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Identification of the chemical constituents of the *Annona muricata* L. seeds with coagulating properties on domestic wastewater

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Abstract

In the same vision of the valorization of the species *Annona muricata* L. (Annonaceae) and with regard to the previous scientific data collected on this plant species, a study was initiated to search for chemical constituents of seeds of this plant which could have the clarifying (coagulant) properties of domestic wastewater. The results obtained show that these seeds contain carbohydrates, proteins, lipids, terpenes and sterols in its almonds and integuments ; flavonoids and tannins in its integuments with significant contents of organic compounds (66.15 %) as well as mineral compounds (00.97 %) and volatile compounds (32.88 %). The vegetable oil (lipids) extracted is 30.56 % in these seeds. The evaluation of the clarifying activity of soursop seeds on samples of domestic wastewater with turbidities of 160 and 259 NTU showed that these seeds have a reduction rate of colloids or suspended matter of 95.40 % with the solution of its almonds and 97.58 % with the solution of its almond cakes at respective doses of 4800 mg powder/L and 3200 mg powder/L in 30 minutes. The results obtained prove that these seeds contain 35.59 % of other organic compounds (proteins, amino acids, carbohydrates and starch) which are responsible for this water clarifying activity. It is important to note that to our knowledge, the indications given on the activity of clarifying wastewater by the seeds of this fruit have not yet been the subject of scientific work making it possible to identify some of the compounds responsible for this activity in these glitches.

Keywords: Chemical constituents; Coagulant property; Seeds; Wastewater; *Annona muricata*

1. Introduction

Sustainable access to drinking water resources is a major concern in the world. On the other hand, current water resources are affected by several factors including the growing demand for water by the population, the phenomenon of pollution of groundwater and surface water by different human activities [1, 2]. The reuse of wastewater, sources of water-borne diseases, presents itself today as an alternative solution to limit its shortage because the latter factor could satisfy agricultural, industrial or domestic needs and resolve environmental problems [3-5].

Before their use or consumption by the population, water treatment is imperative [6]. Current studies are limited to concluding on the quality of water treated with synthetic coagulants (aluminum or iron chloride, alum etc.), because the most effective and used water treatment methods induce in these treated waters several toxic compounds such as halogenated compounds and its derivatives which are sometimes harmful to health and the environment [7, 8]. However, the use of plants is currently one of the alternative ways of replacing synthetic coagulants in the treatment of wastewater because these plants contain several compounds with different properties, some of them need to be identified and revealed. In general, these natural coagulants are food or medicinal plants which have a clarifying power for cloudy water ; this requires an adjustment of the pH of this water which leads to a consumption of alkalinity and

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increases the levels of organic compounds or phytonutrients likely to degrade in this water. However, the presence of these organic compounds in water promotes the development of microorganisms responsible for the biological treatment of said water [9, 10]. The limits of application of these natural coagulants have not yet been clearly established to date [3-5, 11].

In order to preserve the quality of reused wastewater for consumption around the world, it is important to continue research into new natural coagulants capable of treating wastewater without negative impact on living beings and the environment [10, 12-14]. In the same perspective of the discovery of *Moringa oleifera* L. as one of the currently known natural coagulants and a plant with multiple uses (traditional and modern) in the world [8, 13, 15] that we were interested in the seeds of the *Annona muricata* L. fruit. Indeed, this fruit is designated in the literature by numerous vernacular names, namely : corrosol (French), soursop (English), guanabana (Spanish), graviola (Portuguese),….[16-21]. Once consumed, these seeds are thrown into nature and generally become part of household waste, which is one of the current problems in waste management around the world. Nevertheless, several studies focusing on the phytochemical composition of these *Annona muricata* seeds have demonstrated the presence of different compounds with multiple biological properties [22-30]. Depending on the chemical composition of soursop, a study was initiated to search for chemical constituents of the seeds of this plant which could have the clarifying (coagulant) properties of domestic wastewater.

2. Material and methods

2.1. Preparing the extracts

The extracts used were prepared separately from almond and integument powders obtained of soursop seeds. These extracts were vegetable oils, ashes and other extracts obtained from different solvents with increasing polarities.

The extraction of the oils was carried out in hexane using Soxlhet [31, 32]. A concentrated liquid (lipid) and dry solid (almond cake) were obtained and weighed after evaporation of the solvents.

