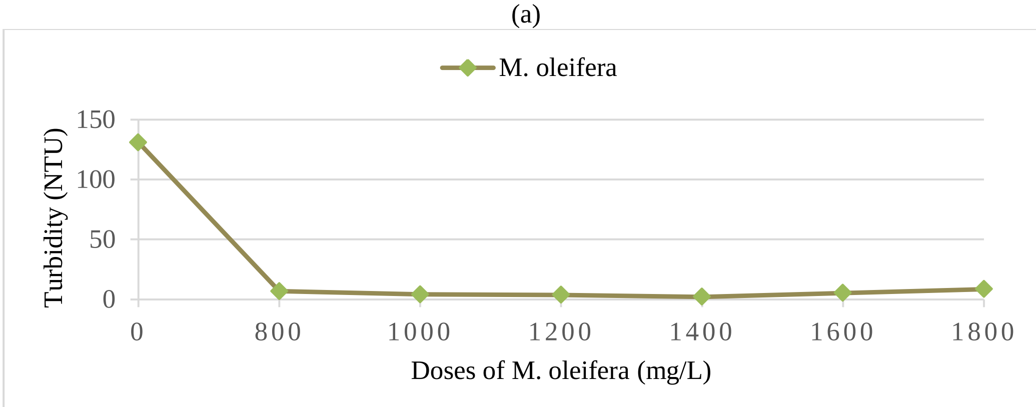
These results show the elimination of turbidity from water samples treated with the seed powder solutions of *A. hypogaea a*nd *C. mannii*. The powder solutions of the seeds of the both plants caused coagulation, which is the neutralization of the colloidal particles responsible for the turbidity of the water. These results therefore demonstrate the coagulant activity of seeds of *A. hypogaea* and *C. mannii* in the clarification of surface water. These results also show the value of seeds of *A. hypogaea* and *C. mannii* in the clarification of surface water. In fact, the percentages of reduction of the turbidity of the solutions of the powders of the seeds of these two plants are close to those of the solution of powder of the seeds of M. oleifera, which is a natural organic coagulant proven at scientific level[20,21] and aluminum sulphate solution which is the most widely used mineral coagulant for the treatment of water intended for human consumption[22]. In the literature, the coagulant activity of *A. hypogaea* has been mentioned by Mbogo and Prasad [27,28].

**Characterization of coagulant solutions: Characterization of the protein and peptide composition:** The results of the analyzes by SE-HPLC are presented in Tables-1 and 2 and in Figure-3 and 4. Observation of Table-1 shows that the percentage of protein constituents having a molecular mass of less than 10kDa is 94.4 %, 75.4% and 14.1%; respectively for the coagulating solutions of *M. oleifera*, *C. mannii* and *A. hypogaea*. The percentage of the protein constituents having a molecular mass between 10kDa and 18kDa is 16.1%, 11.1% and 1.0%; respectively for the coagulating solutions of *C. mannii*, *A. hypogaea* and *M. oleifera*. Table-1 also shows high molecular masses for the protein constituents of the coagulating solution of the powders of the seeds of *A. hypogaea*, of which 25.4% between 100kDa and 300kDa, 16.8% between 300kDa and 500 kDa and 16.8% greater than 500 kDa.

