

Full Length Research Paper

Lectin content of seeds and physical stability of coagulating solutions from the seeds of *Arachis hypogaea* L., *Cucumeropsis mannii* Naud., and *Moringa oleifera* Lam.

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This study assessed the lectin content in the seeds of *Arachis hypogaea* L., *Cucumeropsis mannii* Naud. and *Moringa oleifera* Lam. The physical stability of the coagulating solution of the seed powders of each plant species was also assessed. The lectin content was determined using the haemagglutination assay method. The physical stability of the coagulating solutions of the seed powders of the three plant species was analysed using a Turbiscan. The coagulating activity of the seeds was assessed by Jar-Test. The results of the determination of the lectin content of the seeds showed that the three samples had a negligible lectin content, less than 0.4 HAU/mg. Analysis with Turbiscan for 30 min revealed that the stability index of the aqueous extract of *C. mannii* Naud. Seeds were constant and equal to 0; that of the aqueous extract of *A. hypogaea* L. seeds increased from 0 to 13.5. The stability index of the aqueous extract of *M. oleifera* Lam. seeds increased from 0 to 23 during the first 7 min. Evaluation of the coagulant activity of the three plant species with raw water samples of turbidity ranging from 135.90 to 31.89 Nephelometric Turbidity Units (NTU) revealed turbidity reduction percentages of over 80%.

Key words: *Arachis hypogaea*, *Cucumeropsis mannii*, *Moringa oleifera*, seeds, coagulants, waters, lectins, Turbiscan.

INTRODUCTION

Drinking water is essential to life. Despite its importance, it is not available everywhere. According to one of the World Health Organizations (WHO) reports, in 2019 more than 33 countries in sub-Saharan Africa will have more than 8 million people without a drinking water supply, and these will be living in rural areas (WHO, 2020; United Nations Children's Fund [UNICEF], 2020). The water, sanitation and hygiene (WASH) targets of Sustainable Development Goal 6 (SDG 6) call for universal access to drinking water

services, emphasising the need to focus on people living in rural areas, particularly the vulnerable (United Nations Development Programme [UNDP], 2019; WHO, 2020). For rural areas in developing countries, research into endogenous techniques for treating water for domestic use is an alternative solution to the water problem. Existing endogenous techniques include the clarification of surface water using *Moringa oleifera* Lam. seeds (Jayalakshmi et al., 2017; Sulaiman et al., 2017), a plant whose

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coagulant activity is due to a cationic polypeptide polyelectrolyte (Atakpama et al., 2014). Other researchers have also shown that the proteins in the seeds of certain plants are effective coagulants for coagulating water (Muthuraman et al., 2013; Bodlund et al., 2014; Arunkumar et al., 2019).

There is an interest in finding products of plant origin with coagulant activity, to contribute to providing populations in rural areas of developing countries with simple processes for treating raw water at household level using natural coagulants derived from plants found in their environment. In this study, Seeds of *Cucumeropsis mannii* Naud. and *Arachis hypogaea* L. *M. oleifera* Lam. was used in this study as a reference plant, for its scientifically proven coagulant activity (Bichi, 2013; Salazar Gámez et al., 2015). Various proteins were extracted from the seeds of these three plant species (Ntalani et al., 2021). Among the proteins, lectins, also known as phytohemagglutinins, have various applications (Sharon and Lis, 2004; Hamid et al., 2013; Santos et al., 2014) and can have coagulant activity in water treatment (Santos et al., 2008). However, some lectins can also be toxic (Zubcevic et al., 2016; Cuq, 2006). It would be interesting to assess the lectin content of the seeds of these three plant species. The physical stability of the aqueous extract of the seeds of each plant species can also be assessed, for their use as natural coagulants in the treatment of surface water.

MATERIALS AND METHODS

Plant

The plant material consisted of seeds of *C. mannii*, *A. hypogaea*, and *M. oleifera*. Harvesting was carried out in the southern zone of the Republic of Congo in November 2018. The *C. mannii* and *A. hypogaea* seeds were collected near the village of Boulankio, 45 km from Brazzaville, and the *M. oleifera* seeds were collected at the Institute for Research in Exact and Natural Sciences, located in arrondissement No. 1 (Makélékélé) in the city of Brazzaville.