The ashes of the almonds and integuments of the soursop seeds were obtained by incineration of these plant organs at 600 °C for 8 hours in a CERADEL brand electric ceramic oven, located within the premises of the National School of Fine Arts, Brazzaville-Congo [31, 32].

The other extracts from the almonds and integuments of soursop seeds were obtained successively by extraction with hexane, chloroform, methylethylacetone (MEC), ethanol and water.

2.2. Chemical screening of metabolites

The search for seed metabolites was carried out by Thin Layer Chromatography (TLC) using aluminum plates in silica gel 60 F254 (Merck) and polyester plates in silica gel UV254 as support. The chemical families sought are lipids, carbohydrates, nitrogen compounds, polyphenols, terpenes and sterols using elution systems and specific developers. The methods used are those described in the literature [33-35].

2.3. Determination of chemical constituents contents of the seeds

2.3.1. Water and volatile compounds content

The water and volatile compound content was determined by drying at room temperature for 01 hour and followed by steaming at 104 ± 1 °C for 24 hours of 148.91 g of the soursop seed samples [31, 32]. The water and volatile compounds content (TEC) was calculated according to the following formula :

$$T_{EC} = \frac{m_i - m_f}{m_i} * 100 \qquad (1)$$

With : m_i, mass of seed samples before drying (initial mass) and m_f, mass of seed samples after drying (final mass)

2.3.2. Ash content

The sum of the masses of the ashes obtained after incineration at 600 °C for 8 hours of 61.32 g of almond powders and 38.26 g of integument powders made it possible to determine the ash content (Tc) of the seeds according to the following formulas [32] :

$$Tc(E) = \frac{m_1}{m_0} * 100 \quad (2)$$

With : m_0 : total mass of test portion (almonds and integuments) ; m_1 : total mass of these samples after incineration and E, sample.

2.3.3. Organic compounds content

The organic compounds content (T_{CO}) in the seeds was calculated by the formula :

$$T_{CO}(S) = 100 - (T_C + T_{EC}) \quad (3)$$

With : T_{EC} : water contents and volatile compounds ; Tc : ash content ; S: seeds.

2.3.4. Lipid content

The lipid content (T_L) was determined from the sum of the masses of almond powders (61.30 g) and integument powders (38.30 g) used and those obtained after extraction of these vegetable oils according to the following formula [31, 32] :

$$T_L = \frac{m_1}{m_0} * 100 \quad (4)$$

With : m_o, total mass of the samples (almonds and integuments) before extraction ; m_1, total mass of the vegetable oils obtained of these samples.

2.4. Evaluation of the coagulant activity of soursop seeds

2.4.1. Preparation of coagulant solutions

The coagulating solutions of almond powders and its cakes, integument powders and ashes of soursop seeds were each prepared in distilled water at a concentration of 20 g/L [35]. After 24 hours of decantation, the coagulating solutions (aqueous phases) were each filtered using filter paper.

The aqueous solution of 10 g/L of ferric chloride was also prepared and used as a reference coagulant solution.

2.4.2. Domestic wastewater sampling and collection area

The wastewater samples used in this work were taken in September 2022 from one of the sewers located at the intersection between Bayas Street (District 4 Poto-Poto) and the Madoukou River located south of the city of Brazzaville. These wastewater samples were taken in 10 L plastic bottles.

2.4.3. Clarification of raw water samples taken

Clarification of wastewater or raw water samples was carried out by Jar-test using a Lovibond ET 740 flocculator [36, 37]. In a 1000 mL beaker containing 500 mL of raw water samples was added an optimal volume of the coagulant solution. The medium obtained was subjected to rapid stirring at 140 rpm for 5 minutes, then slow stirring at 10 rpm for 10 minutes. After stirring, the reaction medium was decanted for 30 minutes [39]. The optimal volume was modified until an adequate minimum treatment rate was obtained by balancing the acidity of the medium at a pH = 3.3 with the hydrochloric acid solution of concentration 1 mol/L and that of sodium hydroxide with a concentration of 10 g/L [40]. The same wastewater clarification operation was carried out with the reference coagulant solution.

2.4.4. Determination of physicochemical parameters, treatment rate and clarification yield

The physicochemical parameters measured before and after water treatment are turbidity measured using a Thermo Scientific Orion AQ3010 turbidimeter ; the absorbance (λ = 350 nm) measured using an Aqualitic AL800 UV-Visible spectrophotometer and the temperature measured using a Blacklights EZ-9909-SP multi-parameter.