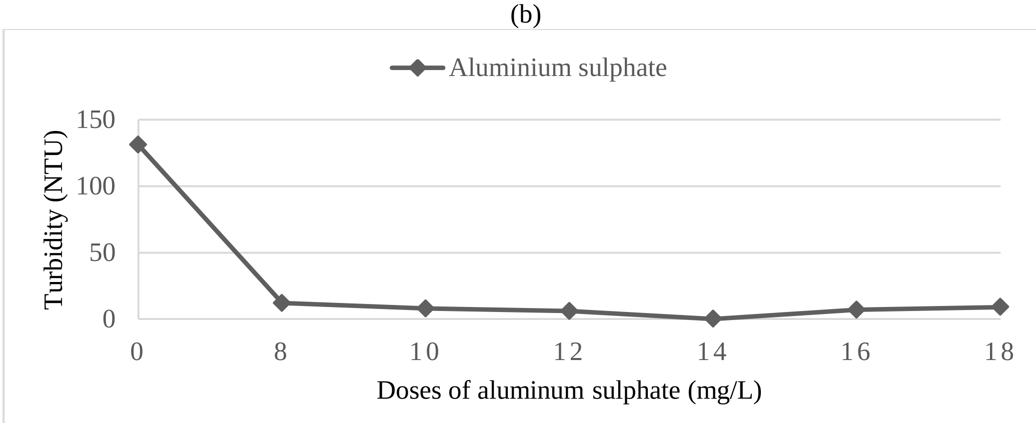
The observation of Figures-3 and 4 shows that for the coagulating solutions of the powders of the seeds of *C. mannii* and of *M. oleifera*, the majority peaks are obtained around 40 minutes of elution on the Shodex column and 45 to 50 minutes of elution on the Superdex column; which corresponds to peptides with molecular masses of 500Da to 1500Da (Table-2). Some small proteins are also present in the coagulating solutions of the powders of the seeds of these 2 plants. The coagulating solution of the powders of the seeds of *A. hypogaea* also contains some peptides and some small proteins, but the majority of the constituents are large proteins with molecular masses greater than 10kDa (Table-2) and times of shorter elution around 25 minutes on the Shodex column and 30 minutes on the Superdex column (Figures-3 and 4). These results are in agreement with the SDS PAGE analyzes (Figure 5), which show for the coagulating solutions of the powders of the seeds of *C. mannii* and *M. oleifera*, significant bands around 10kDa, which rather corresponds to peptides and small proteins of the albumin type. Less significant bands are also observed at 18kDa and 30kDa. For the coagulating solution of the powders of the seeds of *A. hypogaea*, significant bands are observed around 18kDa, 35kDa and 60kDa, which correspond respectively to the subunits of acid arachine, basic arachine and to conarachine which are proteins of globulin type. Small amounts of proteins with a molecular weight of 10kDa are also present. Survey of the literature has shown the coagulant activity of proteins extracted from plant species, in the clarification of surface water[29,30]. Bodlund et al, demonstrated coagulant proteins with molecular masses 6.5kDa and 9kDa in Mustard seed extracts[29]. Arunkumar et al isolated a 12kDa coagulant protein in the seeds of *Strychnos potatorum*[30].




(a)




(b)




(c)

**Figure-1**: Variation of the turbidity of the raw water sample of 131 NTU according to the doses of the coagulating solutions.

