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The Coagulant Activity of the Seeds of *Psidium guajava* L. and the Episperm of *Persea americana* Mill. in Samples of Water from the Bogotá River (Chocontá-Villapinzón)

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Abstract

Water is fundamental in all ecosystems and indispensable for different biological processes: anthropic processes have altered the quality and quantity of water and caused imbalances. The water in the upper basin of the river Bogotá shows high levels of turbidity, due to non-dissolved solids like algae, muds, microbes or other particles, caused by the shedding of wastes, which absorb and disperse the light which pierces them, and thus increase the degree of turbidity and reduce the quality of the water. **Aim**: To assess the effect, as natural coagulants, of the seeds of *guayaba* (guava), *Psidium guajava* L, or the episperm of the seeds of avocado, *Persea americana.* **Method**: We used the jar test with parameters of design and control in standardized conditions, different combinations of doses of the coagulant and a rapid mixture time, velocity gradient and sedimentation time for the evaluation of three extracts of each seed in its respective solution of water, one with 10% ethanol and another with 10% acetic acid. Three tannins were used as an organic control: *quebracho* (*Schinopsis balansae*), *mimosa* (*Acacia dealbata*) and *castaño* (chestnut, *Castanea sativa*); and aluminum sulfate () was used as an inorganic control. The water samples were taken from the upper basin of the río Bogotá in La Marinilla sector, near the road between Chocontá and Villapinzón. **Findings**: In all cases, it was found that the use of the extract of the *Psidium guajava* L in a water solution with 10% of acetic acid reduced the levels of turbidity, with an effectiveness of 99.15% and 98.36%. The results obtained from the episperm of the seeds of avocado, *Persea americana* Mill. likewise showed a reduction in the levels of turbidity, with the extract in a solution with 10% of acetic acid the most effective, at 80.45%. Castaño was the tannin which showed the highest yield, with an effectiveness of 93.72%. **Application**: This investigation not only showed benefits in the search for new alternatives for natural coagulants but it also highlighted the importance of being able to reuse industrial residues.

Keywords: Coagulant Activity, *Persea americana* Mill. *Psidium guajava* L

1. Introduction

The contamination of waters is a cause of millions of deaths in the world, which is the reason why it is necessary to make water potable with basic treatments like clarification, disinfection, and chemical and organoleptic treatment. Clarification is an important stage in the potabilization of untreated water: it includes the coagulation-flocculation process, in which the particles found in the water are agglomerated to form small granules

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with a higher specific gravity. Thus, the particles settle (sediment) and the materials in suspension are removed, which enables the water to attain the physical and organoleptic characteristics which make the water apt for human consumption, in accordance with public health standards and norms[1].

When the appearance of the water is unpleasant, it means that it is not free of turbidity and thus indicates its physical-chemical and ecosystemic conditions suffer from alterations which range from a higher temperature or offensive odors to the loss of species. To solve these problems, communities which rely on such waters have used conventional methods of water treatment, like coagulation, which consists of removing the suspended particles with the use of chemical coagulants[2]: there are a wide range of coagulants and flocculants which are commonly used to clarify different types of waters. Generally, they fall into two categories: inorganic and organic. The inorganic ones are usually made up of metallic salts, normally those of aluminum or iron (the most widely used are aluminum sulfate, ferric chloride and iron sulfate), while the organic ones are polymers (polyelectrolytes), which refer to a large variety of macro-molecular compounds – natural or synthetic in origin and soluble in water – which have the capacity to strengthen the flocculation of the suspended particles. The conventional coagulants and flocculants, which are based on aluminum salts and polymers, present some problems, like the need to use a large amount of the substances and the difficulties of storing and handling them and determining the correct doses. However, they have some very severe risks. They may contaminate the environment and be toxic for human beings, causing illnesses like cancer or Alzheimer´s. Other disadvantages associated with them are the production of muds, variations in pH levels and high costs[3]. Due to the latter factor, there has been a need to find new alternatives which would replace the function of the conventional coagulants. These alternatives make use of natural extracts of plants to clarify raw water[4]. Growing studies in this field have used vegetal species like *Moringa oleifera*[5–11], extracts of the seeds of beans[12], toasted grains of maize (*Zea mays*), *Cicer arietinum* and *Dolichos lablab*[13], *Opuntia ficus-indica cactus*[14], *Strychnos potatorum*[15–17], Okra[18], *Manihot esculenta Crantz*[19], *Opuntia cochenillifera*[20] and *Cactus lefaria*[21].