The treatment rate T_t (in g powder/L) is determined according to the following formula :

$$T_t = \frac{V_p * C_p * 1000}{V_e} \quad (5)$$

With : V_p (mL), volume taken from the coagulant solution; C_p (g powder/L), concentration of the coagulant solution equivalent in g of the seed powders used ; V_e (mL), volume of raw water

The evaluation of yield of the turbidity removal efficiency of the extracts of these seeds was made relative to the minimum of the turbidity or the absorbance of each of the curves according to the following formulas :

$$\text{Yield (\%)} = \left(\frac{TU_i - TU_f}{TU_i}\right) * 100 \quad (6) \qquad \text{or} \qquad \text{Yield (\%)} = \left(\frac{Ab_i - Ab_f}{Ab_i}\right) * 100 \quad (7)$$

With : TU_f, final turbidity in NTU ; TU_i, initial turbidity in NTU ; Ab_i, initial absorbance ; Ab_f, final absorbance

The data collected from these domestic water analyzes were inserted and processed with the EXCEL 2016 software. The clarified water analysis curves are constructed according to the treatment rates.

3. Results and discussion

3.1. Highlighting the metabolites of soursop seeds

The results of the Highlighting of the metabolites are presented in Figure 1 and Table 1. The observation of the chromatograms at 365 nm showed two blue fluorescent spots (Rf : 0.81) after revelation with Neu and $AlCl_3$, in the MEC and ethanolic extracts of the integuments (Figure 1a and 1b). These spots are characteristic of polyphenols, in particular phenolic acids [33]. On the other hand, Figure 1c shows a single brown colored spot (Rf : 0.81) of the compounds from the soursop seed extracts after exposure with $FeCl_3$. This color is characteristic of the presence of tannins in the ethanolic extract of the integuments of these seeds [33]. However, no characteristic stains of these polyphenols were observed in the other extracts obtained from these seeds. These results are consistent with those in the literature which show that these seeds contain several compounds including flavonoids and tannins [41, 42]. These compounds are also present in different parts of soursop [20, 21, 43-47]. In addition, the chromatographic profile of the hexane and chloroform extracts of these seeds revealed, with antimony chloride and UV 365 nm, the presence of blue and red spots characteristic of terpenes and sterols in these seeds (Figure 1d, Table 1a) [33]. These results of these metabolites are validated by data from the soursop literature obtained in several countries [42, 43, 48, 49].

Observation of the chromatogram (Figure 1e) showed gray spots after development with Molish reagent and heating (Table 1b). These spots are comparable to carbohydrates, when compared with that of the carbohydrate taken as a control. We note a strong dominance of these compounds in the ethanolic extract of these almonds. The presence of carbohydrates in these almonds is confirmed by the work carried out by Ranisaharivony (2015) which highlighted maltose and glucose in the aqueous extract of the almonds from the seeds of the Madagascar soursop [17].

The presence of brown and purple spots after development with manganese chloride and heating illustrate the chromatographic profile of vegetable oil obtained from these soursop seeds (Figure 1f, Table 1c). These colors are characteristic of the lipids in comparison with those of sunflower oil (control L1) and castor oil (controls L2). These results on the lipids of the almonds and the integuments of these seeds confirm those found by Ranisaharivony (2015) in Madagascar and by Inkalaba and *al*. (2023) in Democratic Republic of Congo which revealed the presence of lipids in soursop [17, 43]. However, Ranisaharivony B. (2015) identified triglycerides and annonacin (a toxic acetogenin molecule) in the oil and dichloromethane extract of soursop organs [17]. Nowadays, the activities of interest of the lipids and the annonacins of these seeds are multiple because these chemical constituents are used in the production of biofuels and have several biological activities [19, 42, 46-48, 50].

The visible observation of orange and brown spots on the chromatogram obtained after development with ninhydrin and heating of the TLC plates indicates the presence of nitrogen compounds characteristic of proteins in these extracts (Figure 1g, Table 1d**)** [33]. These compounds are present in the ethanolic and aqueous extracts of almonds and absent in the integuments of soursop seeds. These results confirm those found by Ranisaharivony (2015) in Madagascar and by Inkalaba and *al*. (2023) in Democratic Republic of Congo which had highlighted nitrogen molecules (amino acids or proteins) in the aqueous extract of these almonds of soursop seeds [17, 43]. In addition, literature data prove that soursop seeds contain proteins with significant contents [19, 42, 47, 48, 50].