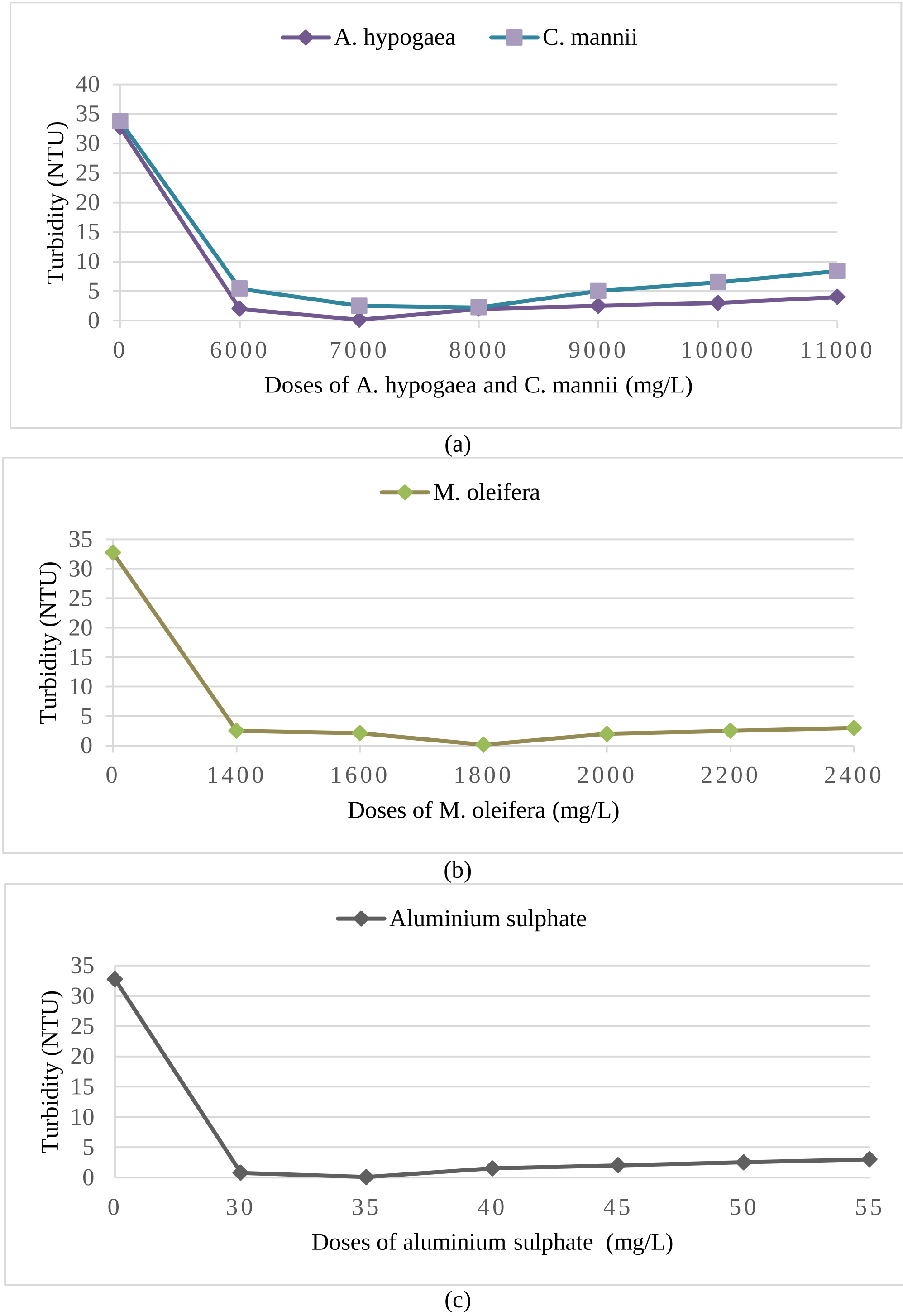



**Figure-2**: Variation of the turbidity of the raw water sample of 32,75 NTU according to the doses of the coagulating solution.

**Characterization of the ionic composition:** The results of the ionic composition of the solutions of the powders of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera* are given in Table-3. The positive ions abundant in the 3 solutions are Calcium and Magnesium ions, with the respective values. 199.17mg/L and 40.32mg/L, for *A. hypogaea*; 151.46mg/L and 30.67mg/L, for *C. mannii*; 512.19mg/L and 103.70mg/L for *M. oleifera*. The contents of aluminum ions are negligible and are 0.15mg/L; 0.68mg/L and 1.52mg/L, respectively for the coagulating solutions of *A. hypogaea*, *C. mannii* and *M. oleifera*. Fer III ions are absent in the coagulating solutions of *A. hypogaea* and *C. mannii*; their negligible content is 0.26mg/L in that of *M. oleifera*.

**Table-1:** Relative distribution (%) of molecular masses between 10 kDa and 500 kDa.

| Molecular weight (kDa) | *C. mannii* | *M. oleifera* | *A. hypogaea* |
|---|---|---|---|
| >500 | 0,2 | 1,3 | 16,8 |
| 300 – 500 | 0,2 | 1,3 | 16,8 |
| 100 – 300 | 3,4 | 0,5 | 25,4 |
| 80 – 100 | 0,8 | 0,1 | 3,2 |
| 60 – 80 | 0,6 | 0,1 | 3,4 |
| 35 – 60 | 1,6 | 0,5 | 5,3 |
| 18 – 35 | 1,8 | 0,8 | 4,1 |
| 10 – 18 | 16,1 | 1,0 | 11,1 |
| <10 | 75,4 | 94,4 | 14,1 |

