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CARICA PAPAYA SEED EXTRACTS AS AN ALTERNATIVE TREATMENT FOR CONTAMINATED WATER

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Abstract

Water is undoubtedly an essential resource for humanity and a key factor in the quality of life of populations worldwide. To decrease water turbidity, aluminum sulfate, an inorganic coagulant is used. However, this compound is unable to meet current demand. Therefore, alternative options from natural plant species are sought. *Objective*: Determine coagulant activity from aqueous, 10% ethanol, 10% acetic acid extracts obtained from dried and fresh papaya seeds (*Carica papaya*), in comparison with positive controls: aluminum sulfate $Al_2(SO_4)_3$, wattle tree (*Acacia mollissima*), willow-leaf red quebracho (*Schinopsis balansae*) and chestnut (*Castanea sativa*). *Methods*: The laboratory flocculation test known as the jar test developed by the Department of Environmental applied microbiology, University of Hungliga Tekniska Högskolan, Stockholm, Sweden was employed. *Results*: All evaluated treatments presented a coagulant effect and were significant in comparison with untreated water ($p < 0.0001$). A greater coagulant effect was observed with dry seed ethanol extract at 2500 ppm, with a 66.45% effectiveness in comparison with aluminum sulfate. *Conclusions*: Obtained extracts from Carica papaya improve water's physico-chemical properties in terms of turbidity reduction.

Keywords: : *Coagulation, Natural Coagulants, Carica papaya, Turbidity, Gallotannins*

Introduction

Water is undoubtedly an essential resource for humanity and a key factor in the quality of life of populations. It is indispensable for ecosystem environmental equilibrium, for regional economic development and productivity, and in general contributes with the wellbeing of communities [1]. However, drinking-water sources are under increasing threat from contamination. Worldwide this is one of the main problems, increasing on a daily basis with negative repercussions on health [2].

The World Health organization (WHO) disclosed most diseases could be prevented if water supply, sanitation, hygiene and proper water resource management was improved. Three out of 10 people (2.1 billion people) lack access to clean and available water in the home, and six out of 10 people (4.5 billion) lack safe sanitation worldwide [3]. None the less, to achieve its potability, elementary treatments are necessary, such as clarification, which includes a unitary process of coagulation-flocculation, where particles present in water are agglomerated forming small granules with a specific weight, greater than water. In this manner, particles sediment and facilitate removal of material in suspension. Hence, water can attain its physical and ideal organoleptic qualities for human consumption, according to public health norms and standards [4].

Among the most employed coagulants are aluminum sulfate $Al2(SO4)3$, yet in humans it is a toxic compound if ingested frequently. Likewise, aluminum is associated with development of diseases, such as Alzheimer's disease [5]. Chemically, it can generate large quantities of alum sludge that has a negative impact on soils and water if not employed as biosolids [6].

Various studies and methodologies have been reported regarding the design of tannin-based bio-flocculants for wastewater treatment (aminomethylation of condensed tannin) [7-9]. The aforementioned, supports the use of tannins from Australian acacia (*Acacia mearnsii*), quebracho (Schinopsis *balansae*), wattle tree (*Acacia mollisima*) and chestnut (*Castanea sativa*) in clarification processes with aluminum sulfate co-treatment. Thus, the objective of the present work was to evaluate coagulant activity from extracts obtained from papaya seeds (*Carica papaya*) on water samples collected from the upper basin of the Bogotá river, Guaymaral-Chía sector.

Methods

Water and seed sample collection

Water samples were collected from the upper basin of the Bogotá river, at the Guaymaral-Chía sector (Cundinamarca-Colombia; N 04°51'56.4" W 074°02'24.9"). Maradol papaya seeds (*Carica papaya*) were obtained in March, 2019 from the Pato Company located in Bogotá,

Extract and coagulant preparation (positive controls)

Carica papaya seed extracts (aqueous, acetic acid or ethanol) were obtained by reflux from 2 g of *Carica papaya* seeds using 100 mL of the following solvents (20000 ppm) water, 10% acetic acid or 96% ethanol. The process was carried out for two hours. Coagulant positive controls, aluminum sulfate and quebracho, wattle tree and chestnut tannins were prepared in water at 20, ppm and 10000 ppm.

Coagulant activity

The study took into account the methodology developed by the Department of Environmental applied microbiology, University of Hungliga Tekniska Högskolan, Stockholm, Sweden. The technique consisted of measuring transmittance of a synthetic water suspension (kaolin and clay) at 500 nm, where the problem sample was compared under the same conditions before and after treatment (Jar test) [10].