These results show that soursop seeds are rich in nitrogen compounds (proteins or amino acids in particular) and lipids. These seeds also contain chemical constituents (sugars, polyphenols, terpenes and sterols) of nutritional and medicinal interest.

3.2. Chemical constituents contents of soursop seeds

The results obtained from drying show that these soursop seeds contain 32.88 % water and volatile compounds. The average ratio obtained from the separation of the almonds and the integuments of these seeds is 01.61. The water and volatile compounds content (32.88 %) obtained after drying the soursop seeds is higher than that (29.90 %) obtained by Ranisaharivony in Madagascar [17]. The incineration of these seeds gives 00.97 % of the ashes (mineral constituents) of which its almonds and its integuments have the respective contents of 00.60 % and 00.37 % compared to the seeds. Which shows that these seeds are mainly made up of organic compounds at a content of 66.15 %. In addition, vegetable oil (lipids) has a content of 30.56 % in 66.15 % of the organic compounds present in these soursop seeds. These lipids are more concentrated in its almonds (29.51 %) than in its integuments (01.05 %) of these seeds. This oil content obtained of these Congolese soursop is better than that obtained (25.10 %) by Ranisaharivony on oils extracted from Madagascar soursop and low than that (40 ± 0.82 %) published by Attanayake and *al*. [17, 47]. This variation in oil content could be due to the nature of the soil, climate, variety of fruits, ripening phase, harvest period, extraction solvent and drying temperature, etc.

These results of quantification of the chemical constituents of soursop seeds prove that these seeds also contain other organic compounds at a content of 35.59 %. This data could be due to the dominance of proteins, amino acids, carbohydrates or starch in these seeds [17, 43].