In many countries, aluminum sulfate ($Al_2(SO_4)_{3(s)}$), commonly known as alumina, or ferric chloride ($FeCl_3$) is used as the traditional coagulant. For years now, organic polymers have been used as aids to coagulation[22] and natural tannins as coagulants[23]. A commercial cationic polymer, based on tannins (TBP), has been used to determine their basic chemical properties and their behavior as a coagulant.

The upper basin of the río Bogota lies between the municipality of Villa Pinzón and the Salto de Tequendama waterfall: it has a surface area of 6000 km^2 and a length of 380 kilometers. The río Bogotá is born in the Páramo (high Andean moor) de Guacheneque, at an altitude of 3,300 masl. It is a crucial part of the lives of a number of towns and the capital of Colombia, Bogotá, which are responsible for economic and social dynamics, which act on the environment as their inhabitants draw on the region´s environmental services. Culture is the factor, which mediates between the socio-economic conditions and the environment, and it is the culture of the region, which determines the use of the environment. In its upper course, the río Bogotá receives organic wastes from a population of around eight million inhabitants and wastes from the many industries there[24].

In view of the abovementioned circumstances, the aim of this study is to assess the effect, as natural coagulants, of the seeds of guayaba, *Psidium guajava* L, or the episperm of the seeds of avocado, *Persea americana*.

2. Methods

2.1 Gathering of the Seeds and Water Samples to be Analyzed

The water samples were taken from the río Bogotá in the sector known as la Marinilla, which lies on the road between Chocontá and Villapinzón, where the respective characterization was made. The *guayaba seeds* (*Psidium guajava* L) were gathered at the Bocadillo Manuel Quiroga factory (Bogotá) and the avocado seeds (*Persea americana*) at the Guacamole Listo S.A.S. factory (Bogotá).

2.2 Preparation of the Coagulants (Controls)

The control samples of aluminum sulfate and tannins (*quebracho, mimosa and castaño*), at 20,000 ppm, were prepared and two grams of each one were weighed and diluted in 100 ml of T1 water.

2.3 Preparation of the Extracts

The extracts of the seeds were prepared at a concentration of 20,000 ppm. To do that, two grams of each sample

(*Persea americana* and *Psidium guajava*) were weighed, and a reflux was undertaken with acetic acid at 10%, ethanol at 10% and TI water as solvents for two hours at boiling point. Later, the liquids were cooled, filtered and diluted to a final volume of 100 mL.

2.4 Jar Test

The analysis was undertaken with the jar test, with parameters of design and control in standardized conditions, with different combinations of coagulant dose, concentration, pH, rapid mixing time, speed gradient, as well as sedimentation time for the turbidity of the supernatant.

The jar test examined concentrations of 50, 100, 200 and 300 ppm, and proceeded in the following way: a) for a concentration of 50 ppm, 1.25mL of the extract and 498.75 mL of the water from the river Bogotá were used; b) for a concentration of 100 ppm, 2.5 mL of the extract and 497. 5 mL of the river water were used; c) for a concentration of 200 ppm, 5 mL of the extract and 495 mL of the river water were used; and (d) for a concentration of 300 ppm, 7.5 mL of the extract and 492.5 mL of the river water were used.

The equipment was programmed for a rapid mixture of 125 rpm for five minutes and a slow mixture of 50 rpm for twenty minutes, followed by 30 minutes during which the mixture was allowed to settle. This procedure was repeated for each of the prepared solutions. Finally, the turbidity was measured with a Hanna Instruments HI88703 turbidity meter, following the standardized methods of the American Public Health Association (APHA)[25]. In all the jar tests, a standard was used to verify the activity of the natural coagulants. The effectiveness of the coagulation of the extracts was determined as a function of the percentage of the removal of turbidity and calculated with the use of the following equation:

Equation 1: %removal of turbidity= {(initial turbidity-final turbidity)/initial turbidity} *100