Granulometric analysis of *A. hypogaea*, *C. mannii* and *M. oleifera* seed powders

100 g of *C. mannii*, *A. hypogaea* and *M. oleifera* seeds were grounded using a GM 200 mill (Retsch); for each powder obtained, the grain size was measured using a laser granulometer (Mastersizer 2000, Malvern). The Mie model was used for the calculation, considering non-spherical particles. A refractive index of 1.49 and an absorption index of 0.1 for the particles were used. The parameters measured were the maximum diameter of 10% of the mass of a sample (D10), the maximum diameter of 50% of the mass of a sample (D50) and the maximum diameter of 90% of the mass of a sample (D90).

Preparation of solutions of *C. mannii*, *A. hypogaea* and *M. oleifera* seed powders and aluminium sulphate

The seeds of *C. mannii*, *A. hypogaea* and *M. oleifera* were dehulled, oven-dried at 50°C for 48 h and ground. For each species, 100 g of the powder obtained was dispersed in 1000 mL of distilled water. After 10 min of vigorous stirring using a Hanna HI190M magnetic

stirrer, the mixture obtained was filtered through an 80 µm sieve. The liquid obtained was decanted into a separating funnel for 24 h and the aqueous phase obtained was used to assess the coagulant activity. The aluminium sulphate solution was prepared at the concentration of 10 g.L⁻¹.

Determination of lectins in *C. mannii*, *A. hypogaea* and *M. oleifera* seeds

Lectin content was determined using the haemagglutination assay, a method based on the exploitation of the hemagglutinating power of lectins on blood cells (Santos et al., 2009; Katre et al., 2008; Zubcevic et al., 2018). This method involves extracting lectins from the sample and then contacting them with rabbit blood cells (TCS Biosciences, UK) over a dilution range. 1 g of sample was added to 20 mL of phosphate buffer, homogenised for 1 min at 5000 rpm and centrifuged. 100 µL of pure product (standard, control: pea, *C. mannii*, *A. hypogaea* and *M. oleifera*) was added to 100 µL of blood in the first well of a microplate. Wells 1 to 10 correspond to a dilution range from 1 (pure sample) to 0.01 (sample diluted 100-fold).

Once the plate has been prepared, it is incubated at 38°C for 1 h and then left at room temperature for 12 h. Haemagglutination is then measured visually. The measurement of Haemagglutinating activity is calculated using the method of Hill (1962). A hemagglutinating unit (1 HAU) is defined as the smallest amount of lectin causing visible hemagglutination. The hemagglutinating activity of the initial sample is calculated by multiplying the reciprocal of the highest dilution causing agglutination by the dilution factor of the sample.

Physical stability of coagulant solutions of *C. mannii*, *A. hypogaea* and *M. oleifera* seed powders

The physical stability of coagulant solutions of *A. hypogaea*, *C. mannii* and *M. oleifera* seed powders was analysed using a Turbiscan (Sun et al., 2019). The three coagulating solutions were placed in three glass cells and then analysed using a Turbiscan. A laser beam scanned the contents of each glass cell vertically every min for 30 min and measured light transmission and backscatter along the glass cell. The dispersion stability index (TSI) was measured for 30 min.

Evaluation of the coagulant activity

Description of the sampling area

As part of this study, Raw water samples were taken from two rivers located to the south of Brazzaville, namely the Djoué and Congo rivers. The geographical coordinates for the Djoué river are: 04°18' 31" South latitude, 015°13' 36" East longitude and 270 m above sea level. Those for the Congo River are as follows: 04°18' 50" South latitude, 015°12' 31" East longitude and 256 m above sea level.

Raw water sampling

The various samples were taken discontinuously. The samples were taken either in the absence of a rainfall event, for a period of at least 24 h prior to sampling, or at least one h after the rainfall event. 21 raw water samples were taken in 10 liters plastic bottles, using the method described by Rodier et al. (2009). After each sampling, the raw water sample was quickly transported to the laboratory for analysis and clarification.

Method for clarifying the raw water samples taken

In this study, the Jar Test method for monitoring turbidity elimination

Table 1. Diameter of seed powders from the three plant species
Lectin content of *A. hypogaea*, *C. mannii* and *M. oleifera* seeds.

Species	Diameter (µm)		
	D10	D50	D90
<i>A. hypogaea</i>	121	370	756
<i>C. mannii</i>	120	950	2260
<i>M. oleifera</i>	64.7	802	2160

Table 2. Lectin content of the seeds of three plant species.