**Table-2:** Relative distribution (%) of molecular masses between 204 Da and 35 000 Da.

| Molecular weight (Da) | *C. mannii* | *M. oleifera* | *A. hypogaea* |
|---|---|---|---|
| > 35 000 | 11,7 | 0,9 | 42,2 |
| 35 000 – 18 000 | 4,1 | 3,3 | 25,2 |
| 18 000 – 10 000 | 6,0 | 8,4 | 10,1 |
| 10 000 – 6 000 | 2,9 | 1,2 | 3,8 |
| 6 000 - 3 000 | 2,1 | 1,7 | 4,0 |
| 3 000 - 2 500 | 0,8 | 0,6 | 0,8 |
| 2 500 - 2 000 | 1,9 | 0,8 | 1,1 |
| 2 000 - 1 500 | 2,3 | 19,1 | 1,3 |
| 1 500 - 1 000 | 15,4 | 37,8 | 1,6 |
| 1 000 - 800 | 24,3 | 10,2 | 0,8 |
| 800 - 500 | 17,6 | 6,1 | 1,7 |
| 500 - 204 | 3,2 | 7,2 | 1,6 |
| < 204 | 7,8 | 2,9 | 5,6 |

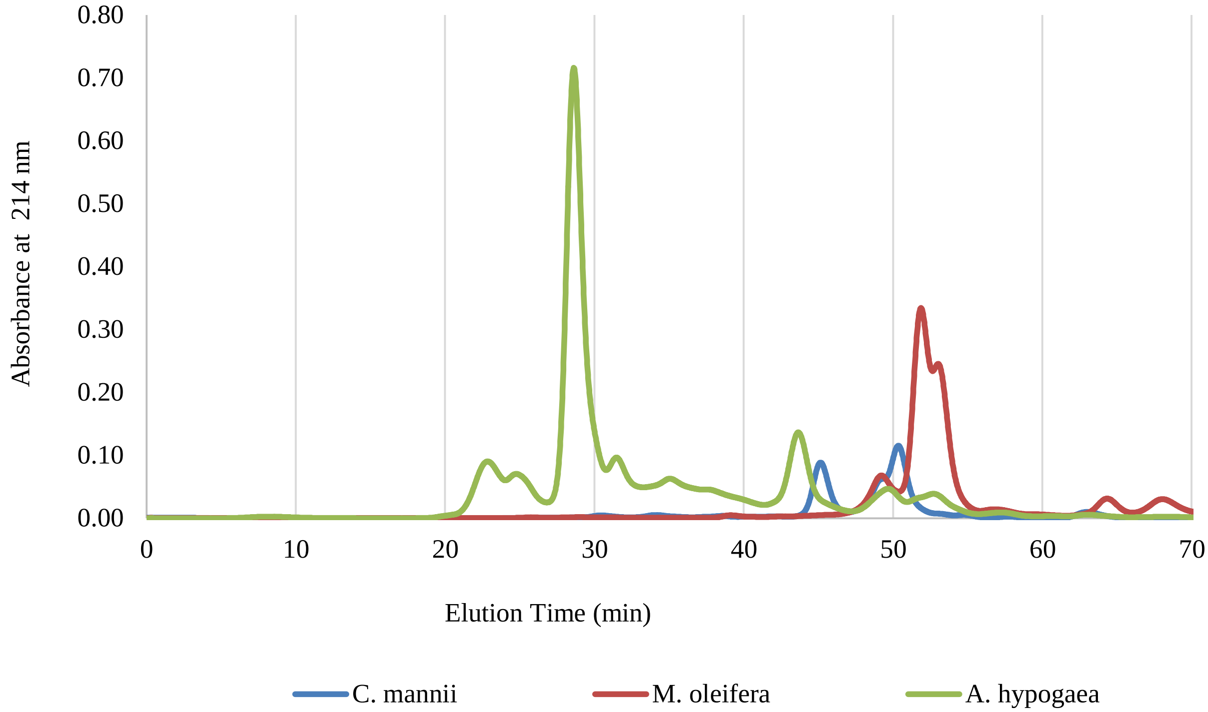



**Figure-3:** SE- HPLC profiles of extracts on Superdex column.

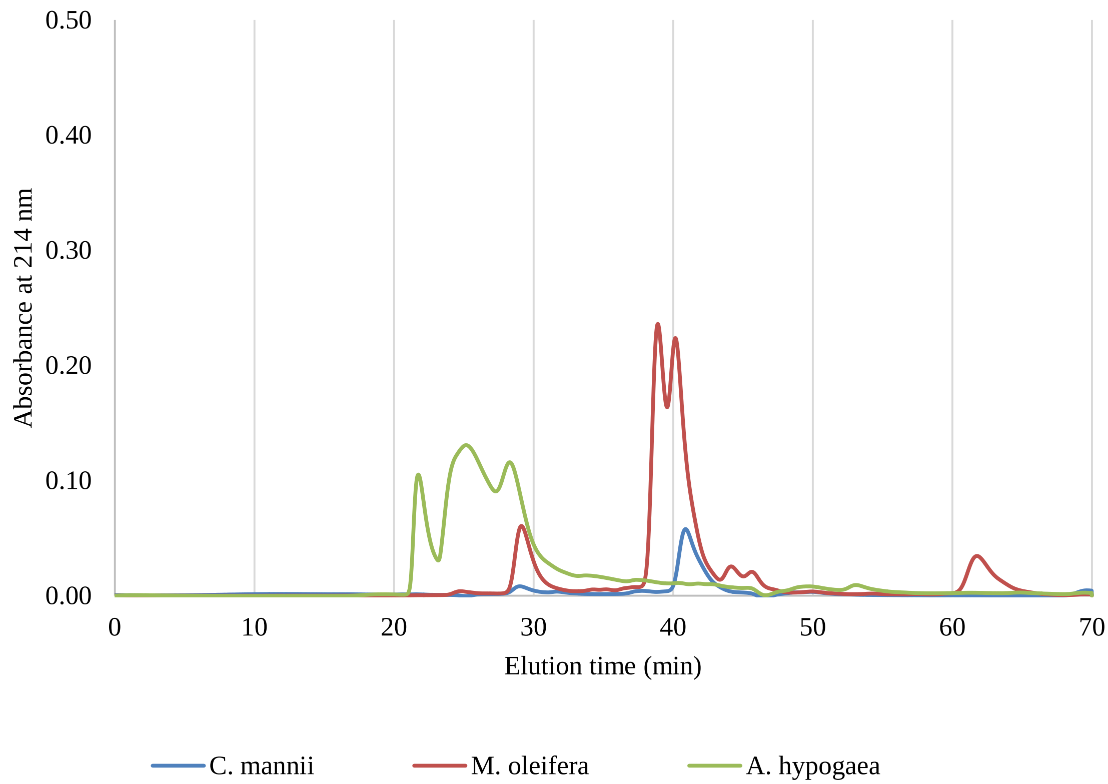



**Figure-4:** SE-HPLC profiles of extracts on Shodex column.

Survey of the literature showed that, the mineral coagulants used in water coagulation treatments are trivalent cationic salts: trivalent fer salts and aluminum salts. Indeed, according to the theory of SCHULZE-HARDY, the efficiency of coagulants is directly linked to the valence of the cations used. And the coagulation is all the more efficient as the valence of the cation is high[2,22]. The results of the ionic composition show similarities in the contents of aluminum ions and fer III, in the coagulating solutions of the powders of the seeds of *A. hypogaea* and *C. mannii*, which are the plant species in this study; and M. oleifera which is used as a reference in this study. Since aluminum and fer III ions are not abundant in the coagulating solutions of the powders of the seeds of the 3 plant species, the similarities noted in this study may allow us to say that the coagulating activity of the solutions of the powders of seeds of *A. hypogaea* and *C. mannii*, is not due to aluminum ions and fer III ions. As for the powder solution of the seeds of *M. oleifera*, whose coagulant activity is due to cationic polyelectrolytes of the polypeptide type[20,21].