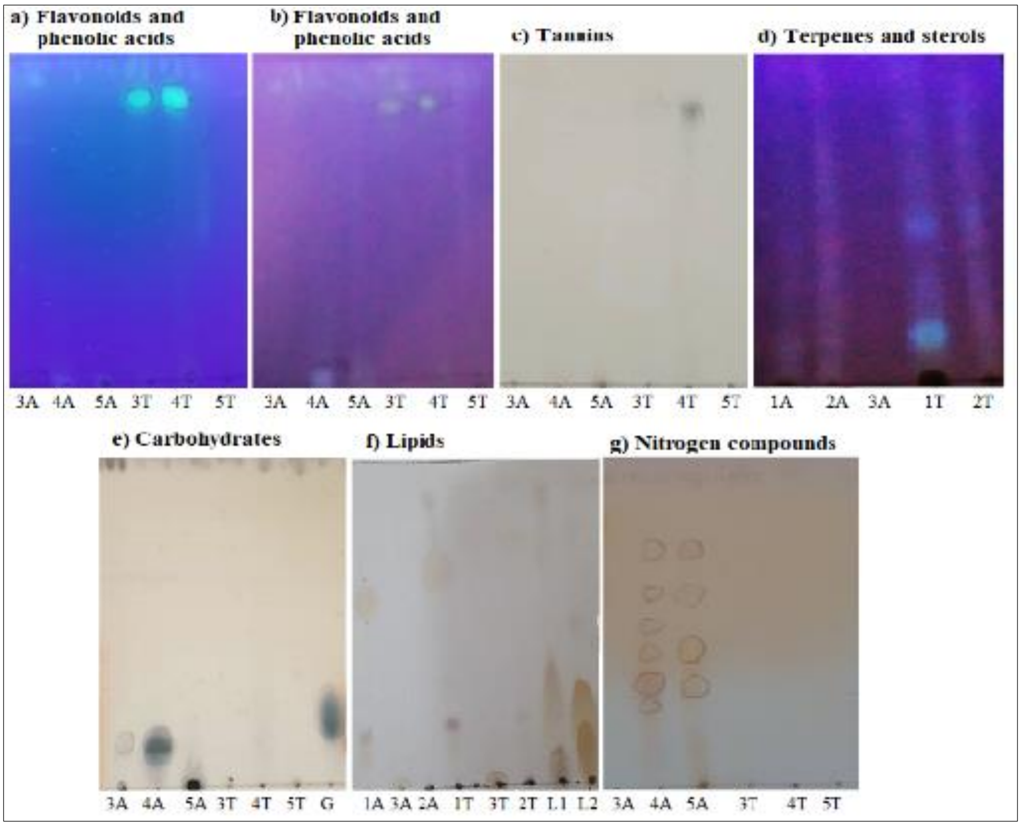

Figure 1 Chromatograms of chemical constituents of soursop seeds

a) Eluent : $CHCl_3$ /AcOEt/EtOH (V/V/V : 2/5/3), Developer : NEU/PEG400, Observation : UV 365 nm ; **b)** Eluent : $CHCl_3$/AcOEt/EtOH (V/V/V : 2/5/3), Developer : $AlCl_3$, Observation : UV 365 nm ; **c)** Eluent : $CHCl_3$ /AcOEt/EtOH (V/V/V : 2/5/3), Developer : $FeCl_3$, Observation : Visible ; **d)** Eluent : Hexane/Acetone (V/V: 10/2), Developer : $SbCl_3$, Observation : UV 365 nm ; **e)** Eluent : AcOEt/HCOOH/H_2O (V/V/V: 6/1.5/1.5), Developer : Molish reagent + heating, Observation : Visible; **f)** Eluent : Hexane/Acetone (V/V: 10/2), Developer : $MnCl_2$ + Heating, Observation : Visible ; **g)** Eluent : AcOEt/HCOOH/H_2O (V/V/V: 6/1.5/1.5), Developer : Ninhydrin + heating, Observation : Visible.
1A : Hexanic extract of almonds, 2A : Chloroform extract of almonds, 3A : MEC extract of almonds, 4A : Ethanolic extract of almonds, 5A : Aqueous extract of almonds, 1T : Hexanic extract of the integuments, 2T : Chloroform extract of the integuments, 3T : MEC extract of the integuments, 4T : Ethanolic extract of the integuments, 5T : Aqueous extract of the integuments, G : Glucose, L1 : Sunflower Oil and L2 : Castor Oil.

Table 1 Frontal ratios and colors of chemical constituents of soursop seeds

a) Terpenes and sterols		
Extract	Rf	Color
	0.43	Brown

c) Lipids		
Extract	RF	Color
	0.58	Brown

d) Nitrogen compounds		
Extract	RF	Color
	0.70	Brown

2A	0.13	Violet
1T	0.47	Blue
	0.30	Blue
	0.15	Blue
2T	0.51	Brown
	0.30	Violet

b) Carbohydrates		
Extract	Rf	Color
3A	0.14	Grey
4A	0.14	Grey
	0.08	Grey
G	0.22	Grey

1A	0.16	Violet
	0.07	Brown
1T	0.88	Brown
	0.67	Brown
	0.19	Violet
	0.14	Brown
2T	0.76	Brown
	0.19	Violet
L1	0.89	Brown
	0.73	Brown
	0.23	Brown
	0.05	Brown
L2	0.22	Brown
	0.08	Brown

4A	0.60	Brown
	0.50	Brown
	0.40	Orange
	0.31	Orange
	0.25	Orange
5A	0.70	Brown
	0.60	Brown
	0.40	Orange
	0.31	Orange

3.3. Evaluation of the coagulant activity of soursop seeds

Figures 2 to 9 illustrate the photos of samples of the raw wastewaters with turbidities 160 and 259 NTU and the water clarified by the different coagulating solutions obtained of the extracts of soursop seeds. Figures 5 and 7 show the variation in turbidity and absorbance as a function of different rates of treatment with coagulant solutions obtained of almond powders from these seeds.

Figure 3 shows the quality of raw wastewater with turbidity 259 NTU treated with the solution of the vegetable oil obtained from soursop seeds. Despite the low miscibility of oils in water, this vegetable oil contains water-soluble organic compounds. These compounds increase the turbidity of this wastewater after treatment. It is important to note that these chemical constituents (lipids) must be eliminated in these seeds because their compounds greatly increase the turbidity of the treated water.

The change in wastewater turbidity from 259 to 11.90 NTU after treatment with the solution obtained of almond powders of these soursop seeds in 30 minutes of settling shows a strong reduction in turbidity with a removal efficiency of colloids or suspended matter of 95.40 % at an optimal dose of 4800 mg powder/L (Figure 5).