Vegetable species	<i>A. hypogaea</i>	<i>C. mannii</i>	<i>M. oleifera</i>
Lectin content (HAU/mg raw sample)	< 0.4	< 0.4	< 0.4

*HAU: Haemagglutinating activity unit.

was used to assess coagulant activity (Degrémont, 2005). Clarification tests were carried out by jar-test (Cardot, 2010), using a Lovibond ET 740 flocculator; firstly, to highlight the coagulant activity of *C. mannii* and *A. hypogaea* seeds; secondly, to carry out a comparative study of the coagulant activity of the seeds of these two plants with that of *M. oleifera* seeds and aluminium sulphate. The four beakers of a Lovibond ET 740 flocculator with four stirrers were each filled with 500 mL of raw water sample, followed by the addition of increasing volumes in mL of aqueous extracts of *A. hypogaea*, *C. mannii*, *M. oleifera* seeds and aluminium sulphate solution. This was followed by rapid agitation at 180 rpm for 3 min and slow agitation at 18 rpm for 20 min. After each jar-test, the treated water was decanted for 30 min and filtered.

Statistical analysis

A statistical analysis was carried out on the residual turbidity values of the water resulting from the treatment of different raw water samples with initial turbidity ranging from 135.90 to 31.89 Nephelometric Turbidity Units (NTU), taken from the Djoué river and the Congo river. For each volume of coagulant, the average of three values was used to calculate the standard deviation, using Microsoft Excel 2013 software.

RESULTS AND DISCUSSION

Granulometric analysis of *A. hypogaea*, *C. mannii* and *M. oleifera* seed powders

The results of the granulometric analysis are presented in Table 1. This table shows that the seed powders of the three plant species are mainly composed of grains with a diameter of 756 µm, 2160 µm and 2260 µm, corresponding to *A. hypogaea*, *M. oleifera* and *C. mannii*, respectively. These results also show a similarity between the powder grains of *C. mannii* and *M. oleifera* seeds, whose D50 and D90 size differences are close. Table 2 shows the results of determining the lectin content of *A. hypogaea*, *C. mannii* and *M. oleifera* seeds. Observation of this table shows that the three seed samples have a negligible lectin content, less than 0.4 HAU/mg. By way of comparison (Cuq, 2006), the lectin content of raw peas and lentils is around 10

HAU/mg, raw kidney beans 100 HAU/mg and raw soya 700 HAU/mg.

Stability of coagulating solutions of *C. mannii*, *A. hypogaea* and *M. oleifera* seed powders

The stability of coagulating solutions was analysed using a Turbiscan. This device measures destabilisation phenomena (sedimentation, creaming, flocculation) and assigns a stability index that is higher the more unstable the product (Meunier, 1994). The results of the stability index of the three coagulating solutions over time are shown in Figure 1. Observation of this figure reveals that during the 30 min of analysis, the stability index of the coagulating solution of the *C. mannii* seed powder is constant and equal to 0, while that of the coagulating solution of the *A. hypogaea* seed powder increases from 0 to 13.5. The stability index for the coagulating solution of *M. oleifera* seed powder increased from 0 to 23 during the first 7 min, then remained constant at 23 from 8 to 30 min. These results indicate a high stability of the coagulating solution of the *C. mannii* seed powder; a rapid destabilisation, that is. instability of the coagulating solution of the *M. oleifera* seed powder, with a plateau reached after 5 min, and an intermediate behaviour of the coagulating solution of the *A. hypogaea* seed powder. The instability of the coagulating solution of *M. oleifera* seed powder is explained by the presence of an unstabilised lipid phase in the form of an emulsion, causing rapid phase separation. The presence of a lipid phase is visible at the top of the tube. For the other two coagulating solutions, the fats are either absent or stabilised in the form of an emulsion, giving the suspension a milky appearance (Figure 2).