Five assays were made of each of the concentrations tested with the different coagulant extracts. The results were averaged and adjusted to linearity for the analysis. Based on the data obtained on the percentage of turbidity caused by the aluminum sulfate, a reference curve was built to verify the lineal dependence of the percentage of the removal of the turbidity compared to the concentration (ppm) of the aluminum sulfate. With the linear equation y=a x + b, the 50 turbidity standard (T_{50}) was calculated, which yielded 62.97 for the aluminum sulfate. The relative turbidity (RT) of the samples, compared to the standard (aluminum sulfate), was determined with the following equation:

Equation 2: Relative turbidity = (T_{50} sample/ T_{50} standard) * 100

Assessment of the presence of tannins in the *Psidium guajava* L seeds and the episperm of the *Persea americana.*

To determine the tannins in the seeds, thin-layer chromatography was used, with solvents like BAW (butanol + acetic acid+ water; 6:4.1) and methanol + acetic acid (1:1), developed with ferric chloride ($FeCl_3$). It revealed a blue color, which indicates the presence of tannins, derived from gallic acid and a green color where there are tannins derived from protocatecholic acid.

2.5 Statistical Analysis

The turbidity standard (T_{50}) was calculated with a linear regression. To determine whether there were significant differences in the turbidity removal percentage of the different concentrations, a two-way ANOVA analysis was made. The difference were regarded as significant for a $p<0.05$. The GraphPad prism 6.0 program was used for the analysis.

3. Results and Discussion

Table 1 shows the parameters of pH, temperature and conductivity of the samples of water gathered from the river Bogotá. When the abovementioned methodology was used, with the percentages for the removal of turbidity shown in Figure 1, the % of turbidity was found and a lineal adjustment was made. Figure 1 then shows the turbidity standard (T_{50}) and the relative turbidity, which is the value the concentration needs to reduce the turbidity of the water samples by 50%. The coagulant activities of the extracts of the seeds were compared with the coagulant activity of the aluminum sulfate and tannins (*quebracho, castaño* and *mimosa*) in samples of water gathered from the upper basin of the river Bogotá, in the sector known as la Marinilla. A greater reduction of the levels of turbidity with the *Psidium guajava* L was seen in the extracts in 10% acetic acid and T1 water, with an effectiveness of 99.15% and 98.36%, respectively, as shown in Table 2 for the episperm of *Persea americana,* the watery

Table 1. Characteristics of the water sample

Parameter	Value	Unit
pH	6.85	$[H^+]$
Temperature	26	**°C**
Conductivity	728	**µS**

extract was the most effective, at 82.60%. By contrast, the extract, which showed the lowest yield, was the ethanol one, with an effectiveness of 80.07%. The *Psidium guajava* extract in acetic acid showed the greatest effectiveness – 99.15% -- followed by the watery extract, at 98.36%. In general, a reduction of the levels of turbidity was seen; in most cases, the 50 ppm concentration yielded the best results (Figure 1). The two-way variance analysis shows that there are significant statistical differences ($p<0.0001$) when the samples of water from the river Bogotá are subjected to different treatments, but that the concentration, as a source of variance, does not have an influence on the percentage of the removal of turbidity, which implies that the 50 ppm concentration is the ideal one. The development of the chromatographic plate with ferric chloride indicates the presence of tannins in the two kinds of seeds, specifically derived from gallic acid (gallotannins). Gallic acid is the base unit of many types of tannin, which are polyelectrolytes with a heavy molecular weight whose involvement in the coagulation processes has already been demonstrated[26]. Thus, those compounds may be related to the coagulant activity obtained from the *Psidium guajava* L seeds, which showed higher yields than the episperm of the *Persea americana.*

The behavior of the tannins (*quebracho, castaño*and *mimosa*) was different from what was expected, taking into account their use as controls. The effectiveness of the *quebracho*was 85.31%, which was quite different from that of the aluminum sulfate used as a positive control. It is important to mention the yield obtained from the extract of *Psidium guajava* L in water, since it was not expected that an extraction in a watery medium would have higher yields than the extraction in 10% of ethanol or that in 10% of acetic acid. It was even higher than the three tannins in question and in some concentrations; its effectiveness was very close to that of the aluminum sulfate as Figure 2 shows, the extract in acetic acid of the seeds of *Psidium guajava* was 87% more effective than the extracts and preparations which are commonly used as coagulants, as was the watery extract, which was 78% more effective. This is evidence of the important potential