The variation in the turbidity of domestic wastewater from 160 to 3.87 NTU after treatment with the solution obtained of almond cakes of these seeds in 30 minutes of decantation shows a very strong decrease in turbidity with a rate of reduction of colloids and/or materials in suspension of 97.58 % at an optimal dose of 3200 mg powder/L (Figure 7). This significant turbidity value obtained after treatment of wastewater with this solution obtained of these almond cakes could be explained by the elimination of lipids in these seeds which increase the availability of other coagulating substances in this solution.

We note that the turbidities, absorbances and treatment rates obtained after treatment of domestic wastewater with the solution these almond powders are less significant than that obtained with the solution of ferric chloride taken as the reference coagulant (3,82 NTU) at a dose of 1600 mg powder/L. However, the treatment of this domestic wastewater with the solution obtained of its almond cakes improves these treatment parameters which present results similar to those obtained in 30 minutes with this solution of ferric chloride.

The visible observation of Figure 8 obtained after treatment of wastewater with the solution obtained of integument powders of these seeds in 30 minutes of decantation shows that the addition of different doses of this coagulating solution caused a slight reduction in the turbidity of the wastewater sample. However, the addition of different doses of solutions of almond and integument ashes did not cause any reduction but rather a slight increase in the turbidity of the raw water samples after treatment in 30 minutes (Figure 9).

In view of these results, it follows that the soursop seeds do indeed have a coagulating activity in the clarification of domestic wastewater, because a reduction in turbidity particularly with its almonds is observed. This clarifying activity of wastewater is more remarkable with almond cakes and not with other constituents of soursop seeds. Which explains that this activity is preferentially due to the organic constituents contained in its almonds, namely carbohydrates, proteins, amino acids or starch [15].

Also, the turbidity value (3.87 NTU in 30 minutes) of water clarified by the almonds of this plant seeds is also close to those obtained by Andréameva in Madagascar on *Moringa* seeds (4.10 NTU) [16].

These results prove that these almond cakes have an elimination rate of colloids or suspended matter of 97.58 % at a dose of 3200 mg powder/L, a value close to that (98.52 %) expressed by ferric chloride at a dose of 1600 mg powder/L. The residual turbidity values obtained in this work are in accordance with the turbidity value recommended by the WHO for drinking water which should be less than or equal to 5 NTU [3, 4].

To our knowledge, there is no document in the literature relating to the coagulant activity of soursop seeds on water clarification. It is therefore important to know the level and composition of organic or inorganic compounds carried by these seeds in the treated water before any reuse such as drinking water.

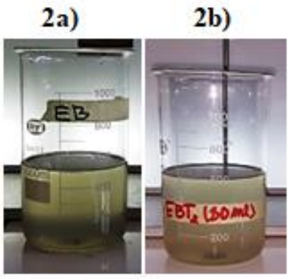

Figure 2 Domestic raw water samples

2a) Sample of wastewater 259 NTU,
2b) Sample of wastewater 160 NTU**.**

Figure 3 Sample of 259 NTU treated with the solution obtained of vegetable oil from soursop seeds

Figure 4 Sample of domestic wastewater of 259 NTU treated with the solution obtained of almond powders from soursop seeds

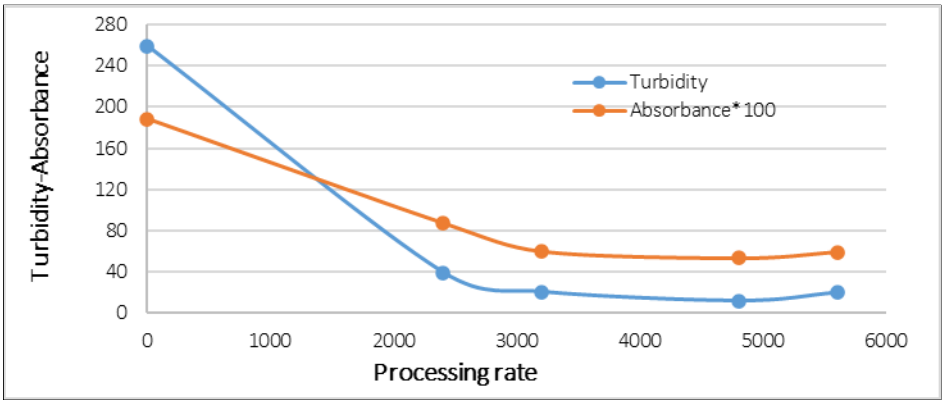

Figure 5 Variation in turbidity and absorbance of domestic wastewater 259 NTU as a function of treatment rates of solution obtained of almond powders in 30 min of decantation