Coagulant activity

Evaluation of the coagulant activity of *A. hypogaea* and *C.*

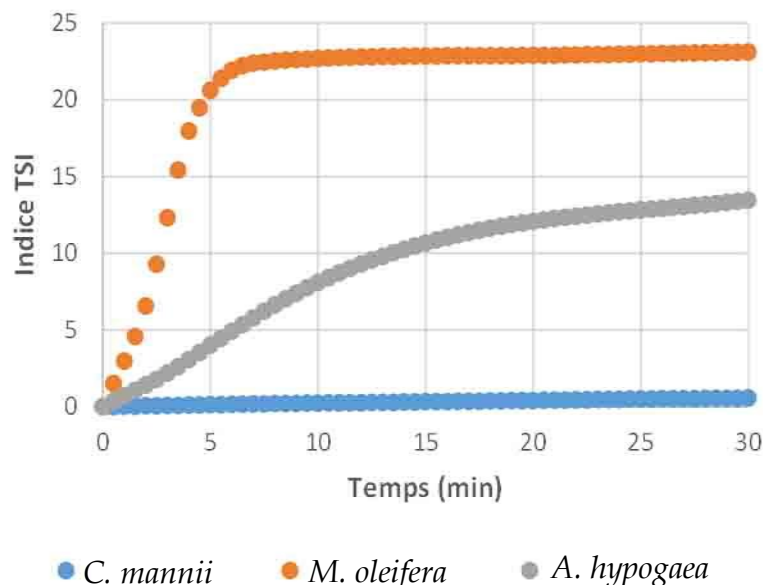


Figure 1. Stability index of coagulating solutions of seed powders of the three plants species over time.



Figure 2. Appearance of the coagulating solutions of the seed powders of the three plants species after 30 min in Turbiscan.

mannii seeds was carried out on 21 raw water samples taken from the Djoué river and the Congo river. The initial turbidity values of these raw water samples are given in Table 3. The results of clarification tests on these raw water samples using aqueous extracts of *A. hypogaea* and

C. mannii seeds are presented in Tables 4 and 5. Observation of these tables shows turbidity reduction percentages greater than 80% after decantation and 95% after decantation and filtration, for the 10 raw water samples coagulated with *C. mannii* (Table 4). The

Table 3. Turbidity values for various raw water samples taken from the Djoué River and the Congo River (Congo).

No.	1	2	3	4	5	6	7	8	9	10	11	12
Turbidities (NTU) of water samples taken from the Djoué river	135.90	133	132	130	117.20	90	76.25	54.39	52.64	52.60	46.54	44.18
Turbidities (NTU) of water samples taken from the Congo river	53.36	45.00	38.10	37.20	34.48	34.26	32.31	32.29	31.89	-	-	-

Table 4. Residual turbidity, percentage of turbidity elimination for 10 samples of water coagulated with *C. mannii*.

Initial turbidity (NTU)		Residual turbidity (NTU)		Percentage of turbidity elimination		Optimum volumes (mL)
Raw water		Coagulated and decanted water	Coagulated, decanted and filtered water	Coagulated and decanted water	Coagulated, decanted and filtered water	
Raw water samples taken from the Djoué River						
132		9.29	2.01	92.67	98.48	80
130		9.27	0.60	93	99.54	80
117.20		9.26	1.99	92.10	98.30	80
54.39		8.34	1.93	83.82	96.45	90
52.64		8.75	0.53	83.38	98.99	90
52.60		8.71	0.53	83	98.99	90
Raw water samples taken from the Congo River						
34.48		1.77	0.20	94.87	99.42	80
34.26		1.79	0.34	94.78	99.12	80
32.31		1.80	0.43	94.43	98.67	80
31.89		1.83	0.45	94.26	98.59	80

percentages of turbidity reduction were greater than 85% after decantation and 95% after decantation and filtration, for the 10 samples of raw water coagulated with *A. hypogaea* (Table 5). The results obtained from the comparative study between the coagulating solutions of *C. mannii*, *A. hypogaea* and *M. oleifera* seed powders and aluminium sulphate, with a raw water sample of 37.20 NTU, are shown in Figure 3. The residual turbidities after coagulation and decantation were 3.33, 1.80, 2.30 and 1.50 NTU respectively. These results also showed that the relative efficiency of destabilization of the colloids in the raw water

samples by the solutions of *C. mannii* seed powder (91.04%) and *A. hypogaea* seed powder (95.16%) was close to that of the solution of *M. oleifera* seed powder (93.81%) which is a scientifically proven natural coagulant, and that of aluminium sulphate (95.96%), which is the mineral coagulant most commonly used in the treatment of water intended for human consumption. All residual turbidity values obtained after coagulation, decantation and filtration of treated water samples comply with WHO recommendations (WHO, 2017). The coagulant activity of *A. hypogaea* seeds in surface water clarification was also mentioned by Prasad