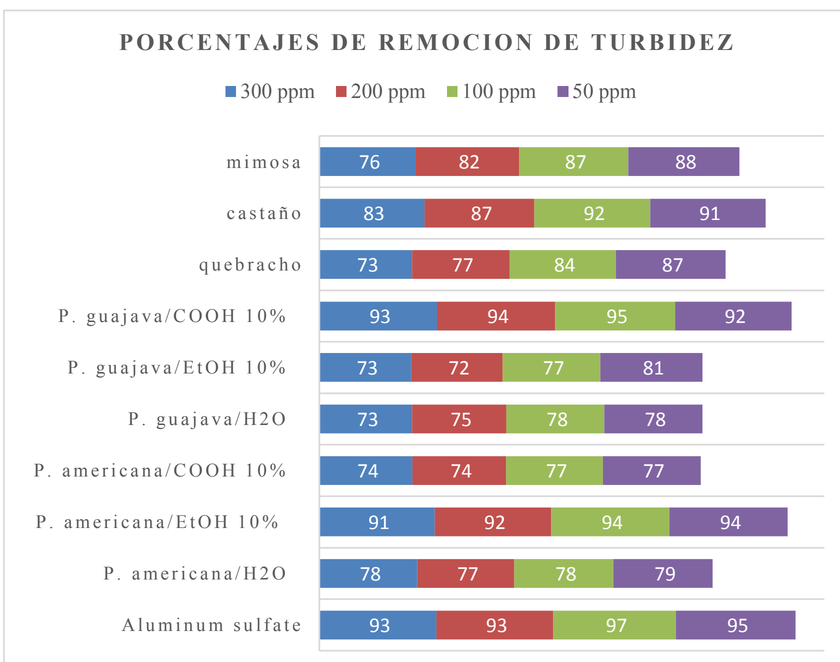

Figure 1. Percentages of the removal of turbidity by *Psidium guajava* L and *Persea americana* at different stages of the experiments.