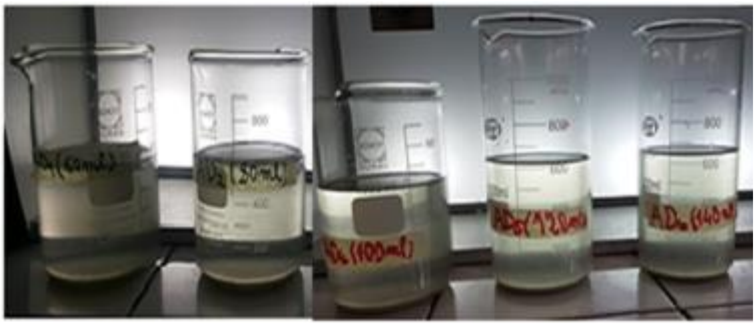

Figure 6 Sample of domestic wastewater of 160 NTU treated with the solution obtained of almond cakes from soursop seeds

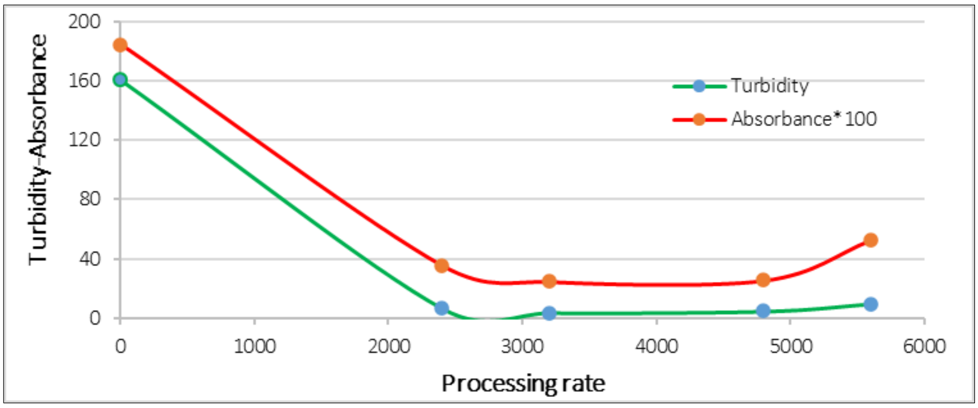

Figure 7 Variation in turbidity and absorbance of domestic wastewater 160 NTU as a function of treatment rates of solution obtained of almond cakes in 30 min of decantation

Figure 8 Sample of domestic wastewater of 160 NTU treated with the solution obtained of integument powders from soursop seeds

EBT: Samples of raw water treated with the solution of integuments from soursop seeds

Figure 9 Sample of domestic wastewater of 160 NTU treated with the solutions obtained of almond and integument ashes from soursop seeds

EBAC: Raw water samples treated with the solution obtained of almond ash, EBTC: Raw water samples treated with the solution obtained of integument ash.

4. Conclusion

This work focused on the identification of chemical constituents with coagulant properties of *Annona muricata* L. (Soursop) seeds in the treatment of domestic wastewater. The results obtained revealed the presence of carbohydrates, proteins, lipids, terpenes and sterols in almonds and integuments ; flavonoids and tannins in the integuments of these soursop seeds. These seeds presented high contents of organic compounds (66.15 %) as well as mineral compounds (00.97 %) and volatile compounds (32.88 %). It is noted that these soursop seeds contain 30.56 % lipids. These results prove that these seeds also contain other organic compounds at a content of 35.59 %. These compounds could be proteins, amino acids, carbohydrates or starch.

The evaluation of the clarifying activity of soursop seeds on domestic wastewater samples with turbidities of 160 and 259 NTU showed that these seeds have a removal rate of colloids and/or suspended matter of 95.40 % with its almond powders and 97.58 % with its almond cakes at respective doses of 4800 mg powder/L and 3200 mg powder/L in 30 minutes.

It is important to note that to our knowledge, the indications given on the activity of clarifying wastewater by soursop seeds have not yet been the subject of scientific work making it possible to identify the compounds responsible for this activity. However, before using these seeds, it is therefore important to extract the oil from these seeds because it contains compounds that increase turbidity. This separation of vegetable oil from the almonds cakes improves the clarifying activity of domestic wastewater.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare that they have no conflict with any third party.

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