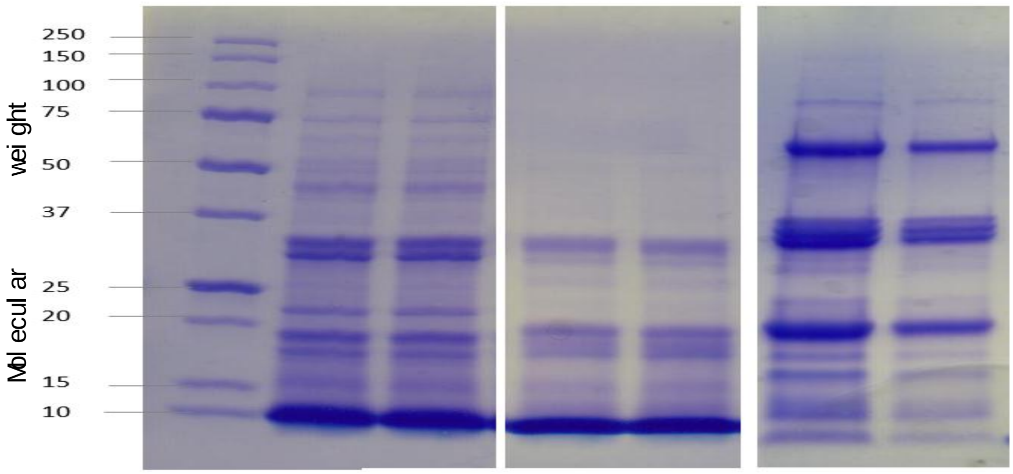



**Figure-5:** SDS PAGE Profiles of Extracts.

**Table-3:** Ionic composition of extract of *A. hypogaea*, *C. mannii* and *M. oleifera.*

| Ion composition (mg.L$^{-1}$) | *A. hypogaea* | *C. mannii* | *M. oleifera* |
|---|---|---|---|
| Composition of positives ions | | | |
| Aluminium | 0.15 | 0.68 | 1.52 |
| Fer III | 0.0 | 0.0 | 0.26 |
| Fer II | 0.26 | 0.13 | 0.10 |
| Calcium | 199.17 | 151.46 | 512.19 |
| Magnésium | 40.32 | 30.67 | 103.70 |
| Zinc | 0.40 | 0.30 | 1.05 |
| Sodium | 12.80 | 61.93 | 206.50 |
| Potassium | 80.30 | 9.75 | 32.90 |
| Composition of negatives ions | | | |
| Nitrate | 0.0 | 0.0 | 0.0 |
| Sulfate | 8.06 | 14.86 | 1210.70 |
| Carbonate | 0.0 | 0.0 | 0.0 |
| Bicarbonate | 730.45 | 555.46 | 370.08 |
| Chloride | 177.72 | 128.52 | 455.02 |

**Physico chemical characteristics:** Table-4 showed the results of the physicochemical parameters of the powder solutions of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera*. Examination of this table reveals that all three solutions are acidic, with pH values of 6.65; 6.52 and 5.01 respectively for the powder solutions of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera*. The conductivity and general mineralization are higher for the powder solution of *M. oleifera* seeds, with values of 3500µS/cm and 2.975g/L, respectively.

**Table-4:** Physico-chemical parameters of the seed solutions of the three plants.

| Physico-chemical parameters | *A. hypogaea* | *C. mannii* | *M. oleifera* |
|---|---|---|---|
| pH at 20℃ | 6.65 | 6.52 | 5.01 |
| Conductivity at 20℃ (µS/cm) | 1361 | 1035 | 3500 |
| General mineralization ($g.L^{-1}$) | 1.30 | 0.981 | 2.975 |
| Turbidity (NTU) | 84 | 52.82 | 62.75 |
| Color (mg/L Pt Co) | 00 | 10 | 30 |
| Density at 15℃ | 1.008 | 1 .003 | 1.010 |

**Statistical analysis:** Statistical analysis of the clarification test results of 131NTU and 32.75NTU raw water samples showed a dispersion of 0.01 to 0.02 for turbidity for *A. hypogaea*. For *C. mannii*, the dispersion is 0.02 to 0.03 for turbidity.

## Conclusion

This study allowed highlighting proteins in the coagulating solutions of the powders of the seeds of *A. hypogaea*, *C. mannii* and *M. oleifera*. The coagulating solutions of *C. mannii* and *M. oleifera* mainly contain small proteins of which 75.4% and 94.4% have molecular masses less than 10kDa. The coagulating solution of *A. hypogaea* mainly contains large proteins of which 25.4% have molecular masses between 100kDa and 300kDa, 16.8% between 300kDa and 500kDa and 16.8% have molecular masses greater than 500kDa.

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