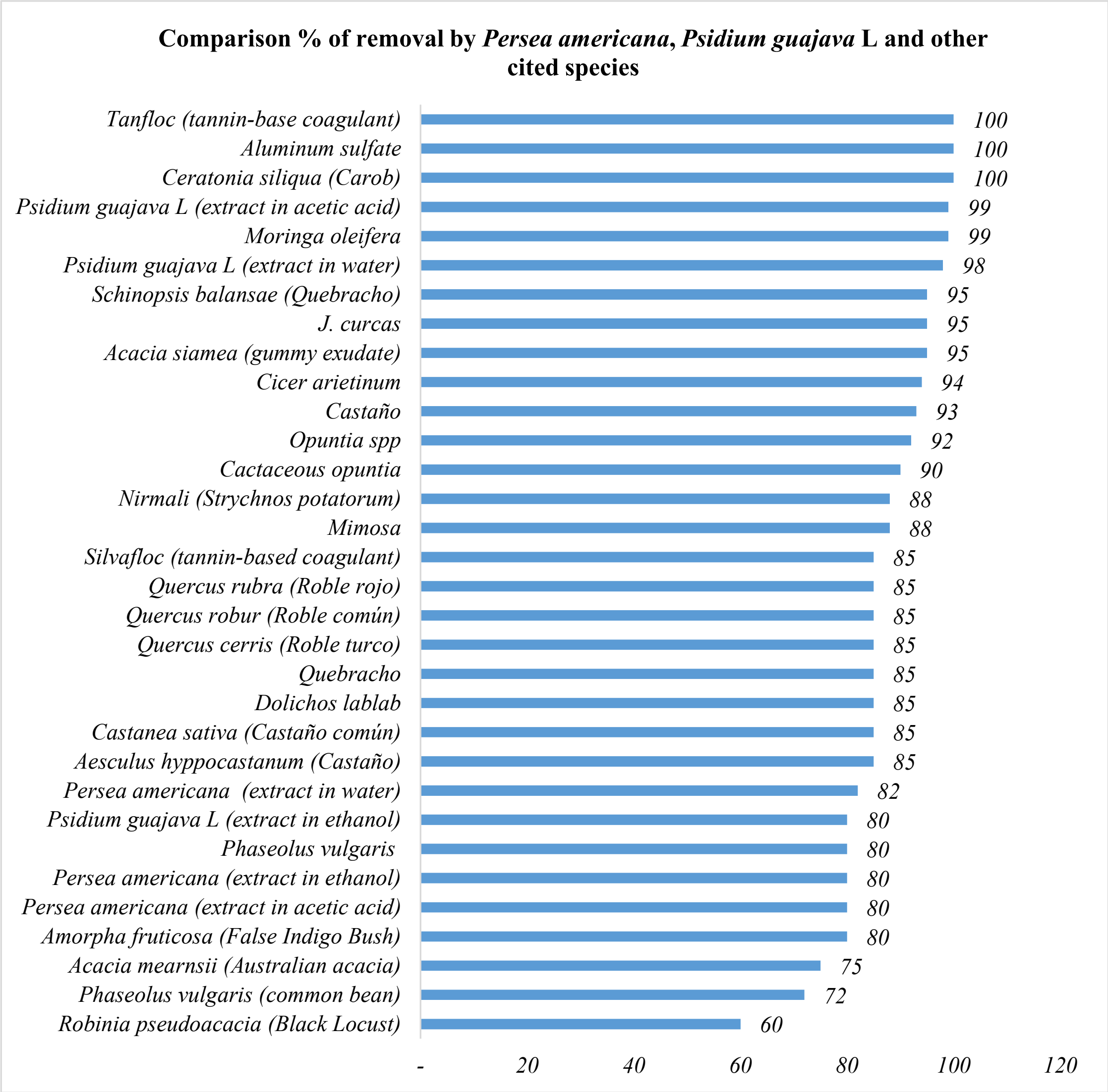

Figure 2. Comparative % of removal by *Persea americana*, *Psidium guajava* L and other cited species.[13,16,17, 27–36]

Table 2. T_{50}, RT and % of effectiveness in water samples from the river Bogotá of the coagulant substances and extracts *** p<0,001, ANOVA, Tukey`s post hoc test

Evaluated Substance	T_{50}	Relative Turbidity	% Effectiveness
Aluminum Sulfate	62.97	1.00	100.00
Persea americana (Extract in water)	76.23	1.21	82.60
Psidium guajava L (Extract in water)	64.02	1.01	98.36***
Persea americana (Extract in ethanol)	78.64	1.24	80.07
Psidiumguajava L (Extract in ethanol)	78.29	1.24	80.43
Persea americana (Extract in aceticacid)	78.27	1.24	80.45
Psidium guajava L (Extract in acetic acid)	63.50	1.00	99.15***
Quebracho	73.81	1.17	85.31
Castaño	67.18	1.06	93.72
Mimosa	71.41	1.13	88.18

of the extracts of these seeds as possible alternative agents for the purification of contaminated waters.

4. Conclusion

On the basis of the results obtained and their subsequent analysis, we conclude that of the six extracts which were evaluated, the seeds of *Psidium guajava* L in 10% of acetic acid were the most effective when compared with the aluminum sulfate control. In terms of the percentage of removal of turbidity, the study showed that the effectiveness of the extracts in acetic acid and in water of the seeds of *Psidium guajava* did not differ from that of the aluminum sulfate used as a positive control. It is worth highlighting the benefits of this study, not only in the search for new alternatives to conventional coagulants, but also because of the importance of being able to reuse the residues generated by the industries which use fruits with seeds which have hydrolysable tannins as a raw material, as in the case of the Manuel Quiroga SA factory of *bocadillos* (guava jelly sweets) and the guacamole factory, Guacamole Listo, S.A.S. This may pave the way for future investigations of residues, which may have the capacity to be reused as coagulants. It is also important to highlight the results obtained from the seeds of *Psidium guajava* L in water, whose percentages of yield were lower than those of the extracts of *Psidium guajava* L in acetic acid, but even so, continue to show excellent results, which indicates the strong coagulant capacity of this extract and thus implies its feasibility in that respect, since one would only need the seeds and little water to obtain an effective and economic coagulant, especially in vulnerable communities like those of the small farmers of Colombia.

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