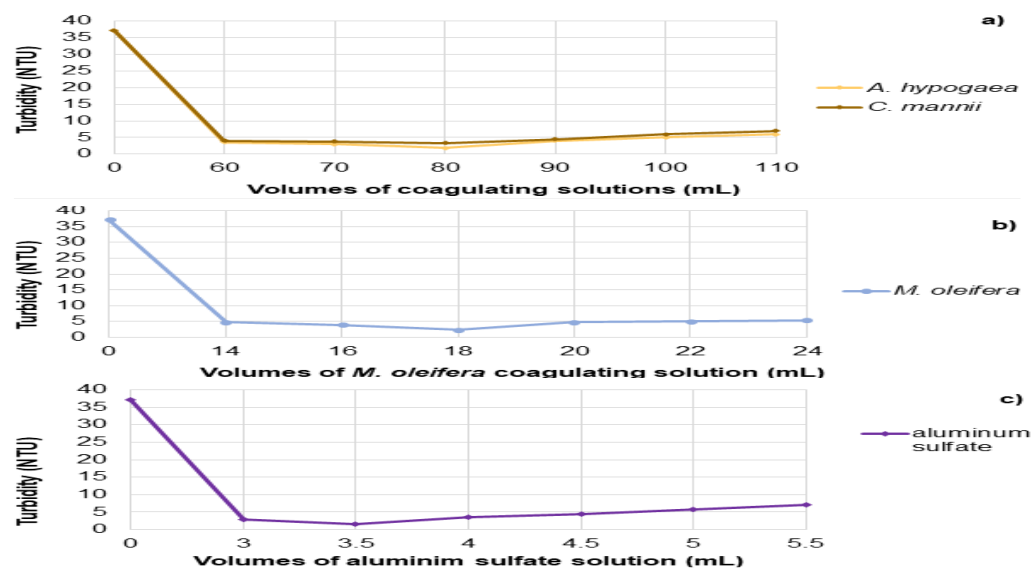
et al. (2017), with a percentage turbidity reduction of 83%. However, Documents in the literature that mention the coagulant activity of *C. mannii* seeds was not found.

Statistical analysis

Statistical analysis of the results of the turbidity reduction during the clarification trials of the *C. mannii* different raw water samples with initial turbidity ranging from 135.90 to 31.89 NTU showed a dispersion of 0.05 to 0.1 for the treatments

Table 5. Residual turbidities, percentages of turbidity elimination for 10 water samples from coagulation with *A. hypogaea*.

Initial turbidity (NTU)	Residual turbidity (NTU)		Percentage of turbidity elimination		Optimum volumes (mL)
Raw water	Coagulated and decanted water	Coagulated, decanted and filtered water	Coagulated and decanted water	Coagulated, decanted and filtered water	
Raw water samples taken from the Djoué River					
135.90	13.81	2.26	89.84	98.34	70
133	13.80	2.00	89.62	98.50	70
90	9.06	1.99	89.93	97.79	70
76.25	3.48	0.21	95.00	99.72	80
46.54	3.24	0.17	93	99.68	80
44.18	3.24	0.60	92.67	98.64	80
Raw water samples taken from the Congo River					
53.36	1.33	0.02	95.51	99.96	80
45	1.34	0.54	97.02	98.80	80
38.10	1.79	0.58	95.30	98.48	80
32.29	4.10	0.71	87.30	97.80	70

**Figure 3.** Variation in turbidity of the raw water sample of 37.20 NTU as a function of the volumes of coagulating solutions.

carried out with the coagulating solution of *A. hypogaea* seed powder. The dispersion was 0.06 to 0.2 for treatments with the coagulating solution of *C.* was also mentioned by Prasad et al. (2017), with a percentage turbidity reduction of 83%. However, Documents in the literature that mention the coagulant activity of *C. mannii* seeds was not found. carried out with the coagulating solution of *A. hypogaea* seed powder. The dispersion was 0.06 to 0.2 for treatments with the coagulating solution of *C. mannii* seed powder. seed powder.

Conclusion

This study showed that lectin levels in *C. mannii* and *A. hypogaea* seeds are negligible, below 0.4 HAU/mg, as in *M. oleifera* seeds. This study also made it possible to assess the physical stability of the coagulating solutions of the seed powders of these three plant species in Turbiscan. The results show a high stability of the coagulating solution of the *C. mannii* seed powder, rapid destabilisation, that is. instability of the coagulating solution of the *M. oleifera* seed powder, with a plateau reached after 5 min, and intermediate behaviour of the coagulating solution of the *A. hypogaea* seed powder. Evaluation of the coagulant activity of the three plant species with raw water samples of turbidity ranging from 135.90 to 31.89 NTU showed turbidity reduction percentages of over 80%.

CONFLICT OF INTERESTS

The authors have not declared any conflicts of interests.

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