Extracts were assayed at the following concentrations 200, 500, 1000, 1500, 2000 and 2500 ppm. Extracts and water were mixed with magnetic stir bar agitation for 30 minutes. Following, the samples were allowed to decant for two hours. The volume of the mix was taken to a final volume of 5 mL, maintaining the concentrations of the assayed coagulants.

Transmittance, as a turbidity measurement, was recorded before and after treatment employing a UV-Visible spectrophotometer (Milton Roy Spectronic 21D). The same procedure was performed on positive controls. Percentage coagulant activity was calculated using equation

Coagulant activity (%) = ((inicial turbidity-Final turbidity))/(Inicial turbidity) x100

Gallotannin determination

To quantify gallotannin content in *Carica papaya* seeds the rhodanine method was used. This method consists of gallotannin hydrolysis under anaerobic conditions, where gallic acid reacts with rhodanine, producing a pink chromophore [11].

Sample preparation

5 mL of 1 M H_2SO_4 were mixed with 1 ml *Carica papaya* seed extract in 70% acetone in a test tube with screw lid. The sample was heated for 26 h at 100°C. Following, the sample was cooled and brought to 50 mL with deionized water

Procedure

1 mL of sample and 1.5 mL reactant (0.667% rhodanine solution in methanol) were mixed. After 5 minutes 1 mL 0.5 M KOH was added. Last, the solution was diluted with water until 25 mL was attained. Absorbance was quantified at 520 nm. A gallic acid standard curve (1.000, 0.500, 0.250, 0.125 and 0.0625 mg/mL gallic acid in methanol) was prepared to calculate gallic acid equivalents. Results are presented as gallic acid equivalents.

Statistical analysis

Transmittance values are presented as mean ± SEM. A two-way ANOVA was used to determine significant differences in comparison with untreated water. Independent variables: treatments and concentrations. A Tukey post-hoc test was performed to establish differences among groups. A $p < 0.05$ was considered significant.

Results and Discussion

This study evaluated *Carica papaya* seed extract coagulant capacity (prepared in either water, 10% acetic acid or 96% ethanol) and compared them with frequently used coagulants (aluminum sulfate and tannins). In table 1 and figure 1 coagulant activities are presented for different treatments and controls, as turbidity removal efficiency (mean ± SEM).

Effect of different *Carica papaya* seed extract as a function of concentration in comparison with untreated water is illustrated in figure 2. For untreated water the same dilution procedure was used as for the rest of the treatments.

Carica papaya seed extracts gallotannines quantification was determined by the rhodanine method. From the calibration curve (absorbance vs. concentration) a concentration value of 0.046 mg/mL was calculated from the absorbance read. Taking into consideration the dilution factor and *Carica papaya* dry seed weight a 3.54% of gallotannins was obtained expressed as gallic acid equivalents.

According to obtained data, all evaluated treatments presented a significant coagulant effect ($p < 0.0001$) in comparison with untreated water. The highest coagulant effect was observed for 2500 ppm *Carica papaya* dry seed ethanol extract with an efficiency of 66.45% in comparison with aluminum sulfate.

In general, when analyzing extract coagulant effect based on seed characteristics (dry or fresh) for most cases it can be highlighted, there was a higher coagulation capacity in extracts prepared from dry seeds.

For all *Carica papaya* seed extracts the transmittance was lineal with respect to different concentrations used (concentration dependent behavior), taking into account results presented in figure 2. For the aqueous extract obtained from fresh seeds, significant differences were observed among concentrations at 1000, 500 and 250 ppm in comparison with the highest concentration (2500 ppm). Hence, suggesting a concentration of 1500 ppm could be used, obtaining the same effect as if it were the highest. For dry plant material in water, acetic acid and ethanol, significant differences were observed from 1500 to the lowest concentration, presenting a similar behavior between 2500 and 2000 ppm. The same analysis for fresh seed extract revealed that a significant difference between concentrations was only observed for acetic acid extract at the highest concentration in comparison to the rest. For fresh seed ethanol extract, the same coagulant effect was observed from 1,000 ppm to the highest concentration. Furthermore, a higher coagulant activity was observed for ethanol extract with dry seeds.

Different studies have analyzed natural's products coagulant activity in waters with kaolin and clay [12,13] , among them *Moringa oleífera*, [14-16] , one of the most effective natural coagulants studied in the field, as well as *Phaseolus vulgare* [17] and certain asteraceaes [18].

Gallotannin percentage found in *Carica papaya* seeds (3.54%) exceeded that obtained from plants used as natural coagulants, such as those reported for *Quercus rubra* and *Diospyros virgiana*, with values of 0.88% and 0.36%, respectively [11]. Presence of these compounds derived from gallic acid and some polymers could explain, in part, papaya seed extract coagulant effect. In a study performed to evaluate *Moringa oleífera* coagulant's effect, extract rich in tannins, an 80% efficiency was observed [19-28] .

Likewise, in a study performed by [29], where *Psidium guayava* L and *Persea americana* seed extract was obtained to analyze coagulant activities under similar extracts and conditions, it was evidenced aqueous, acetic and ethanol extracts presented higher percentage efficiency in comparison to *Carica papaya* seed extracts employed in this study

In general, water sample treatment, collected from the upper basin of the Bogotá river in the Guaymaral-Chía sector, with *Carica papaya* seed extract improved its physicochemical conditions, in terms of turbidity reduction. A greater coagulant effect was observed for the dry seed ethanol extract at 2500 ppm, with an efficiency of 66.45% in comparison with aluminum sulfate. This coagulant effect could be associated with the presence of hydrolyzable tannins.

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Table 1. Average Turbidity Removal Efficiency

Treatments	Concentration (ppm)					
	2500	2000	1500	1000	500	250
Fresh seed aqueous extract	71.3 ±1.33	63.4±079	60.6±2.46	53.9±0.14	41.3±3.34	41.8±4.20
Dry seed aqueous extract	65.8±1.43	58.5±3.36	52.9±2.16	49.5±3.74	47.0±5.17	45.1±3.59
Fresh seed acetic acid extract	70.5±0.54	47.3±6.44	37.9±5.61	27.2±0.42	23.1±0.71	25.6±2.18
Dry seed acetic acid extract	63.7±1.02	56.4±1.31	49.9±5.29	45.5±11.3	28.4±8.13	25.9±2.83
Fresh seed ethanol extract	65.9±2.84	58.2±0.55	55.7±3.92	55.5±2.71	51.8±3.28	43.8±6.86
Aluminum sulfate	88.9±0.13	88.0±0.40	87.0±0.35	86.2±0.52	85.1±0.30	84.4±0.08
Wattle tree	88.7±0.06	86.3±0.77	77.9±0.25	74.1±1.10	70.9±0.86	65.3±2.82
Chestnut	79.1±1.73	75.7±2.82	72.0±0.80	65.0±3.99	61.6±3.10	55.1±3.57
Quebracho	85.9±0.43	82.5±0.85	81.1±0.90	79.7±0.79	77.8±0.62	75.4±0.86

Figure 1. Turbidity removal percentage

Turbidity removal percentage compared with untreated water and positive controls. Turbidity removal efficiency of the 10 treatments at 2500, 2000, 1500, 1000, 500 and 250 ppm, respectively. Dry seed ethanol extracts were the treatment with the highest coagulation effect for all concentrations evaluated (bars boxed in red). **** p < 0.0001 (Two-way ANOVA).

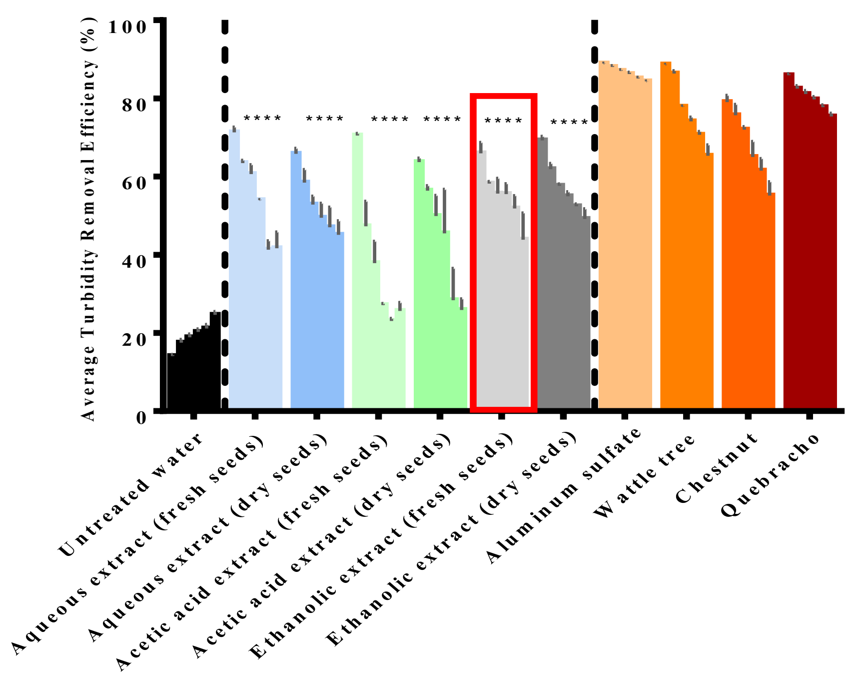

Figure 2. Extract concentration effect. *Carica papaya* seed extract in aqueous solution, 10% acetic acid or 10% ethanol extract at different ppm concentrations.